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## Mouse *Samd9l* is not a functional paralogue of the human *SAMD9*, the gene mutated in normophosphatemic familial tumoral calcinosis

Qiaoli Li<sup>1</sup>, Haitao Guo<sup>1</sup>, Hirotaka Matsui<sup>2</sup>, Hiroaki Honda<sup>3</sup>, Toshiya Inaba<sup>2</sup>, John P. Sundberg<sup>4</sup>, Eli Sprecher<sup>5</sup>, and Jouni Uitto<sup>1</sup>

<sup>1</sup>Department of Dermatology and Cutaneous Biology, Jefferson Medical College, and Jefferson Institute of Molecular Medicine, Thomas Jefferson University, Philadelphia, PA

<sup>2</sup>Department of Molecular Oncology, Research Institute for Radiation Biology & Medicine, Hiroshima University, Hiroshima, Japan

<sup>3</sup>Department of Developmental Biology, Research Institute for Radiation Biology & Medicine, Hiroshima University, Hiroshima, Japan

<sup>4</sup>Jackson Laboratory, Bar Harbor, Maine, USA

<sup>5</sup>Department of Dermatology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel

### Abstract

Normophosphatemic familial tumoral calcinosis, characterized by ectopic mineralization of skin, is caused by mutations in the *SAMD9* gene located on human chromosome 7q21, next to a paralogous gene, *SAMD9-like (SAMD9L)*. Mouse does not have a *SAMD9* orthologue, *Samd9*, because it has been deleted during evolution due to genomic rearrangements. It has been suggested that the mouse *Samd9l* gene serves as a functional paralogue of human *SAMD9*. In this study we examined *Samd9l* knockout mice with respect to ectopic mineralization. We also crossed these mice with *Abcc6<sup>tm1Jfk</sup>* mice, a model system to study pseudoxanthoma elasticum, to see if the absence of the *Samd9l* gene modifies the mineralization process. Necropsy analysis of *Samd9<sup>tm1Homy</sup>* mice revealed no evidence of ectopic mineralization, and deletion of the *Samd9l* gene in mice failed to modify the mineralization process on the *Abcc6<sup>tm1Jfk</sup>* background. Collectively, the results suggest that mouse *Samd9l* is not a functional paralogue of human *SAMD9*.

### Keywords

Ectopic connective tissue mineralization; mouse models of skin diseases; familial tumoral calcinosis; pseudoxanthoma elasticum

### Background

Familial tumoral calcinosis (MIM610455) is a group of heritable disorders manifesting with ectopic mineralization of connective tissues (1). The normophosphatemic variant (NFTC) is

Address for Correspondence: Jouni Uitto, M.D., Ph.D., Department of Dermatology and Cutaneous Biology, Jefferson Medical College, 233 S. 10<sup>th</sup> Street, Suite 431 BLSB, Philadelphia, PA 19107, Tel: 215-503-5785, Fax: 215-503-5788, Jouni.Uitto@Jefferson.edu.

### Conflict of interest

The authors have declared no conflicting interests.

characterized by extensive mineralization of the skin and mucous membranes, associated with preceding inflammation. We have recently demonstrated that mutations in the sterile alpha motif domain-containing 9 gene (*SAMD9*) underlie NFTC (2,3). The *SAMD9* gene is located in the human genome in chromosomal region 7q21, and next to it in head-to-tail orientation is a paralogous gene, *SAMD9*-like (*SAMD9L*). Both genes have similar structures consisting of a large, ~5–6 kb coding exon (4). Both human genes encode respective proteins of ~170–190 kD, with 56% sequence homology.

An intriguing observation is that an orthologous gene of human *SAMD9* has been deleted in mouse genome as a result of unique genomic rearrangement during evolution (4). However, mice do have the orthologue of human *SAMD9L*, but relatively little is known of the functional relationships of the *SAMD9*/*SAMD9L* gene/protein interactions either in human or mouse. Since the *SAMD9* orthologue has been deleted in mouse, it has been suggested that the *SAMD9L* paralogue might functionally substitute for the *SAMD9* gene (4,5). Thus, one could postulate that the deletion of the *Samd9l* gene in mouse would result in ectopic mineralization similar to that noted in NFTC patients.

