ORIGINAL ARTICLE

WILEY

Novel *SLC30A2* mutations in the pathogenesis of transient neonatal zinc deficiency

Taichiro Muto[1]* | Yuriko Kawase[2]* | Kaori Aiba[3]* | Miyuki Okuma[4] | Naoya Itsumura[5] | Shuangyu Luo[5] | Namino Ogawa[5] | Tokuji Tsuji[5] | Taiho Kambe[5]

[1]Department of Pediatrics, Aichi Medical University Hospital, Nagakute, Japan
[2]Department of Dermatology, Toshiba Central Hospital, Tokyo, Japan
[3]Department of Pediatrics, Toyohashi Municipal Hospital, Toyohashi, Japan
[4]Department of Pediatrics, Toshiba Central Hospital, Tokyo, Japan
[5]Department of Applied Molecular Biology, Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto, Japan

Correspondence
Taiho Kambe, Division of Integrated Life Science, Graduate School of Biostudies, Kyoto University, Kyoto 606-8502, Japan.
Email: kambe.taiho.7z@kyoto-u.ac.jp

*These authors contributed equally to this work.

Funding source
This work was supported by a Grant-in-Aid for Scientific Research on Innovative Areas "Integrated Bio-metal Science" (MEXT KAKENHI Grant Number JP19H05768) from the Ministry of Education, Culture, Sports, Science, and Technology, and a Grant-in-Aid for Scientific Research (B) (JSPS KAKENHI Grant No. JP19H02883) from the Japan Society for the Promotion of Science (to Taiho Kambe).

Received: 17 October 2022
Accepted: 18 January 2023

ABSTRACT

Importance: Transient neonatal zinc deficiency (TNZD) occurs in breastfed infants due to abnormally low breast milk zinc levels. Mutations in the solute carrier family 30 member 2 (*SLC30A2*) gene, which encodes the zinc transporter ZNT2, cause low zinc concentration in breast milk.

Objective: This study aimed to provide further insights into TNZD pathophysiology.

Methods: *SLC30A2* sequencing was performed in three unrelated Japanese mothers, whose infants developed TNZD due to low-zinc milk consumption. The effects of the identified mutations were examined using cell-based assays and luciferase reporter analysis.

Results: Novel *SLC30A2* mutations were identified in each mother. One harbored a heterozygous missense mutation in the ZNT2 zinc-binding site, which resulted in defective zinc transport. The other two mothers exhibited multiple heterozygous mutations in the *SLC30A2* promoter, the first mutations in the *SLC30A2* regulatory region reported to date.

Interpretation: This report provides new genetic insights into TNZD pathogenesis in breastfed infants.

KEYWORDS

Dermatology, Breastfeeding, Transient neonatal zinc deficiency (TNZD), Low-zinc breast milk, *SLC30A2/ZNT2*, Promoter, Zinc-binding motif

INTRODUCTION

A large amount of zinc is secreted into the breast milk (1–3 mg zinc/day) during lactation to support the normal growth and development of the infant.[1,2] Thus, reduction of the breast milk zinc concentration causes breastfed infants to be in a state of zinc deficiency, which manifests as a broad range of symptoms, including persistent diarrhea,

DOI: 10.1002/ped4.12366

erythematous and erosive dermatitis, hair loss, alopecia, immune insufficiency, and growth restriction.[3,4] In the case of transient neonatal zinc deficiency (TNZD) (Online Mendelian Inheritance in Man [OMIM] 608118), these symptoms can be alleviated by oral zinc supplementation, and the affected infant can successfully thrive without zinc supplementation after weaning.[5–7] Accordingly, TNZD is a definitively distinct form of zinc deficiency from acrodermatitis enteropathica (AE) (OMIM 201100), which is caused by the intestinal malabsorption of zinc, resulting in zinc deficiency in the infant after weaning.[8,9]

