A patient with congenital hypothyroidism due to a PAX8 frameshift variant accompanying a urogenital malformation

Kanako Tanase-Nakao1, Koji Muroya2, Masanori Adachi2, Kiyomi Abe3, Tomonobu Hasegawa3, and Satoshi Narumi1, 3

1Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan
2Department of Endocrinology and Metabolism, Kanagawa Children’s Medical Center, Yokohama, Japan
3Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

Abstract. PAX8 is a transcription factor that is expressed in the thyroid gland and kidneys. Monoallelic loss-of-function PAX8 variants cause congenital hypothyroidism (CH), and urogenital malformations are infrequent complications seen in less than 10% of PAX8 variant carriers. Herein, we report the case of a 3-yr-old female patient with CH who was diagnosed during newborn screening. She was treated with levothyroxine, and she showed normal growth and development at a minimal dose (0.7 µg/kg/d of levothyroxine at 3 yr of age). At 5 mo of age, she visited an emergency department for fever and was incidentally found to have differently sized kidneys by ultrasonography, which was subsequently diagnosed as unilateral multicystic dysplastic kidney. Her serum creatinine and cystatin C levels were normal. Next-generation sequencing-based genetic analysis revealed that the patient was heterozygous for a PAX8 frameshift variant (p.Thr320ProfsTer106) and a DUOX2 missense variant (p.Arg885Gln). Our patient is the first truncating PAX8 variant carrier to have a urogenital malformation with CH. Genetic analysis for PAX8 should be considered in patients with CH and urogenital malformations.

Key words: congenital hypothyroidism, PAX8, frameshift mutation, urogenital abnormality

Highlights

- We report the first truncating PAX8 variant carrier to have a urogenital malformation with CH.
- Genetic analysis of PAX8 should be considered in patients with CH accompanied by urogenital malformations.
Introduction

Congenital hypothyroidism (CH) is the most common congenital endocrine disorder, affecting 1 in 2,000–4,000 live births (1). Clinically, CH is classified as syndromic or isolated according to the presence or absence of associated extrathyroidal malformations. The syndromic forms of CH include Bamforth–Lazarus syndrome (OMIM 241850) due to biallelic variants in FOXE1, brain-lung-thyroid syndrome (OMIM 610978) due to monoallelic NKKX2-1 variants, and Pendred syndrome (OMIM 274600) due to biallelic variants in SLC26A4. CH due to monoallelic PAX8 variants accompanying urogenital malformations has also been reported (2). Information on the associated conditions helps specify the genetic cause of CH.

PAX8 is a transcription factor indispensable for thyroid organogenesis and the regulation of thyroid-specific genes such as TG and TPO (3). CH due to monoallelic PAX8 variants accounts for approximately 2–3% of all CH cases (4–7). The reported clinical phenotypes range from overt CH with thyroid hypoplasia to subclinical CH with normal-sized thyroid glands. Here, we report a patient with CH and an incidentally discovered urogenital malformation who was subsequently found to carry a novel PAX8 frameshift variant.

Patient Description

The patient, a 3-yr-old Japanese girl, was born at 39 + 2 gestational weeks to an unrelated healthy couple. Her birth weight and length were 2,915 g and 46.6 cm, respectively. She received medical attention because of the elevated blood-spot TSH level at the time of newborn screening for CH (initial 10.0 mU/L and 11.2 mU/L at retest). At the first visit, when she was 22 d old, the physical examination showed no signs related to CH or other abnormalities. Her serum TSH level was high (14.2 mU/L; reference, 1.7–9.1) with a low serum free T4 level (0.63 ng/dL; reference, 0.9–2.3). The serum thyroglobulin levels were not measured. Levothyroxine supplementation was started at a dose of 30 µg/d and was decreased to 25 µg/d due to TSH suppression (0.76 mU/L) at 1 mo of age, and the patient was referred to us for further follow-up at 2 mo of age. When she visited the emergency department of a nearby hospital for fever at 5 mo of age, an imbalance in the terms of serum TSH elevation as compared to missense variant carriers showed milder phenotypes in (6, 13). The translation of the frameshift variant p.Thr320ProfsTer106 identified in our patient would generate a truncated PAX8 protein lacking the C-terminus (360–400 aa) (13). To date, 93 patients with CH caused by a novel PAX8 frameshift variant (c.957del, p.Thr320ProfsTer106) (Fig. 2). She also carried a DUOX2 variant (c.2654G>A, p.Arg885Gln) in a heterozygous state. These two variants were absent in gnomAD (https://gnomad.broadinstitute.org/), the 1000 Genomes Browser (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/), and 14KJPN (https://jmor.p.megabank.tohoku.ac.jp/202112/variants). The PAX8 variant has not yet been reported in literature. The DUOX2 variant has been described in a patient with transient CH in a compound heterozygous state, along with another DUOX2 missense variant (12). The parents denied to get their genes analyzed.

