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CASE REPORT

Compound heterozygous *KCTD19* variants in a man with isolated nonobstructive azoospermia

Yuki Muranishi[1,2] | Yuko Katoh-Fukui[1] | Atsushi Hattori[1] | Yoshitomo Kobori[3] | Akiyoshi Osaka[3] | Hiroshi Okada[3] | Toshiyuki Iwahata[3] | Masafumi Kon[2] | Nobuo Shinohara[2] | Maki Fukami[1]

[1]Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan

[2]Department of Renal and Genitourinary Surgery, Hokkaido University Graduate School of Medicine, Sapporo, Japan

[3]International Center for Reproductive Medicine, Dokkyo Medical University Saitama Medical Center, Koshigaya, Japan

Correspondence
Maki Fukami, Department of Molecular Endocrinology, National Research Institute for Child Health and Development, Tokyo, Japan.
Email: fukami-m@ncchd.go.jp

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Abstract

Case: A 40-year-old Japanese man with nonobstructive azoospermia (NOA) was found to carry rare variants in *KCTD19*, a newly identified causative gene for spermatogenic failure. This patient was identified through mutation screening of *KCTD19* in 97 men with etiology-unknown isolated NOA.

Outcome: The patient had two heterozygous variants in *KCTD19* that affect consensus sequences of splice-donor sites [c.300+2T>A and c.2667C>T (p.E889E)]. Both variants were predicted to cause exon skipping. Long-read sequencing confirmed the compound heterozygosity of the variants. The patient exhibited small testes and a mildly elevated level of follicle-stimulating hormone but no other phenotypic abnormalities. Testicular histology showed borderline findings between spermatocyte maturation arrest and severe hypospermatogenesis.

Conclusion: These results provide evidence that biallelic loss-of-function variants of *KCTD19* represent rare causes of isolated NOA.

KEYWORDS
azoospermia, compound heterozygosity, long-read sequencing, maturation arrest, mutation

1 | INTRODUCTION

Nonobstructive azoospermia (NOA) is a multifactorial disorder caused by several genetic and environmental factors.[1] Copy number variations in the Y chromosomal azoospermia factor (AZF) region and damaging variants in more than 160 genes have been implicated in the development of isolated (non-syndromic) NOA.[2,3] However, these factors can explain only less than 20% of the cases,[3] indicating that hitherto unrecognized genetic variants are also involved in isolated NOA.

KCTD19/Kctd19 is a testis-specific gene that encodes a potassium channel tetramerization domain protein.[4] Recently, *Kctd19* was implicated in spermatogenesis in mice.[5] *Kctd19* knockout mice exhibited small testes with spermatocyte meiotic arrest without other phenotypic abnormalities.[4,5] Subsequently, homozygous variants in *KCTD19* (NM_001100915) were identified in nine men from seven families with spermatogenic failure.[6–8] All variant-positive individuals, except for one young man who did not undergo sperm examination, exhibited NOA, oligozoospermia, or oligoasthenozoospermia.[6–8] In contrast, heterozygous carriers of these families retained normal fertility.[6–8] These results suggested that biallelic *KCTD19* variants are novel causes of spermatogenic failure in humans. However, since most of the reported patients were born to consanguineous parents, the clinical significance of *KCTD19* variants

as the cause of isolated NOA remains uncertain. Here, we report a man with etiology-unknown NOA who carried compound heterozygous variants of *KCTD19*.

2 | CASE REPORT

The case was a 40-year-old Japanese man who visited our hospital because of infertility. He was subjected to our previous mutation analysis for 115 patients with isolated NOA and normal 46,XY karyotype.[3] That study was approved by the Institutional Ethics Committee of the National Research Institute for Child Health and Development, and written informed consent was obtained from all patients. In that study, we identified pathogenic CNVs in the AZF region or mutations in major NOA-related genes in 18 patients. Thus, we searched for *KCTD19* variants in the remaining 97 patients using their whole exome sequencing data. Consequently, we identified rare damaging variants only in one patient (the present case).

The patient was found to carry two heterozygous variants, c.300+2T>A and c.2667C>T (p.E889E), in *KCTD19*. Sanger sequencing confirmed the presence of the two variants (Figure 1). These variants were absent or extremely rare in the general population (Table 1). We examined the allelic positions of these variants by haplotype phasing using a long-read sequencer (Oxford Nanopore Technology, UK). The methods were described previously.[9] The results suggested that c.300+2T>A and c.2667C>T were located on different alleles of the patient (Figure S1). Both variants have not been identified in patients with spermatogenic failure and were estimated as variants of unknown significance (VUS) according to the American College of Medical Genetics guidelines (c.300+2T>A, PM2+PM4+PP3; and p.E889E, PM4+PP3). Notably, c.300+2 is the second nucleotide of intron 2, and c.2667C is the last nucleotide of exon 15. Hence, both the c.300+2T>A and c.2667C>T variants affected consensus sequences of splice-donor sites (Figure 1). In silico analyses using Alternative Splice Site Predictor (http://wangcomputing.com/assp/), Human Splicing Finder (http://umd.be/Redirect.html), Splice Site Prediction by Neural Network (https://www.fruitfly.org/seq_tools/splice.html), and SpliceAI (https://spliceailookup.broadinstitute.org/) suggested that the two variants exert negative effects on splice-donor site activities (Table 1). Furthermore, Splice Vault (https://kidsneuro.shinyapps.io/splicevault/) predicted aberrant splicing of the two variants (Figure 1, Table 1). The c.300+2T>A variant was assumed to induce skipping of exon 2 leading to inframe deletion of 99 amino acids or skipping of exons 2 and 3 leading to premature termination due to frameshift. Likewise, the c.2667C>T variant was predicted to cause skipping of exon 15 leading to in-frame deletion of 34 amino acids or skipping of exons 14 and 15 leading to premature termination due to frameshift.