AE is caused by mutations in the solute carrier family 39 member 4 gene (*SLC39A4*), also known as ZRT and IRT-like protein 4 (*ZIP4*), which results in impairment of zinc absorption in the small intestine,[10,11] whereas TNZD is caused by mutations in the solute carrier family 30 member 2 gene (*SLC30A2*), which encodes Zn transporter 2 (ZNT2).[12–21] ZNT2 is a critical zinc transporter that mobilizes zinc into the secretory vesicles in mammary epithelial cells, which is ultimately secreted into breast milk during lactation. Hence, pathogenic *SLC30A2* mutations cause mothers to produce zinc-deficient breast milk. Since the first heterozygous mutation was identified in *SLC30A2* in a nursing mother in 2006,[12] many loss-of-function mutations have been identified to be responsible for TNZD pathogenesis. The majority of such cases involved heterozygous mutations in *SLC30A2*, except for one case with a homozygous mutation[21] and one case with compound heterozygous mutations.[14]

Here, we report novel heterozygous *SLC30A2* mutations from three mothers whose infants suffered from TNZD. One is a novel loss-of-function mutation identified in one mother, and the other is the same set of multiple mutations in the *SLC30A2* promoter region identified in two unrelated mothers. The missense mutation was found in the zinc-binding HDHD motif, which is conserved among ZNT transporter family members.[22,23] Moreover, to our best knowledge, this is the first identification of *SLC30A2* mutations in the promoter. Our results thus provide additional crucial genetic information to offer insight into TNZD pathogenesis in breastfed infants.

METHODS

Ethical approval

This study was approved by the Ethics Committee of Kyoto University Graduate School and Faculty of Medicine (No. G760). Written consent was obtained from each patient's mother.

Patients

Three full-term breastfed infants were diagnosed with TNZD by their pediatricians based on clinical presentations. All three infants showed low serum zinc levels and presented with skin symptoms of zinc deficiency. Zinc levels in the breast milk of all three mothers were also lower than the average level expected during lactation. However, all mothers had blood zinc levels within the normal range. Polaprezinc [INN: $(C_9H_{12}N_4O_3Zn)_n$, Zeria Pharmaceutical, Tokyo, Japan] oral zinc replacement therapy was administered, which relieved the symptoms in all infants. Table 1 summarizes the clinical data of the patients.

SLC30A2 sequencing

NucleoSpin Blood Kit (Macherey-Nagel, Düren, Germany) was used for isolating genomic DNA from the mothers' whole blood. All exons containing coding regions, and the *SLC30A2* promoter and flanking regions were directly sequenced in both directions using fragments amplified from the isolated genomic DNA. To sequence the fragment derived from each allele, the fragment was subcloned in the pZero2 vector (Invitrogen, Carlsbad, CA, USA), and then the inserted fragment was sequenced. Primer information is described in our previous report.[14]

Zinc-transport activity of ZNT2 in zinc-sensitive cells against high extracellular zinc concentrations

Chicken B lymphocyte-derived DT40 cells deficient in the *Znt1*, *Znt4*, and metallothionein (*Mt*) genes ($Znt1^{-/-}Mt^{-/-}Znt4^{-/-}$ cells) were used to evaluate the zinc-transport activity of ZNT2 protein carrying the identified mutations, as described previously.[14,24] $Znt1^{-/-}Mt^{-/-}Znt4^{-/-}$ cells fail to grow in the presence of at least 60 μM $ZnSO_4$, which is reversed by stable expression of zinc-transport competent wild-type (WT) ZNT2, but not by a zinc-transport incompetent ZNT2 mutant.[25] The D227A mutant was used as a control of the null mutant identified in the HDHD motif because the substitution of an aspartic acid residue (Asp, D) to an alanine residue (Ala, A) in the HDHD motif is known to impair zinc transport activity.[26] Cell viability was determined using the alamarBlue assay (Trek Diagnostic Systems, Westlake, OH, USA) after culturing the cells for 48 h. The detailed procedures to establish $Znt1^{-/-}Mt^{-/-}Znt4^{-/-}$ cells stably expressing WT or mutant ZNT2 were described in our previous works.[14,24] To confirm that ZNT2 was expressed at the same level in the cells with WT or mutant ZNT2, immunoblotting was performed using the total cellular lysate as described previously.[27]

TABLE 1 Symptoms of transient neonatal zinc deficiency in the infants of this study