Discussion

In the present report, we described a patient with CH caused by a novel PAX8 frameshift variant and a multicystic dysplastic kidney. PAX8 consists of 450 amino acids comprising a DNA-binding paired domain at the N-terminus (9-133 aa) and a transactivation domain at the C-terminus (360–400 aa) (13). To date, 93 patients from 41 families carrying a total of 29 PAX8 disease-causing variants with experimental verification of their loss of function have been reported (5, 6, 13–32). Of the 29 PAX8 variants, 22 were missense variants located in the DNA-binding paired domain, one was a single amino acid deletion in the paired domain, and the remaining six were truncating variants (6, 13, 17, 25). The translation of the frameshift variant p.Thr320ProfsTer106 identified in our patient would generate a truncated PAX8 protein lacking the C-terminus portion, which contains the entire transactivation domain (Fig. 2); it is therefore expected to be non-functional. Previously reported truncating PAX8 variant carriers showed milder phenotypes in terms of serum TSH elevation as compared to missense PAX8 variant carriers (13). Our patient required only a small amount of levothyroxine to achieve a euthyroid state, which is comparable to the relatively mild phenotype. One possible explanation for this phenotypic
difference is the dominant-negative effect of missense PAX8 variants in vivo, which has been observed in vitro only in a limited number of reports (19, 20) and has not been fully established.

Among the 93 experimentally verified loss-of-function PAX8 variant carriers reported to date, six had urological malformations (i.e., kidney agenesis, ureterocele, and horseshoe kidney) (6, 15, 22, 24). All of the six patients had PAX8 missense variants in the paired domain, and our case is the first patient harboring a truncating PAX8 variant to present with kidney malformation (Table 1). Outside the thyroid, PAX8 is expressed in the developing human kidney (33). In mice, Pax2+/− embryos displayed hypoplastic kidneys (34), whereas Pax8−/− embryos displayed normal kidney development (35). However, Pax2+/−Pax8+/− embryos showed more severe kidney size reduction than Pax2+/−embryos, suggesting a synergistic effect of Pax8 and Pax2 on kidney formation (35). Congenital anomalies of the kidney and urinary tract affect 3–7 of 1,000 live births (36), which is significantly lower than the frequency observed in patients with PAX8 variants (7 of 98). This indicates an association between PAX8 variants and the occurrence of malformations, although it is unclear why only a subset of patients has the malformations. A limitation of this study is that we sequenced only the 13 causal and candidate causal genes for primary CH. More than 20 genes, including PAX2, are reported to involve in the development of congenital urogenital malformations (37). Additional genetic factors may have been detected in our patient if we had performed comprehensive analyses such as whole-exome sequencing.

Our patient harbored a monoallelic variant (p.Arg885Gln) of DUOX2, which encodes dual oxidase 2 (DUOX2). DUOX2 produces H2O2, which is indispensable for thyroid hormone synthesis, at the apical membrane of the thyroid follicular cells. Biallelic loss-of-function DUOX2 variants cause CH at an estimated prevalence of approximately 1:20,000 in Japan (38), which makes the calculated monoallelic carrier frequency as high as 1
in 70. Thus, it is reasonable to assume that our patient harbored the \textit{DUOX2} variant by chance, with a modest modification of thyroid function at most.

In conclusion, we described the first patient with \textit{CH} due to a \textit{PAX8} frameshift variant with a multicystic dysplastic kidney. Genetic analysis of \textit{PAX8} should be considered in patients with \textit{CH} accompanied by urological malformations.

**Acknowledgements**

We thank Dr. Kazuyuki Yamamoto and Dr. Saki Noda (Ichinomiya Municipal Hospital) for providing the clinical information.

This work was supported by JSPS KAKENHI grant Number 22K16420.

