

## DATA REPORT

A novel *SLC34A2* mutation in a patient with pulmonary alveolar microlithiasisHiroyuki Izumi<sup>1</sup>, Jun Kurai<sup>1</sup>, Masahiro Kodani<sup>1</sup>, Masanari Watanabe<sup>1</sup>, Akihiro Yamamoto<sup>1</sup>, Eiji Nanba<sup>2</sup>, Kaori Adachi<sup>2</sup>, Tadashi Igishi<sup>1</sup> and Eiji Shimizu<sup>1</sup>

Pulmonary alveolar microlithiasis (PAM) is a rare autosomal recessive disease caused by mutations in *SLC34A2* and characterized by intra-alveolar accumulation of microliths. We diagnosed a case of PAM in a 27-year-old Japanese female and identified a novel mutation in *SLC34A2* (c.1390 G>C [G464R] in exon 12).

*Human Genome Variation* (2017) 4, 16047; doi:10.1038/hgv.2016.47; published online 26 January 2017

Pulmonary alveolar microlithiasis (PAM) is a rare autosomal recessive disease that is characterized by intra-alveolar accumulation of microliths in the absence of a known calcium metabolism disorder.<sup>1</sup> Solute carrier family 34 member 2 (*SLC34A2*) is a type IIb sodium phosphate co-transporter (NaPi-IIb) that is primarily expressed in alveolar type II cells and is responsible for the uptake of phosphate released from phospholipids during pulmonary surfactant recycling. *SLC34A2* is the only known sodium-dependent phosphate transporter expressed in the lung and has a key role in clearing phospholipids from the alveolar space by transporting phosphate ions into alveolar type II cells. Mutations in *SLC34A2* that cause impaired function or deficiency could therefore result in local accumulation of phosphate in the alveolar space. In Corut *et al.*<sup>2</sup> first reported that *SLC34A2* mutations cause PAM, and subsequent studies have also described mutations in this gene in patients with PAM.<sup>3,4</sup> Here, we report a case of PAM with a compound heterozygous mutation in *SLC34A2* that includes a novel mutation in exon 12.

A 27-year-old Japanese female from a non-inbred family presented to our hospital after indication of an abnormal shadow on a chest X-ray was observed during a medical examination. She had no previous medical history and did not present any complaints, such as dyspnea on exertion or cough. A chest X-ray and computed tomography scan showed a diffuse infiltration of fine sand stones in both lungs, mainly in the lower fields and lobes (Figure 1a,b), and the patient was admitted to our hospital for diagnosis of the lung shadow. Physical examinations, including chest auscultation, were normal. Laboratory tests were almost normal and did not suggest the presence of any metabolic disease, sarcoidosis or amyloidosis. Histopathological examination of the lung was performed in the hospital on day 4 and showed lamellar microliths deposited in the alveolar spaces (Figure 1c). On the basis of thoracic imaging and the pathological findings, she was diagnosed with PAM. Furthermore, molecular diagnosis was performed using genomic DNA extracted from the peripheral blood of the patient after obtaining informed consent. Molecular testing on family members was not performed because informed consent could not be obtained. After a 1-year follow-up,

the patient had no symptoms and there was no change in the lung shadow.

Genomic DNA was extracted from the peripheral blood of the patient. Using Primer3 software online (Genetyx software, Genetyx, Shibuya, Japan), 12 pairs of primers were designed to amplify *SLC34A2* from coding exons 2–13, as well as the intronic flanking sequences. Polymerase chain reaction using a standard protocol was used to amplify the DNA. Direct sequencing was performed for sequencing of polymerase chain reaction products using a 3,500×1 Genetic Analyzer (Thermo Fisher Scientific, Japan). The obtained sequence variants were tested for pathogenicity using Polyphen2<sup>5</sup> and MutationTaster2.<sup>6</sup>