Patient (*SLC30A2/ZNT2* mutation identified in the mother)	Zinc levels in breast milk†	Age of onset in infant	Zinc levels in infant serum‡	Symptoms
1. (c.679G>A)	23 μg/dl (7 months)	2–3 months	19 μg/dl (7 months)	Erythematous and erosive dermatitis, particularly around the mouth, face, diaper region, and fingers, could not be improved by topical anti-inflammatory drugs, including corticosteroids. Persistent diarrhea and poor weight gain.
2. (c.-309C>T, c.-307G>T, c.-304T>A, c.-303T>A, c.-302T>A, c.-301T>A)	22 μg/dl (5 months)	2 months	4 μg/dl (5 months)	Erythematous and erosive dermatitis with crusting, particularly in the diaper region, face (around the mouth and eyes), and extremities, which could not be improved by topical corticosteroids. Poor weight and height gain, developmental delay.
3. (c.-309C>T, c.-307G>T, c.-304T>A, c.-303T>A, c.-302T>A, c.-301T>A)	21 μg/dl (5 months)	4 months	16 μg/dl (5 months)	Erythema erosive dermatitis on the back of the head and fingers.

†Normal reference level: 80 ± 30 μg/dl at 4–6 months.
‡Normal reference level: 70–120 μg/dl.

Reporter assay

SLC30A2 promoter-Cypridina luciferase reporter plasmids, in which WT or mutant *SLC30A2* promoter (from −363 to −32 with reference to the adenine base of the start codon methionine as +1) is inserted into the multiple cloning site of pMCS-Cypridina Luc (Thermo Scientific, Waltham, MA, USA), were transiently transfected into A549, MCF7, or MDA-MB-231 cells, as described previously;[28] Renilla luciferase expression plasmid was simultaneously transfected. The activities of Cypridina and Renilla luciferase were measured using Pierce Cypridina Luciferase Glow Assay Kit (Thermo Fisher Scientific, Rockford, IL) and Renilla-Glo Luciferase Assay System (Promega, Madison, WI, USA), respectively, using a Synergy H1 Hybrid multimode microplate reader (BioTek, Winooski, VT, USA). Cypridina luciferase activity was divided by that of Renilla luciferase for normalization of transfection efficiency.

Statistical analyses

All data are presented as the mean ± standard deviation. Statistical significance was determined by Student's *t*-test and accepted at $P < 0.01$.

RESULTS

Identification of a heterozygous mutation in the zinc-binding site of ZNT2

We found a novel missense mutation, c.679G>A, in exon 5 of *SLC30A2* (with reference to the adenine base of the start codon methionine as +1) in the affected mother of patient 1 (Table 1), which introduces an asparagine residue (Asn, N) in place of an Asp, D (p.D227N) in ZNT2 (Figure 1A, B). Stable expression of WT ZNT2 rescued the zinc-sensitive phenotype of *Znt1*$^{-/-}$*Mt*$^{-/-}$*Znt4*$^{-/-}$ cells. However, stable expression of both the D227N and D227A (positive control) ZNT2 mutants failed to recover growth in $ZnSO_4$ (Figure 1C, D). These results indicated that the D227N substitution is a loss-of-function missense mutation of ZNT2.

Heterozygous mutations in the *SLC30A2* promoter

We found no mutations in any of the *SLC30A2* exons in the mothers of patients 2 and 3 but identified the same novel multiple heterozygous mutations in the *SLC30A2/ZNT2* promoter in both mothers (Figure 2A): c.-309C>T, c.-307G>T, c.-304T>A, c.-303T>A, c.-302T>A, c.-301T>A (also with reference to the adenine of the start codon methionine as +1). These mutations alter the "CGGGCTTTT" sequence, located from nucleotides −309 to −301 of the *SLC30A2* promoter, to "TGTGCAAAA" (the positions of the mutations are underlined) (Figure 2B–2D). However, reporter analysis using plasmids in which luciferase expression is under the control of the WT or mutant *SLC30A2* promoter did not show any differences (Figure S1).