**References**

1. Ford G, LaFranchi SH. Screening for congenital hypothyroidism: a worldwide view of strategies. Best Pract Res Clin Endocrinol Metab 2014;28: 175–87. [Medline]  [CrossRef]
2. van Trotsenburg P, Stoupa A, Léger J, Rohrer T, Peters C, Fugazzola L, \textit{et al}. Congenital hypothyroidism: A 2020-2021 consensus guidelines update—an ENDO-European reference network initiative endorsed by the European Society for Pediatric Endocrinology and the European Society for Endocrinology. Thyroid 2021;31: 387–419. [Medline]  [CrossRef]
3. Di Palma T, Nitsch R, Mascia A, Nitsch L, Di Lauro R, Zannini M. The paired domain-containing factor Pax8 and the homeodomain-containing factor TTF-1 directly interact and synergistically activate transcription. J Biol Chem 2003;278: 3395–402. [Medline]  [CrossRef]
4. Lanzerath K, Bettendorf M, Haag C, Kneppe C, Schulze E, Grulich-Henn J. Screening for Pax8 mutations in patients with...
hypothyroidism and a high proportion of affected individuals. Horm Res Paediatr 2016;86: 137–42. [Medline] [CrossRef]

29. Vilain C, Rydlewski C, Duprez L, Heinrichs C, Abramowicz M, Malvaux P, et al. Autosomal dominant transmission of congenital thyroid hypoplasia due to loss-of-function mutation of PAX8. J Clin Endocrinol Metab 2001;86: 234–8. [Medline]

30. Zou H, Chai J, Liu S, Zang H, Yu X, Tian L, et al. A De novo PAX8 mutation in a Chinese child with congenital thyroid dysgenesis. Int J Clin Exp Pathol 2015;8: 11434–9. [Medline]

31. Vincenzi M, Camilot M, Ferrari E, Teofoli F, Venturi G, Gaudino R, et al. Identification of a novel pax8 gene sequence variant in four members of the same family: from congenital hypothyroidism with thyroid hypoplasia to mild subclinical hypothyroidism. BMC Endocr Disord 2014;14: 69. [Medline] [CrossRef]

32. Camats N, Baz-Redón N, Fernández-Cancio M, Clemente M, Campos-Martorell A, Jaimes N, et al. Phenotypic variability of patients with PAX8 variants presenting with congenital hypothyroidism and eutopic thyroid. J Clin Endocrinol Metab 2021;106: e152–70. [Medline] [CrossRef]

33. Trueba SS, Augé J, Mattei G, Etchevers H, Martinovic J, Czernichow P, et al. PAX8, TITF1, and FOXE1 gene expression patterns during human development: new insights into human thyroid development and thyroid dysgenesis-associated malformations. J Clin Endocrinol Metab 2005;90: 455–62. [Medline] [CrossRef]

34. Porteous S, Torban E, Cho NP, Cunliffe H, Chua L, McNoe L, et al. Primary renal hypoplasia in humans and mice with PAX2 mutations: evidence of increased apoptosis in fetal kidneys of Pax2(1Neu) +/- mutant mice. Hum Mol Genet 2000;9: 1–11. [Medline] [CrossRef]

35. Bouchard M, Souabni A, Mandler M, Neubüser A, Busslinger M. Nephric lineage specification by Pax2 and Pax8. Genes Dev 2002;16: 2958–70. [Medline] [CrossRef]

36. Birth Defects Monitoring Program (BDMP)/Commission on Professional and Hospital Activities (CPHA) surveillance data, 1988-1991. Teratology 1993;48: 658–75. [Medline] [CrossRef]

37. Capone VP, Morello W, Taroni F, Montini G. Genetics of congenital anomalies of the kidney and urinary tract: the current state of play. Int J Mol Sci 2017;18: 18. [Medline] [CrossRef]

38. Abe K, Narumi S, Suwanai AS, Adachi M, Muroya K, Asakura Y, et al. Association between monoallelic TSHR mutations and congenital hypothyroidism: a statistical approach. Eur J Endocrinol 2018;178: 137–44. [Medline] [CrossRef]

39. Tanaka T, Yokoya S, Kato N, Ito Y, Tachibana K, Sugihara S, et al. Basic approach to the evaluation of height and weight in Japanese children. J Jpn Ass Hum Auxo 2011;17: 84–99 (in Japanese).

40. Delvecchio M, Salerno M, Vigone MC, Wasniewska M, Popolo PP, Lapolla R, et al. Levothyroxine requirement in congenital hypothyroidism: a 12-year longitudinal study. Endocrine 2015;50: 674–80. [Medline] [CrossRef]