## Questions addressed

To explore the possibility that mouse *Samd9l* could serve as a functional paralogue of human *SAMD9*, the gene mutated in NFTC, we have now examined targeted mutant *Samd9l* (“knockout”, KO) mouse, *Samd9l<sup>tm1Homy</sup>*. These KO mice were first examined with respect to tissue mineralization up to eight months of age. The *Samd9l<sup>tm1Homy</sup>* mice, as well as their heterozygous or wild type counterparts were also crossed with *Abcc6<sup>tm1JfK</sup>* mice, an animal model for another ectopic mineralization disorder, pseudoxanthoma elasticum (PXE) (6), to examine whether the absence of *Samd9l* functional protein may accelerate the mineralization noted in *Abcc6<sup>tm1JfK</sup>* mice.

## Experimental design

*Samd9l<sup>tm1Homy</sup>* mouse model was developed by homologous recombination resulting in replacement of part of exon 2 in the *Samd9l* gene by the *LacZ* gene (7). These mice were maintained on the pure C57BL/6N background (N10). Complete necropsies were performed at approximately 8 months of age (8).

In another set of experiments, *Samd9l<sup>tm1Homy</sup>* mice were crossed with *Abcc6<sup>tm1JfK</sup>* mice, which have been shown to develop ectopic connective tissue mineralization at ~5–6 weeks of age (9–11). The *Abcc6<sup>tm1JfK</sup>* mice were congenics on C57BL/6J background (N10). The targeted mutant *Samd9l* mice (*Samd9l<sup>tm1Homy</sup>*), the corresponding heterozygote carriers (*Samd9l<sup>+/tm1Homy</sup>*) or their wild type counterparts (*Samd9l<sup>+/+</sup>*), all on *Abcc6<sup>tm1JfK</sup>* background, were placed at weaning (4 weeks of age) on diet that was previously shown to accelerate the ectopic mineralization process in *Abcc6<sup>tm1JfK</sup>* mice (12,13). Biopsies from the muzzle skin containing the vibrissae were obtained at 4 and 8 weeks after initiation of the acceleration diet, and mineralization of the connective tissue sheath of vibrissae, an early site of mineralization which serves as a biomarker of the entire mineralization process, was determined either semi-quantitatively by histopathologic examination or quantitatively by chemical assay of calcium and phosphate (10–13).

## Results

### Necropsy of *Samd9l<sup>tm1Homy</sup>* mice – no evidence of ectopic mineralization

Homozygous (*Samd9l<sup>tm1Homy</sup>*) and heterozygous (*Samd9l<sup>+/tm1Homy</sup>*) mice, together with their wild type counterparts, were subjected to complete necropsy approximately at the age

of 8 months, with focus on ectopic mineralization of soft connective tissues. Careful examination of histopathologic sections failed to reveal any evidence of ectopic mineralization in these mice. The pathology in the homozygous and heterozygous KO mice consisted primarily of age associated changes similar to those noted in wild type mice, including focal areas of granulomatous inflammation of the skin, mild focal dermal fibrosis, alopecia and dermatitis. However, one of the heterozygous mice had signs of alterations in the hematopoietic system, consistent with lymphoma. It should be noted that both the heterozygous and homozygous mice naturally develop acute myeloid leukemia at high frequency after the mice have reached 20 months of age (7).