DISCUSSION

We here report novel mutations of *SLC30A2* causing TNZD in three infants. The first mutation was a missense mutation (c.679G>A in exon 5 of *SLC30A2*), resulting in the D227N substitution at the crucial intramembranous zinc-binding site of ZNT2. This mutation is at an important position that is essential for zinc transport and is thus conserved

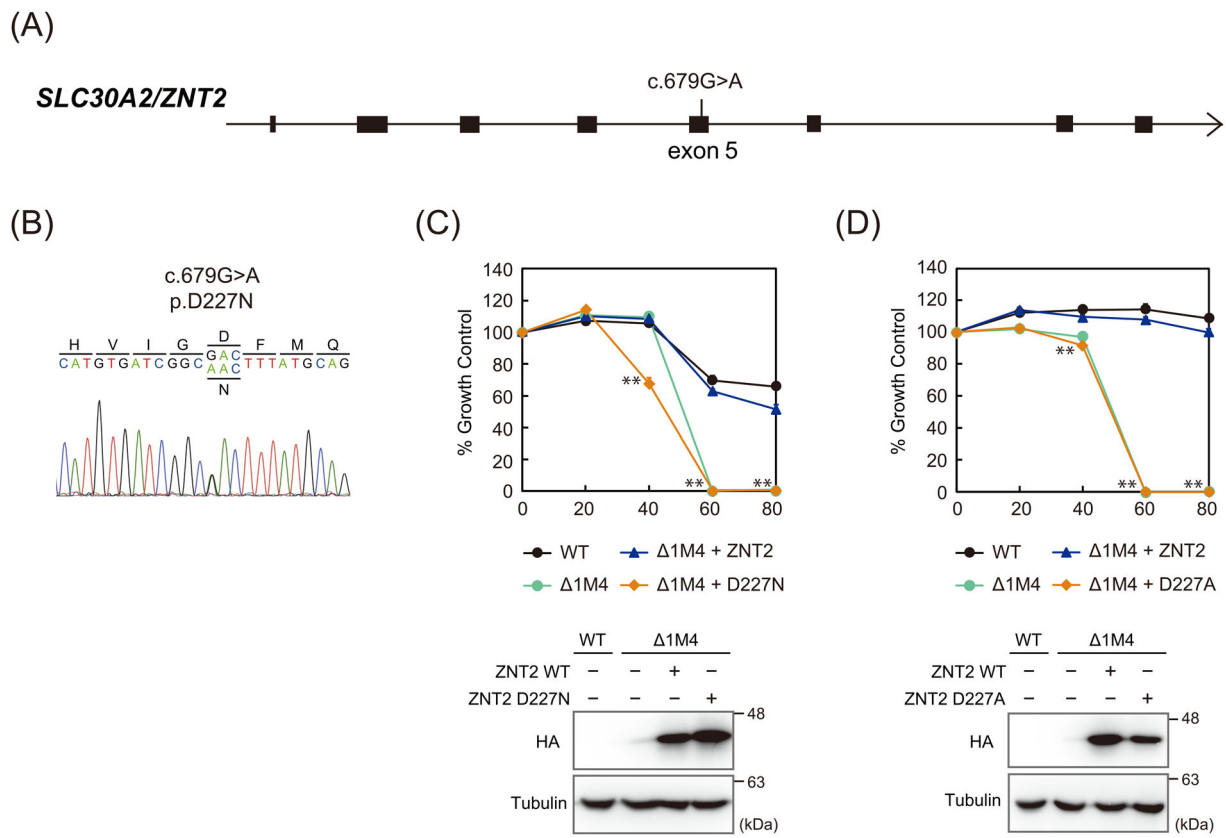

FIGURE 1 A novel missense mutation in solute carrier family 30 member 2 (*SLC30A2*) identified in the nursing mother of an infant with transient neonatal zinc deficiency. (A) The position of the mutation (c.679G>A in exon 5) in the schematic of the *SLC30A2* gene. (B) Electropherogram showing the c.679G>A mutation in exon 5, which results in a D227N substitution. (C) Evaluation of the zinc transport activity of the D227N ZNT2 mutant using *Znt1*$^{-/-}$*Mt*$^{-/-}$*Znt4*$^{-/-}$ cells. The cells were grown in the presence of the indicated concentrations of $ZnSO_4$ for 48 h, and the number of living cells was measured using the alamarBlue assay (plotted as a percentage of living cells at 0 μM $ZnSO_4$ for each group of cells). (D) Evaluation of the zinc transport activity of the D227A ZNT2 mutant as described in (B). In (C) and (D), WT means wild-type DT40 cells, Δ1M4 indicates *Znt1*$^{-/-}$*Mt*$^{-/-}$*Znt4*$^{-/-}$ cells, Δ1M4 + ZNT2 indicates *Znt1*$^{-/-}$*Mt*$^{-/-}$*Znt4*$^{-/-}$ cells stably transfected with WT ZNT2, and Δ1M4 + D227N indicates *Znt1*$^{-/-}$*Mt*$^{-/-}$*Znt4*$^{-/-}$ cells stably transfected with D227N-mutant ZNT2. ZNT2, tagged with HA at the C-terminus, was used. Each value represents the mean ± standard deviation of triplicate experiments (upper graph). **Significant difference between the cells expressing WT ZNT2 and mutant ZNT2 proteins ($P < 0.01$). Twenty micrograms of the total cellular proteins prepared from the respective cells were immunoblotted to demonstrate ZNT2 protein expression in the respective cells (lower panels). Tubulin was used as a control. Each experiment was performed at least thrice, and representative results from independent experiments are presented.

