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Hypomorphic mutation in the hairless gene accelerates pruritic atopic skin caused by feeding a special diet to mice

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Background

Atopic dermatitis (AD) is a common skin disease characterised by dry skin, impaired barrier and pruritus. AD is thought to result from complex interactions between multiple genetic and environmental factors (1). We have shown that HR-1 hairless mice that are fed a special diet (called HR-AD) instead of a normal diet develop pruritic dermatitis resembling human AD (2). Previous studies have shown that skin barrier dysfunction could be involved in itch-related scratching in HR-AD-fed HR-1 mice (2,3). Furthermore, we have recently determined that dietary deficiencies of polyunsaturated fatty acids (PUFAs) and starch are responsible for diet-induced AD (4,5). However, it has not been established whether such pruritic dermatitis can be replicated in other mouse strains; therefore, genetic influences on diet-induced AD remain unclear.

The hairless (*Hr*) gene is highly expressed in the mammalian skin and appears to play a critical role in skin homeostasis (6). HR-1 hairless mice carry a hypomorphic mutation in the *Hr* gene (7), but it remains unclear whether this mutation affects the development of diet-induced AD.

Questions addressed

In this study, using five different strains of mice, we examined the influence of genetic background on diet-induced AD. Furthermore, using a newly generated hairless congenic strain carrying a hypomorphic *Hr* mutation, we also examined the impact of *Hr* mutation on the development of pruritic atopic skin caused by feeding HR-AD.

Experimental design

See supplementary information (Data S1).

Results

Five different strains of mice (HR-1, BALB/c, C57BL/6, ddY and ICR) were fed either a normal diet or HR-AD for 12 weeks. Because dry skin, barrier dysfunction and itch-related scratching (as evaluated by prolongation of one scratching bout) are the initial signs of diet-induced AD (2,3), skin hydration, transepidermal water loss (TEWL) and the duration of one scratching bout were measured over time and evaluated as the area under the curve (AUC) during the 4–12 weeks of feeding. Considering these time-courses (Fig. S1 and Data S2), the summarised AUC data (Fig. 1) indicated that although barrier-defective dry skin and itch-related scratching developed in all strains fed HR-AD, the onset was earlier and the severity was greater in HR-1 mice than in the other four strains. For information on gross appearance and histological and immunological analyses at the 12th week, please refer to the supplementary materials (Figs. S2–S4, Table S1 and Data S2).

Of the five strains, only HR-1 mice carry a hypomorphic mutation (Hr^{hr}) in the *Hr* gene (7). Therefore, we hypothesised that this mutation would be involved in the increased susceptibility to diet-induced pruritic atopic skin. To assess this possibility, we used a newly generated hairless congenic strain in which the mutated gene in HR-1 mice was introduced into the low-responder ICR mice. By polymerase chain reaction, we confirmed the presence of Hr^{hr} allele (the 439-bp band) in the genomic DNA of HR-1 and the hairless congenic mice but not in that of ICR mice (Fig. 2a); thereby designating this hairless congenic strain as ICR- Hr^{hr} . When ICR, HR-1 and ICR- Hr^{hr} mice were fed HR-AD for 4 weeks, ICR- Hr^{hr} mice as well as HR-1 mice exhibited barrier-defective dry skin and itch-related scratching (Fig. 2b–e).

Conclusions

Our study showed that the hypomorphic *Hr* mutation was essential for the full development of diet-induced pruritic atopic skin. How does this mutation accelerate the disease development? The *Hr* gene product HR has been shown to function as a corepressor of several nuclear receptors, such as the vitamin D receptor (VDR) (6); HR can not only support VDR-mediated transrepression but also suppress ligand-induced VDR transactivation (s1–s3). Retinoid X receptor (RXR) is unlikely to interact with HR, but can form a heterodimer with VDR to repress the transcription of target genes (s4). Intriguingly, a null (i.e., complete loss-of-function) mutation of HR, VDR or RXR results in a similar skin phenotype including hair loss and abnormal epidermal proliferation and differentiation (s5–s7). Furthermore, Li *et al.* demonstrated that either keratinocyte-selective ablation of RXR α and RXR β or topical application of a VDR agonist calcipotriol, both of which relieved a transcriptional repression of the VDR-RXR heterodimer, induced AD symptoms in mice (8,9). These findings suggest a mechanistic link between HR and the VDR-RXR heterodimer in the development of AD symptoms. However, since the skin of normal diet-fed HR-1 mice was intact, the hypomorphic (i.e., partial loss-of-function) mutation in the *Hr* gene alone is not enough to induce such pruritic atopic skin. As shown recently, HR-AD-induced symptoms were due to deficiencies of PUFAs and starch (4,5). PUFA deficiency leads to an inflammatory skin condition and skin barrier dysfunction (s8). Thus, the *Hr* mutation can synergise with these deficiencies to accelerate disease progression. Accordingly, our recent preliminary experiment showed that topical application of the relatively low dose of calcipotriol, which did not induce any skin lesions in normal diet-fed HR-1 mice, could exacerbate skin symptoms in HR-AD-fed ones (M. Fujii, unpublished observation). Thus, the high susceptibility of diet-induced pruritic atopic skin in *Hr* mutant mice may mediate through increased VDR transactivation. On the other hand, the relationship between increased VDR

transactivation and deficiencies of PUFAs and starch remains unclear and requires further investigation.