### Deletion of the *Samd9l* gene does not accelerate ectopic mineralization in *Abcc6<sup>tm1JfK</sup>* mice

*Abcc6<sup>tm1JfK</sup>* mice serve as a model for PXE, a heritable mineralization disorder, and these mice develop progressive mineralization from ~5–6 weeks on. To examine the possibility that the *Samd9l* gene product might play a role in modulating this mineralization process, *Samd9l* KO mice were crossed with *Abcc6<sup>tm1JfK</sup>* mice, and *Samd9l<sup>+/+</sup>*, *Samd9l<sup>+/tm1Homy</sup>* and *Samd9l<sup>tm1Homy</sup>* mice, all on *Abcc6<sup>tm1JfK</sup>* background, were then generated and examined for mineralization of the connective tissue sheath of vibrissae as well as a number of internal organs. Histopathologic examination of muzzle skin at 8 and 12 weeks of age (4 weeks and 8 weeks after the initiation of the acceleration diet) revealed progressive mineralization of connective tissue sheath of vibrissae by routine hematoxylin-eosin stain, as well as special stains revealing calcium deposits (Alizarin Red and von Kossa), similar to that previously described for the *Abcc6<sup>tm1JfK</sup>* mice (Fig. 1). However, deletion of the *Samd9l* gene, either in homozygous or heterozygous state, did not influence the degree of mineralization at these time points. These histopathologic findings were confirmed by determining quantitatively the percent of vibrissae that were mineralized in each group (Fig. 2a). Finally, skin biopsies from the muzzle skin were used to determine chemically the calcium and phosphate content, a reflection of the mineralization of vibrissae (Fig. 2b). No statistically significant differences either at 4 weeks or 8 weeks on the acceleration diet were noted in mineralization between the genotypically different groups of mice.

A number of internal organs were also examined for evidence of ectopic mineralization in the same mice by histopathologic examination. Aberrant mineralization was noted, in addition to vibrissae, in the kidneys, heart, and the eyes. No evidence for increased mineralization in mice with allelic deletion of the *Samd9l* gene was noted (Supplementary Table 1). No mineralization was noted in any groups of mice in aorta, spleen or lung (not shown).

## Conclusion

The results of this study indicate that deletion of the *Samd9l* gene does not result in ectopic mineralization of mouse tissues nor does it accelerate the mineralization that takes place in *Abcc6<sup>tm1JfK</sup>* mice. Collectively, our observations suggest that the mouse *Samd9l* gene does not serve as a functional paralogue for human *SAMD9*, the gene harboring mutations in NFTC.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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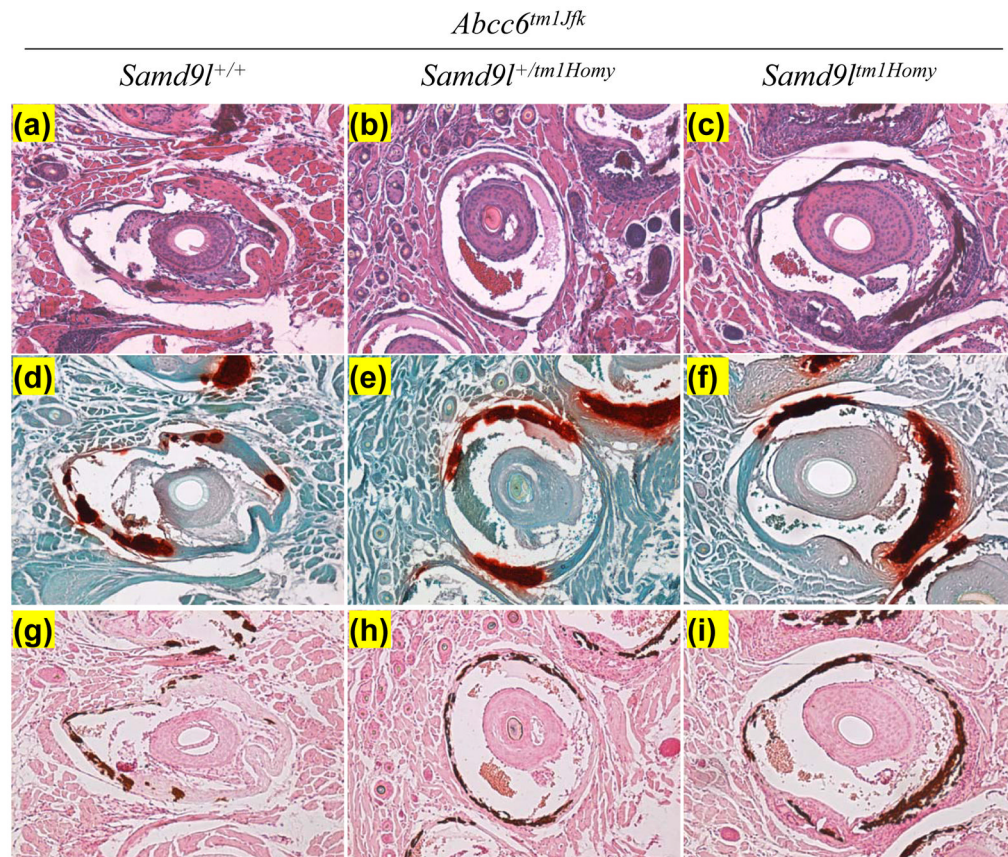
Dr. Li performed the research and analyzed the data; Dr. Guo performed the research; Drs. Matsui, Honda and Inaba contributed essential reagents and tools; Dr. Sundberg performed research and analyzed the data; Dr. Sprecher designed the research study; Dr. Uitto designed the research study, analyzed the data and wrote the paper.