among ZNT family proteins. Specifically, the conserved site is called the HDHD motif, consisting of two histidines (His, H) and two aspartic acids (Asp, D) residues.[22,23] Thus, the HDHD zinc-binding motif is altered to HDHN in the mother (the altered residue is underlined). This alteration was considered to be responsible for the breast milk zinc deficiency in this case; however, the altered motif could have the ability to bind zinc, because a Gln (N) residue can accommodate zinc as a zinc coordination residue, although this is rare.[29] Indeed, functional assays confirmed that this was a loss-of-function mutation. It is unclear whether the loss-of-function D227N heterozygous mutation resulted in a low zinc level in breast milk in a dominant-negative manner or in a haploinsufficient manner. The manifestation of zinc deficiency was found at the age of 2–3 months in patient 1, which is much later compared with the typical postnatal age of 10 days in the case of a homozygous mutation[21] or 13 days in the case of a compound heterozygous mutation.[14] In both of these previously reported cases, the breast milk zinc content was extremely low.[14,21] Considering these points, low zinc content in the breast milk of a mother with the D227N mutation would likely be caused by haploinsufficiency, as reported for other missense mutations causing TNZD.[12,14,16–20] However, further studies are warranted to elucidate the underlying mechanism.

The second mutation, which was identified in two unrelated mothers, was a heterozygous mutation in the *SLC30A2* promoter, representing the first association of *SLC30A2* promoter mutation with TNZD pathogenesis. Interestingly, the multiple mutations overlap with the *SLC30A2* promoter sequence "-TGCACAC-" from position −301 to −295