Mutations in the human *HR* gene have been revealed to be the cause of congenital hair loss disorders such as alopecia universalis congenita and atrichia with papular lesions (s9), but whether this mutation is associated with human AD is yet unknown and should be studied in the future. We also need to investigate whether acquired down-regulation and/or hypofunction of HR occur in AD patients.

Hairless mice such as SKH1 and HR-1 have sometimes been used to create an AD model (s10–s13), probably because depilation is not required before every procedure. Our results here provide evidence that the *Hr* mutant mice have some genetic predisposition to AD. Further elucidation of the protective role of the *Hr* gene in AD not only helps to understanding the pathogenic mechanism of this disease, but also provides clues for developing new therapeutic drugs.

Acknowledgements

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Author contributions

M.F. designed the research study, conducted the research, analysed the data, and wrote the manuscript. F.O., A.I., K.D. and J.T., conducted the research and analysed the data. N.I. established ICR-*Hr^{hr}* mice and critically revised the manuscript. S.K., T.N. and S.O. contributed to the interpretation of the data and critically revised the manuscript.

Conflict of interests

The authors have declared no conflicting interests.

Supporting information

Additional supporting data may be found in the supplementary information of this article.

Figure S1. Time courses of skin hydration, TEWL and spontaneous scratching behaviour.

Figure S2. Skin appearance at the 12th week.

Figure S3. Histological analysis.

Figure S4. Serum IgE levels.

Figure S5. ICR-*Hr^{hr}* mice did not fully replicate mast cell hyperplasia.

Table S1. Statistical comparison between normal diet- and HR-AD-fed mice.

Data S1. Materials and methods.

Data S2. Additional results and discussions.

Appendix S1. Additional references.

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Figure legends

Figure 1. Strain differences in the development of diet-induced pruritic atopic skin. Five different strains of mice (HR-1, BALB/c, C57BL/6, ddY and ICR) were fed a normal diet (open column) or a special diet (HR-AD, closed column) from 4 weeks of age. At 4, 8 and 12 weeks after the start of feeding, each parameter was evaluated and its area-under-the-curve (AUC) was calculated. (a–c) Skin hydration (a), TEWL (b) and the duration of one scratching bout (c). Data represents the mean $\pm$ SD of 8–20 animals. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

Figure 2. ICR-*Hr^{hr}* mice, like HR-1 mice, showed diet-induced pruritic atopic skin. (a) Identification of the *Hr* mutant allele. Hairless congenic ICR mice were established as described in Data S1. Because the 439-bp bands indicate PCR products amplified with the specific primer that identified the hypomorphic *Hr* allele, the hairless congenic strain was named ICR-*Hr^{hr}*. (b–e) Appearance of back skin (b), skin hydration (c), TEWL (d) and the duration of one scratching bout (e) at 4 weeks after feeding HR-AD. Bar represents 2 mm. Data represent the mean $\pm$ SEM (c and d) or SD (e) of 10–13 animals. *** $P < 0.001$, one-way ANOVA with Tukey's multiple comparison test.