## Abbreviations

<b>NFTC</b>	normophosphatemic familial tumoral calcinosis
<b>PXE</b>	pseudoxanthoma elasticum
<b>KO</b>	knockout

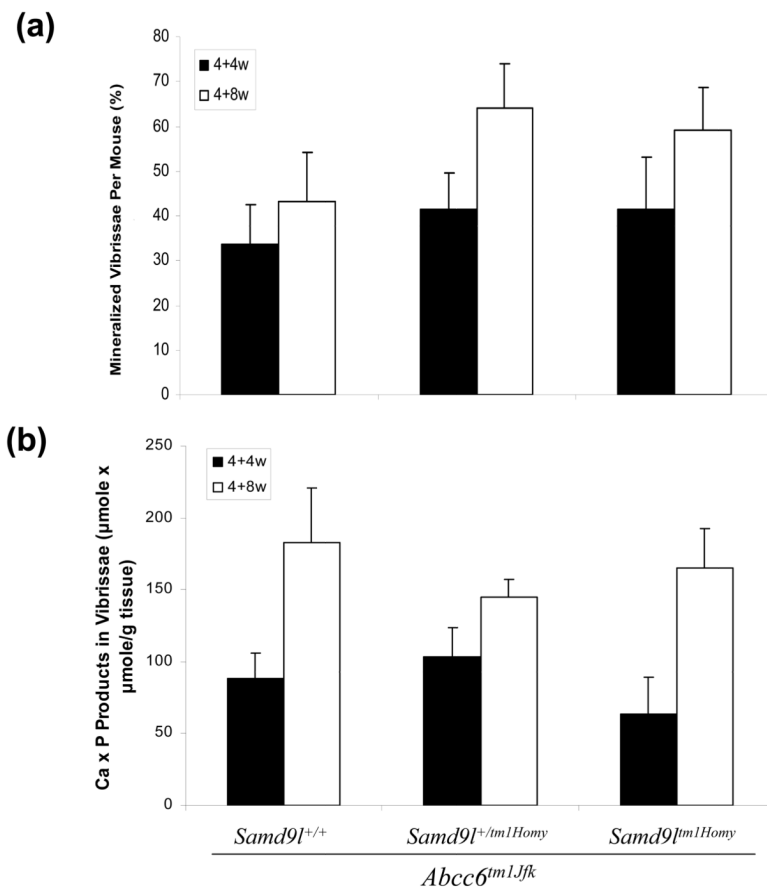
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**Figure 1.**

Histopathologic examination of mineralization of the connective tissue sheath of vibrissae in *Samd9l<sup>tm1Homy</sup>* and *Samd9l<sup>+/tm1Homy</sup>* mice in comparison to their wild type counterpart (*Samd9l<sup>+/+</sup>*), all on *Abcc6<sup>tm1Jfk</sup>* background. The stains used were hematoxylin-eosin (a–c), Alizarin Red (d–f), and von Kossa (g–i).



**Figure 2.** Quantitation of mineralization of the connective tissue sheath of vibrissae in the group of mice shown in Fig. 1 by the count of mineralized vibrissae over total number examined (%) (a), and by chemical assay of calcium and phosphate in the skin biopsy specimens (b), at 4 weeks (4+4w) and 8 weeks (4+8w) of feeding with a diet accelerating the mineralization process in *Abcc6*<sup>m1Jfk</sup> mice. The values are mean + S.E., n = 6–13 mice per group.