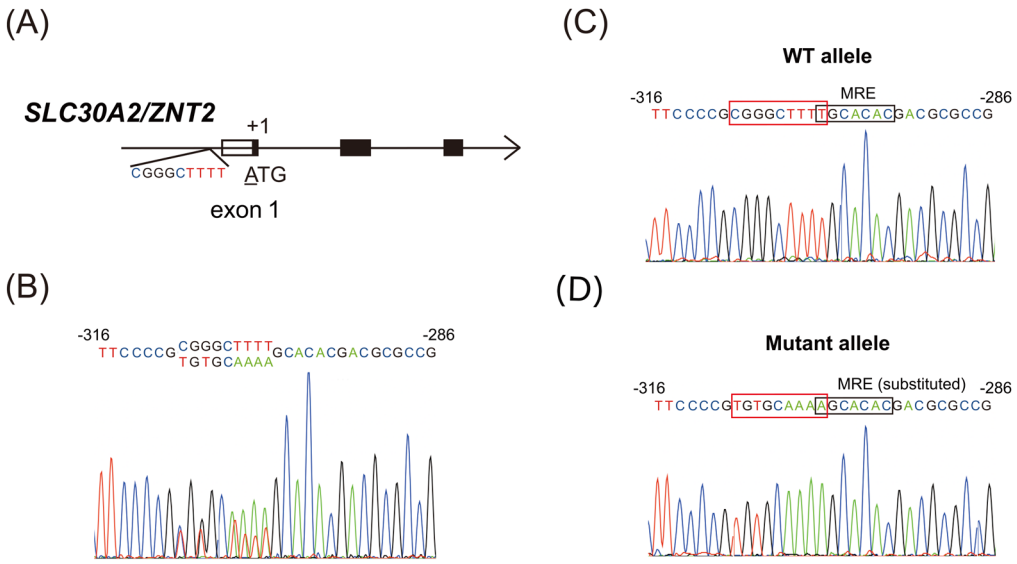

FIGURE 2 Heterozygous mutations in the solute carrier family 30 member 2 (*SLC30A2*) promoter identified in the nursing mothers of infants with transient neonatal zinc deficiency. (A) Schematic of the *SLC30A2* gene including promoter region. The position of "CGGGCTTTT" is shown. (B) Electropherograms showing the mutations c.-309C>T, c.-307G>T, c.-304T>A, c.-303T>A, c.-302T>A, and c.-301T>A in the *SLC30A2* promoter (referring to the adenine of the start codon methionine as +1). (C) Electropherograms of wild-type (WT) allele of the corresponding positions shown in (B). (D) Electropherograms of a mutant allele of the corresponding positions shown in (B). The position of the substitutions and the metal response element (MRE) site is framed in a red or black square in the sequence shown above each electropherogram. The MRE in the mutant allele is substituted (see text).

(with reference to the adenine of the start codon methionine as +1). This sequence was reported as a metal response element (MRE),[30] serving as the binding site of metal-responsive transcription factor 1 (MTF1).[31,32] The MRE site was reported to be crucial for zinc-dependent *SLC30A2* transcription based on a luciferase reporter assay.[30] The overlap of the mutations identified in this study with the MRE site (T at the −301st position) suggests that important regulatory element(s) for *SLC30A2* transcription may reside in or around these positions, and that binding of transcription factor(s) to the element(s) may be disrupted, which may explain the pathogenesis of TNZD in these two infants. One possibility is that MTF1 upregulates *SLC30A2* transcription in cooperation with as-yet uncharacterized lactogenic hormone signaling. However, we do not currently have experimental data to support this possibility (Figure S1). At present, the molecular mechanism underlying the involvement of these novel heterozygous mutations in the *SLC30A2* promoter in the observed phenotype of low zinc breast milk is unclear. However, the fact that the same mutations were found in two mothers who have no direct blood relationship strongly suggests that they are likely to be involved in TNZD pathogenesis in breastfed infants. We are continuing to investigate the molecular mechanism by which multiple mutations in the *SLC30A2* promoter result in low milk zinc secretion, in addition to examining the possibility proposed above, which would further provide a clue into the mechanism by which a large amount of zinc is secreted into the breast milk during lactation.

Overall, the genetic information concerning TNZD pathogenesis remains limited. However, the identification of these novel heterozygous mutations confirms the presence of a larger variety of TNZD-causing *SLC30A2* mutations. The symptoms of TNZD in the infant patients reported herein, which were attributed to low levels of zinc in the mothers' breast milk, were cured by early diagnosis and zinc supplementation therapy and did not reoccur after weaning. The opportunity for early diagnosis and treatment was available owing to the accumulating information on TNZD pathogenesis in breastfed infants in Japan.[14,18,19,24,33] Such information and further insights into TNZD pathophysiology are crucial to enable clinicians to diagnose and treat patients at an early stage, without confusing TNZD and AE. This timely recognition helps to support the normal growth and development of breastfed infants while making appropriate nutritional care available to subsequent children in affected families.