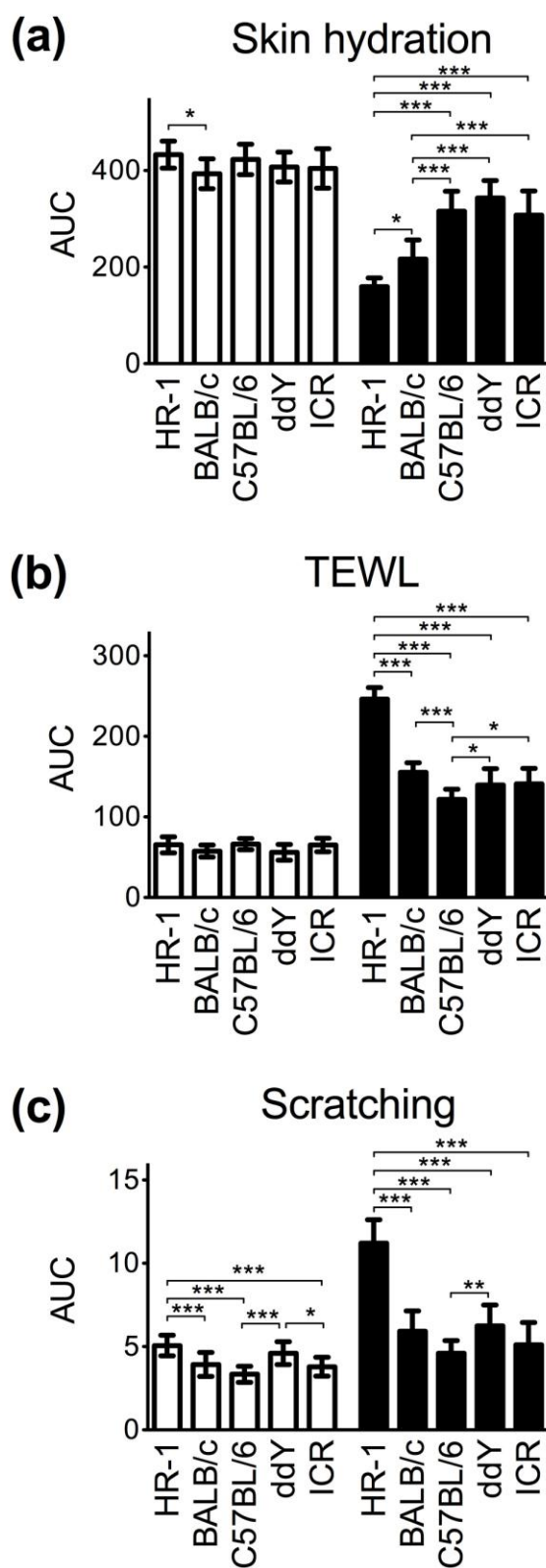


Fig. 1 Fujii et al.

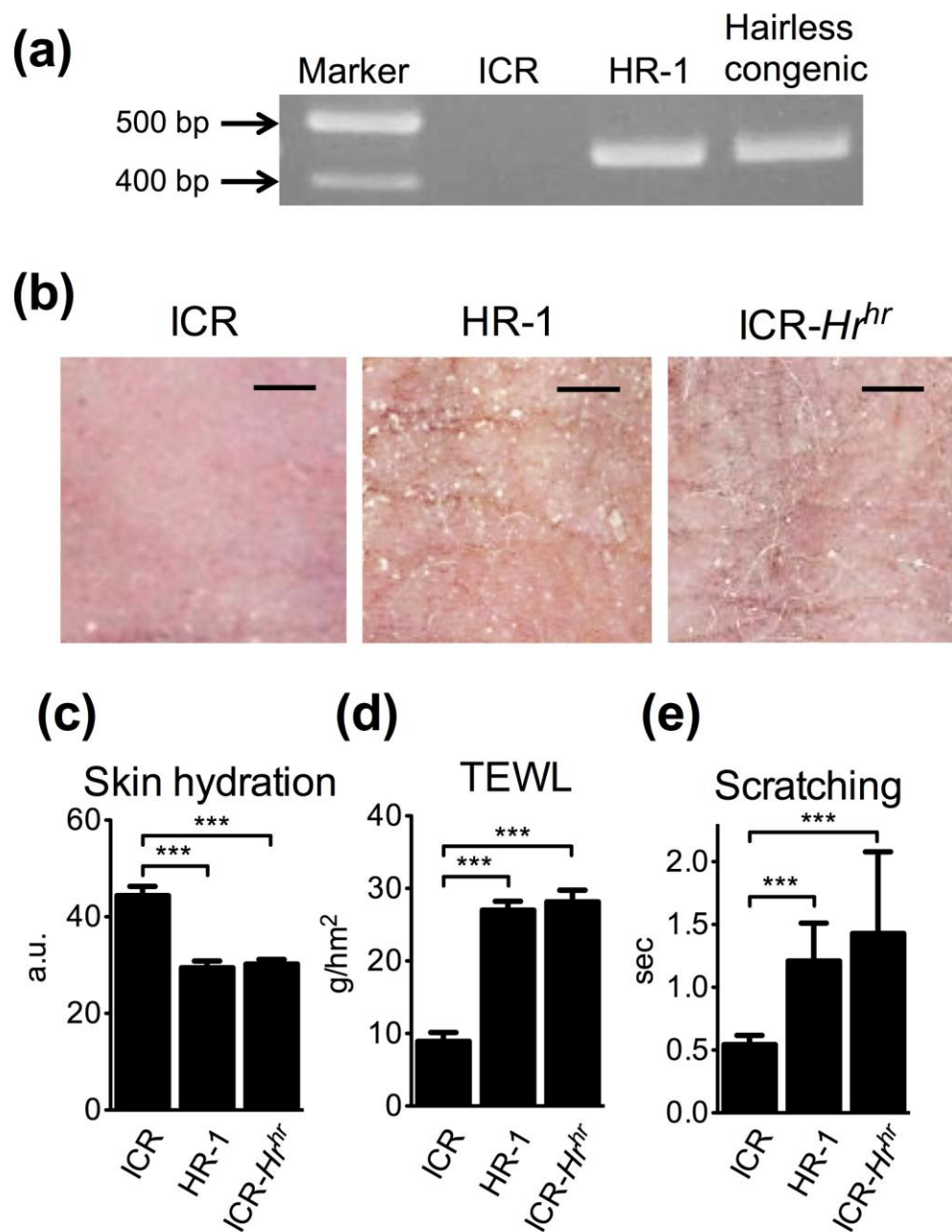


Fig. 2 Fujii et al.