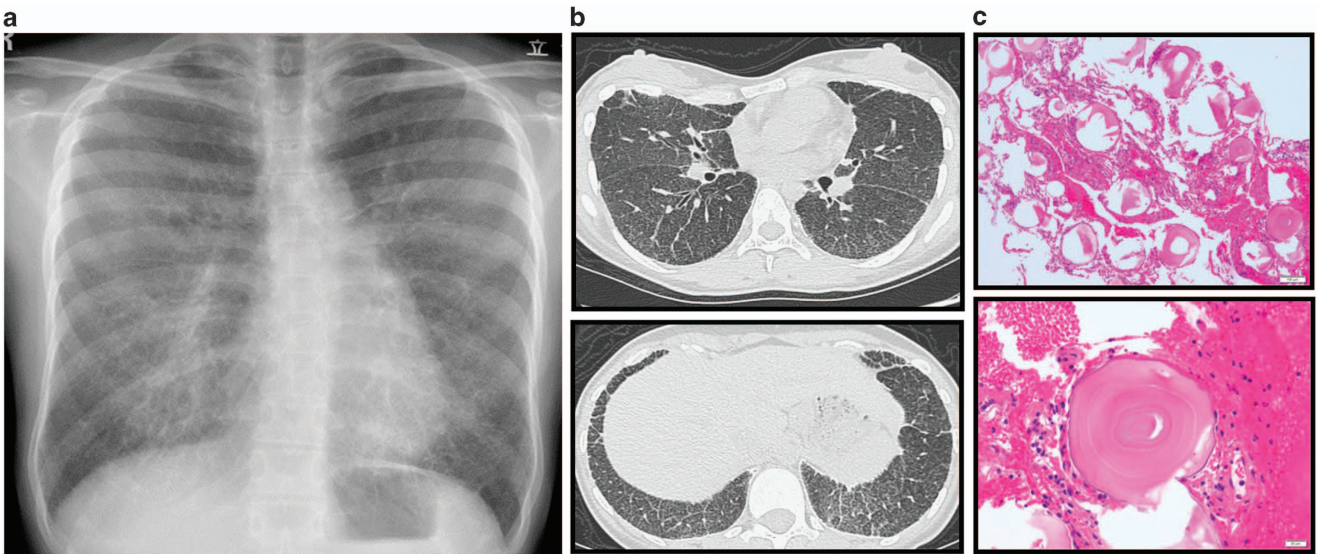
As a result of this genetic analysis, a novel compound heterozygous mutation in *SLC34A2* was identified in the patient (Figure 2a–d). One mutation, c.1048+1 G>A in intron 9, has already been reported and is causative for PAM.<sup>7</sup> A second mutation, c.1390 G>C (G464R), was identified in exon 12. To the best of our knowledge, the latter has not been previously reported. Polyphen2 scores for the novel mutation were 1.0 (sensitivity 0.00, specificity 1.00) using the HumDiv model and 1.0 (sensitivity 0.00, specificity 1.00) using the HumVar model.<sup>5</sup> MutationTaster analysis indicated that the novel mutation was a disease-causing mutation (model:simple\_aae, prob: 0.999999999912954).<sup>6</sup>

The patient was initially diagnosed with PAM according to radiological and pathological findings. Mutations in *SLC34A2* have been detected in almost all studied patients with PAM; therefore, *SLC34A2* is now considered to be the only gene responsible for PAM. We sequenced *SLC34A2* to confirm the diagnosis of PAM. *SLC34A2* consists of 13 exons, the first of which is non-coding, with the remaining exons encoding a 690-amino acid protein, a type 2 phosphate transporter that is expressed in several human tissues of epithelial origin. As previously detected *SLC34A2* mutations were not found within an obvious hotspot region, we sequenced the coding exons and the intronic flanking sequences of *SLC34A2*. Mutations were detected in intron 9 (c.1048+1 G>A) and exon 12 (c.1390 G>C[G464R]), which strongly supported the diagnosis of PAM. Huqun *et al.*<sup>7</sup> reported that the point mutation c.1048+1 G>A results in aberrant splicing and premature termination of the