ACKNOWLEDGMENTS

We thank the patients and their families for their interest and cooperation in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Kelleher SL, Seo YA, Lopez V. Mammary gland zinc metabolism: regulation and dysregulation. *Genes Nutr.* 2009;4:83-94. DOI: 10.1007/s12263-009-0119-4
2. Kelleher SL, McCormick NH, Velasquez V, Lopez V. Zinc in specialized secretory tissues: roles in the pancreas, prostate, and mammary gland. *Adv Nutr.* 2011;2:101-111. DOI: 10.3945/an.110.000232
3. Ackland ML, Michalczyk AA. Zinc and infant nutrition. *Arch Biochem Biophys.* 2016;611:51-57. DOI: 10.1016/j.abb.2016.06.011
4. Golan Y, Kambe T, Assaraf YG. The role of the zinc transporter SLC30A2/ZnT2 in transient neonatal zinc deficiency. *Metallomics.* 2017;9:1352-1366. DOI: 10.1039/c7mt00162b
5. King NY, Gach JE. Transient neonatal zinc deficiency in an infant. *Clin Exp Dermatol.* 2021;46:1650-1652. DOI: 10.1111/ced.14755
6. Kambe T, Fukue K, Ishida R, Miyazaki S. Overview of inherited zinc deficiency in infants and children. *J Nutr Sci Vitaminol.* 2015;61(Suppl):S44-46. DOI: 10.3177/jnsv.61.S44
7. Kasana S, Din J, Maret W. Genetic causes and gene–nutrient interactions in mammalian zinc deficiencies: acrodermatitis enteropathica and transient neonatal zinc deficiency as examples. *J Trace Elem Med Biol.* 2015;29:47-62. DOI: 10.1016/j.jtemb.2014.10.003
8. Maverakis E, Fung MA, Lynch PJ, Draznin M, Michael DJ, Ruben B, et al. Acrodermatitis enteropathica and an overview of zinc metabolism. *J Am Acad Dermatol.* 2007;56:116-124. DOI: 10.1016/j.jaad.2006.08.015
9. Schmitt S, Küry S, Giraud M, Dréno B, Kharfi M, Bézieau S. An update on mutations of the *SLC39A4* gene in acrodermatitis enteropathica. *Hum Mutat.* 2009;30:926-933. DOI: 10.1002/humu.20988
10. Wang K, Zhou B, Kuo YM, Zemansky J, Gitschier J. A novel member of a zinc transporter family is defective in acrodermatitis enteropathica. *Am J Hum Genet.* 2002;71:66-73. DOI: 10.1086/341125
11. Küry S, Dréno B, Bézieau S, Giraudet S, Kharfi M, Kamoun R, et al. Identification of *SLC39A4*, a gene involved in acrodermatitis enteropathica. *Nat Genet.* 2002;31:239-240. DOI: 10.1038/ng913
12. Chowanadisai W, Lönnerdal B, Kelleher SL. Identification of a mutation in *SLC30A2* (ZnT-2) in women with low milk zinc concentration that results in transient neonatal zinc deficiency. *J Biol Chem.* 2006;281:39699-39707. DOI: 10.1074/jbc.M605821200
13. Lasry I, Seo YA, Ityel H, Shalva N, Pode-Shakked B, Glaser F, et al. A dominant negative heterozygous G87R mutation in the zinc transporter, ZnT-2 (SLC30A2), results in transient neonatal zinc deficiency. *J Biol Chem.* 2012;287:29348-29361. DOI: 10.1074/jbc.M112.368159
14. Itsumura N, Inamo Y, Okazaki F, Teranishi F, Narita H, Kambe T, et al. Compound heterozygous mutations in *SLC30A2/ZnT2* results in low milk zinc concentrations: a novel mechanism for zinc deficiency in a breast-fed infant. *PLoS One.* 2013;8:e64045. DOI: 10.1371/journal.pone.0064045
15. Miletta MC, Bieri A, Kernland K, Schöni MH, Petkovic V, Flück CE, et al. Transient neonatal zinc deficiency caused by a heterozygous G87R mutation in the *zinc transporter ZnT-2* (*SLC30A2*) gene in the mother highlighting the importance of Zn^{2+} for normal growth and development. *Int J Endocrinol.* 2013;2013:259189. DOI: 10.1155/2013/259189
16. Lova Navarro M, Vera Casaño A, Benito López C, Fernández Ballesteros MD, Godoy Díaz DJ, Crespo Erchiga A, et al. Transient neonatal zinc deficiency due to a new autosomal dominant mutation in gene *SLC30A2* (ZnT-2). *Pediatr Dermatol.* 2014;31:251-252. DOI: 10.1111/pde.12257
17. Kambe T, Hashimoto A, Fujimoto S. Current understanding of ZIP and ZnT zinc transporters in human health and diseases. *Cell Mol Life Sci.* 2014;71:3281-3295. DOI: 10.1007/s00018-014-1617-0
18. Rokunohe D, Nakano H, Sawamura D. Transient neonatal zinc deficiency (in Japanese). *Jpn J Clin Dermatol.* 2015;69:27-30.
19. Obara T, Komatsu N, Itsumura N, Kambe T, Muto Y, Nishihara, T, et al. Zinc deficiency in low zinc breast milk related to maternal ZnT2 gene mutation (in Japanese). *J Jpn Pediatr Soc.* 2016;120:1649-1656.
20. Liew HM, Tan CW, Ho CK, Chee JN, Koh MJ. Transient neonatal zinc deficiency caused by a novel mutation in the *SLC30A2* gene. *Pediatr Dermatol.* 2017;34:e104-e105. DOI: 10.1111/pde.13065
21. Li Z, Wang J, Yang Y, Wang S. A novel homozygous mutation p.E88K in maternal *SLC30A2* gene as a cause of transient neonatal zinc deficiency. *Exp Dermatol.* 2020;29:556-561. DOI: 10.1111/exd.14099
22. Kambe T. Molecular architecture and function of ZnT transporters. *Curr Top Membr.* 2012;69:199-220. DOI: 10.1016/B978-0-12-394390-3.00008-2
23. Kambe T, Taylor KM, Fu D. Zinc transporters and their functional integration in mammalian cells. *J Biol Chem.* 2021;296:100320. DOI: 10.1016/j.jbc.2021.100320
24. Itsumura N, Kibihara Y, Fukue K, Miyata A, Fukushima K, Tamagawa-Mineoka R, et al. Novel mutations in *SLC30A2* involved in the pathogenesis of transient neonatal zinc deficiency. *Pediatr Res.* 2016;80:586-594. DOI: 10.1038/pr.2016.108
25. Fujimoto S, Itsumura N, Tsuji T, Anan Y, Tsuji N, Ogra Y, et al. Cooperative functions of ZnT1, metallothionein and ZnT4 in the cytoplasm are required for full activation of TNAP in the early secretory pathway. *PLoS One.* 2013;8:e77445. DOI: 10.1371/journal.pone.0077445
26. Tsuji T, Kurokawa Y, Chiche J, Pouysségur J, Sato H, Fukuzawa H, et al. Dissecting the process of activation of cancer-promoting zinc-requiring ectoenzymes by zinc metalation mediated by ZNT transporters. *J Biol Chem.* 2017;292:2159-2173. DOI: 10.1074/jbc.M116.763946
27. Golan Y, Itsumura N, Glaser F, Berman B, Kambe T, Assaraf YG. Molecular basis of transient neonatal zinc deficiency: Novel ZnT2 mutations disrupting zinc binding and permeation. *J Biol Chem.* 2016;291:13546-13559. DOI: 10.1074/jbc.M116.732693