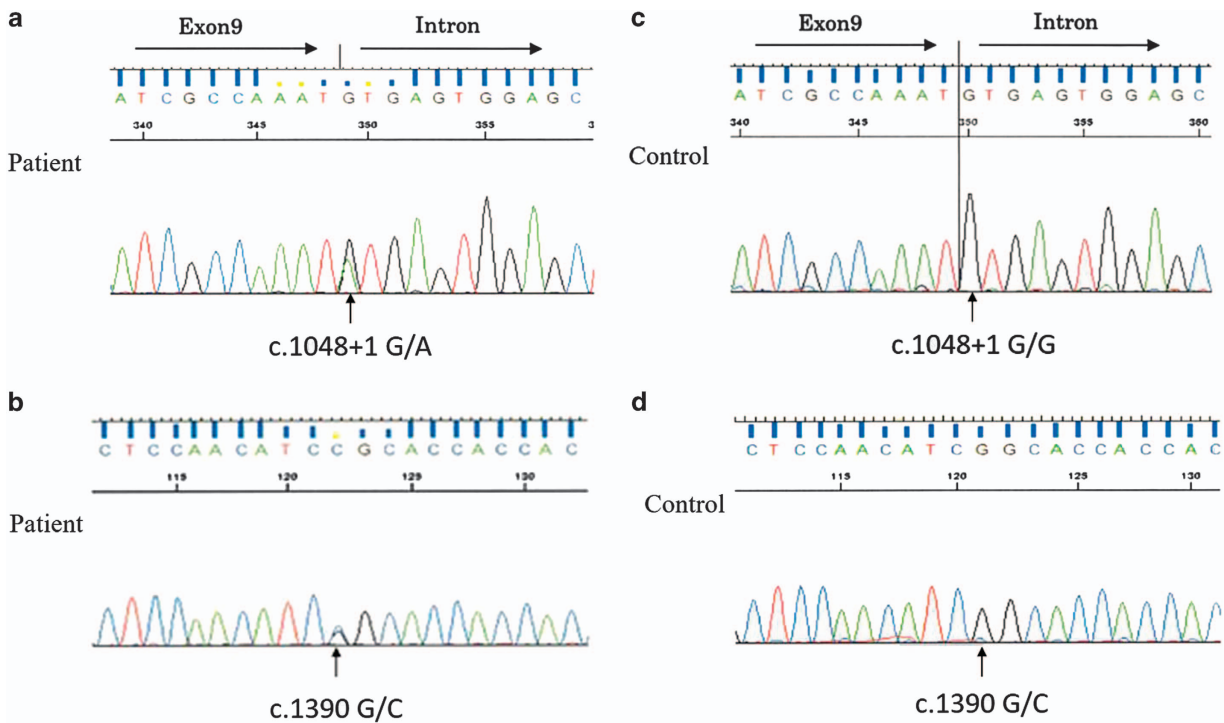
<sup>1</sup>Faculty of Medicine, Division of Medical Oncology and Molecular Respiriology, Tottori University, Yonago, Japan and <sup>2</sup>Division of Functional Genomics, Research Center for Bioscience and Technology, Tottori University, Yonago, Japan.

Correspondence: H Izumi (hiroizu0211@gmail.com)

Received 11 October 2016; revised 7 November 2016; accepted 22 November 2016



**Figure 1.** (a) Chest X-ray and (b) chest computed tomography scan on admission showed a diffused infiltration of fine sand-stones in both lungs mainly in the lower fields and the lower lobe. (c) Hematoxylin and eosin stain of the lung specimen showed lamellar microliths deposited in alveolar spaces (upper; low-power field, below; high-power field).



**Figure 2.** Sequence chromatograms of the pulmonary alveolar microlithiasis (PAM) patient and a control. A heterozygous change, c.1048+1G>A (a) and c.1390 G>C (b) were identified in the patient. (c, d) Healthy controls are wild type at these positions.

transcript. Although mutations in exon 12 have been previously reported e.g., the c.1393T>C point mutation<sup>8</sup> as well as deletions,<sup>9</sup> this case of PAM is the first to be caused by the point mutation c.1390 G>C(G464R). It is unclear how the mutation affects the function of the NaPi-IIb protein, but Polyphen2 and MutationTaster analyses predicted that the novel mutation is detrimental to the protein function.

It was preferable to show that each mutation was located on a different copy of the *SLC34A2* gene with either genomic DNA or complementary DNA. However, exons 9 and 12 of the *SLC34A2* gene are located on different parts of the genomic DNA; thus, it is very difficult to obtain genomic clones containing both exons

9 and 12 from the patient's genomic DNA. Furthermore, the mutation in intron 9 was located on the consensus sequence of the splicing donor site and will likely to lead to nonsense decay. Therefore, the isolation of complementary DNA in which intron 9 is incorrectly spliced is considered to be difficult. In all PAM cases in which *SLC34A2* mutations were detected, both copies of the *SLC34A2* were mutated.<sup>2,3,7,9-16</sup> Therefore, it is highly probable that the two mutations observed in this case are considered to affect both alleles.

More than 1,000 cases of PAM have been reported since the first case was described by Malpighi in 1686,<sup>4</sup> but to date only 33 patients have been diagnosed with mutations in *SLC34A2*,

with a total of 13 mutant alleles.<sup>2,3,7,9–16</sup> Molecular analyses of patients with PAM diagnosed by clinical criteria seem to be important to understand the etiology of PAM and to develop treatments. In conclusion, we identified a case of PAM clinically and genetically and identified a novel heterozygous mutant allele of *SLC34A2* that is causative for PAM.

#### HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.929>.

#### ACKNOWLEDGEMENTS

We acknowledge the patient for her participation.

#### COMPETING INTERESTS

The authors declare no conflict of interest.

#### REFERENCES

- 1 Tachibana T, Hagiwara K, Johkoh T. Pulmonary alveolar microlithiasis: review and management. *Curr Opin Pulm Med* 2009; **15**: 486–490.
- 2 Corut A, Senyigit A, Ugur SA, Altin S, Ozcelik U, Calisir H *et al*. Mutations in *SLC34A2* cause pulmonary alveolar microlithiasis and are possibly associated with testicular microlithiasis. *Am J Hum Genet* 2006; **79**: 650–656.
- 3 Vismara MFM, Colao E, Fabiani F, Bombardiere F, Tamburrini O, Alessio C *et al*. The sodium-phosphate co-transporter *SLC34A2*, and pulmonary alveolar microlithiasis: presentation of an inbred family and a novel truncating mutation in exon 3. *Respir Med Case Rep* 2015; **16**: 77–80.
- 4 Castellana G, Castellana G, Gentile M, Castellana R, Resta O. Pulmonary alveolar microlithiasis: review of the 1022 cases reported worldwide. *Eur Respir Rev* 2015; **24**: 607–620.
- 5 Adzhubei IA, Schmidt S, Peshkin L, Ramensky VE, Gerasimova A, Bork P *et al*. A method and server for predicting damaging missense mutations. *Nat Methods* 2010; **7**: 248–249.
- 6 Schwarz JM, Cooper DN, Schuelke M, Seelow D. MutationTaster2: mutation prediction for the deep-sequencing age. *Nat Methods* 2014; **11**: 361–362.

- 7 Huqun S, Izumi S, Miyazawa H, Ishii K, Uchiyama B, Ishida T *et al*. Mutations in the *SLC34A2* gene are associated with pulmonary alveolar microlithiasis. *Am J Respir Crit Care Med* 2007; **175**: 263–268.
- 8 Proesmans M, Boon M, Verbeken E, Ozcelik U, Kiper N, Van De Casseye W *et al*. Pulmonary alveolar microlithiasis: a case report and review of the literature. *Eur J Pediatr* 2012; **171**: 1069–1072.
- 9 Jönsson ÅLM, Hilberg O, Bendstrup EM, Mogensen S, Simonsen U. *SLC34A2* gene mutation may explain comorbidity of pulmonary alveolar microlithiasis and aortic valve sclerosis. *Am J Respir Crit Care Med* 2012; **185**: 464.
- 10 Ishihara Y, Hagiwara K, Zen K, Huqun, Hosokawa Y, Natsuhara A. A case of pulmonary alveolar microlithiasis with an intragenetic deletion in *SLC34A2* detected by a genome-wide SNP study. *Thorax* 2009; **64**: 365–367.
- 11 Dogan O, Ozsahin SL, Gul E, Arslan S, Koksall B, Berk S *et al*. A frame-shift mutation in the *SLC34A2* gene in three patients with pulmonary alveolar microlithiasis in an inbred family. *Intern Med* 2010; **49**: 45–49.
- 12 Zhong YQ, Hu CP, Cai XD, Nie HP. A novel mutation of the *SLC34A2* gene in a Chinese pedigree with pulmonary alveolar microlithiasis. *Chinese J Med Genet* 2009; **26**: 365–368.
- 13 Ma T, Ren J, Yin J, Ma Z. A pedigree with pulmonary alveolar microlithiasis: a clinical case report and literature review. *Cell Biochem Biophys* 2014; **70**: 565–572.
- 14 Ozbudak IH, Bassorgun CI, Ozbilim G, Luleci G, Sarper A, Erdogan A *et al*. Pulmonary alveolar microlithiasis with homozygous c.316g>c (p.G106R) mutation: a case report. *Turk Patoloji Derg* 2012; **28**: 282–285.
- 15 Proesmans M, Boon M, Verbeken E, Ozcelik U, Kiper N, Van DCW *et al*. Pulmonary alveolar microlithiasis: a case report and review of the literature. *Eur J Pediatr* 2012; **171**: 1069–1072.
- 16 Wang H, Yin X, Wu D, Jiang X. *SLC34A2* gene compound heterozygous mutation identification in a patient with pulmonary alveolar microlithiasis and computational 3D protein structure prediction. *Meta Gene* 2014; **2**: 557–564.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivs 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>

© The Author(s) 2017