28. Wagatsuma T, Shimotsuma K, Sogo A, Sato R, Kubo N, Ueda S, et al. Zinc transport via ZNT5-6 and ZNT7 is critical for cell surface glycosylphosphatidylinositol-anchored protein expression. *J Biol Chem.* 2022;298:102011. DOI: 10.1016/j.jbc.2022.102011
29. Barber-Zucker S, Shaanan B, Zarivach R. Transition metal binding selectivity in proteins and its correlation with the phylogenomic classification of the cation diffusion facilitator protein family. *Sci Rep.* 2017;7:16381. DOI: 10.1038/s41598-017-16777-5
30. Lu YJ, Liu YC, Lin MC, Chen YT, Lin LY. Coordinative modulation of human zinc transporter 2 gene expression through active and suppressive regulators. *J Nutr Biochem.* 2015;26:351-359. DOI: 10.1016/j.jnutbio.2014.11.009
31. Radtke F, Heuchel R, Georgiev O, Hergersberg M, Gariglio M, Dembic Z, et al. Cloned transcription factor MTF-1 activates the mouse metallothionein I promoter. *EMBO J.* 1993;12:1355-1362. DOI: 10.1002/j.1460-2075.1993.tb05780.x
32. Andrews GK. Cellular zinc sensors: MTF-1 regulation of gene expression. *Biometals.* 2001;14:223-237. DOI: 10.1023/a:1012932712483
33. Kodama H, Inamo Y. Infant zinc deficiency due to low zinc concentration in maternal breast-milk (in Japanese). *Endocrinol diabetol Metab.* 2016;43:103-107.

SUPPORTING INFORMATION

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

How to cite this article: Muto T, Kawase Y, Aiba K, Okuma M, Itsumura N, Luo S, et al. Novel *SLC30A2* mutations in the pathogenesis of transient neonatal zinc deficiency. *Pediatr Investig*. 2023;7:6–12. https://doi.org/10.1002/ped4.12366