We examined detailed clinical data of the patient (Table 2). His parents and elder brother retained normal fertility. The patient exhibited normal male-type external genitalia with relatively small testes. He was otherwise healthy and manifested no additional phenotypic abnormalities. Endocrinological examinations showed no abnormalities except for a mildly elevated level of follicle-stimulating hormone (FSH). Semen analyses demonstrated azoospermia, and microdissection testicular sperm extraction (MD-TESE) confirmed the lack of spermatozoa. Testicular histology showed borderline findings between spermatocyte maturation arrest and severe hypospermatogenesis that correspond to Johnsen's scores of 5.8 and 5.5.[10] He was diagnosed with isolated NOA and treated with recombinant FSH (150 IU/m^2/dose/week for three consecutive months followed by 300 IU/m^2/dose/week for another 3 months). This 6-month treatment yielded no improvement in the testicular histology at the second MD-TESE.

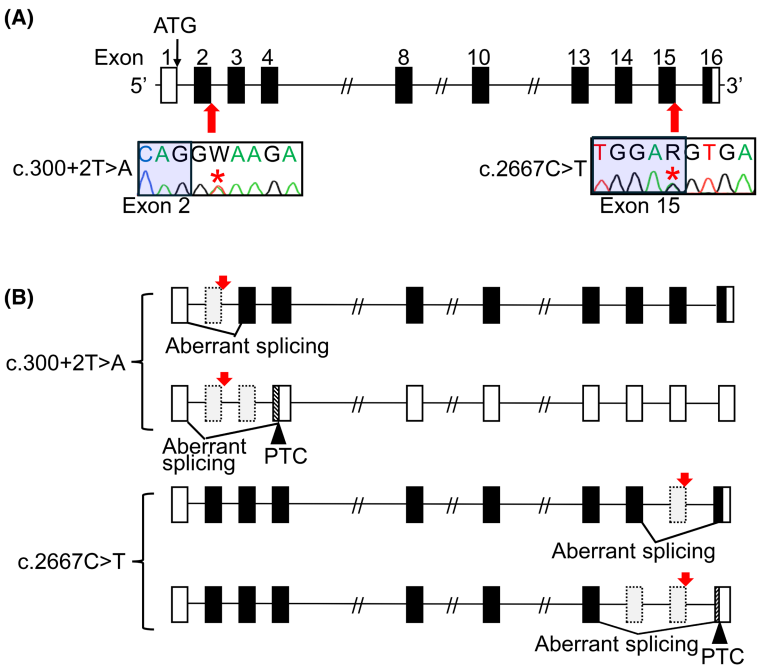

FIGURE 1 *KCTD19* variants identified in the patient. Red asterisks and arrows indicate the mutated nucleotides. (A) The positions of the two variants. Both variants affected consensus sequences at splice donor sites. The black and white boxes represent non-coding and coding regions, respectively. The exons and introns are not drawn to scale. (B) Predicted effects of the two variants on splicing. The c.300+2T>A variant appeared to cause skipping of exon 3 or exons 2 and 3, whereas the c.2667C>T variant was assumed to cause skipping of exon 14 or exons 14 and 15. The gray boxes depict the skipped exons, and the striped boxes indicate exons with frameshift sequences. PTC, premature termination codon.

TABLE 1 *KCTD19* variants identified in the patient.

	c.300+2T>A	Wild-type sequence (c.300+2T)	c.2667C>T	Wild-type sequence (c.2667C)	Threshold
Allele frequency in the general population					
1000G_all[a]	0		0		
ToMMo[b]	0		0		
gnomAD[c]	0		4.80×10^{-6}		
Splice donor site prediction					
ASSP[d]	Not recognized	13.6	8.7	11.0	>4.5
HSF[e]	70.2	97.3	83.4	93.5	Not available
NNSPLICE[f]	Not recognized	1.0	Not recognized	0.7	>0.4
SpliceAI[g]	0.9	0.0	0.7	0.0	<0.5
Exon skip prediction[h]					
Skipped exon	Exon 2 (1.3%), Exons 2 and 3 (2.7%)		Exon 15 (35.3%), Exons 14 and 15 (13.6%)		

[a]1000 Genomes Project (https://www.genome.gov/27528684/1000-genomes-project).
[b]Tohoku Medical Megabank Organization Database (ToMMo, https://www.megabank.tohoku.ac.jp/).
[c]Genome Aggregation Database (gnomAD, https://gnomad.broadinstitute.org/).
[d]Alternative splice site predictor (http://wangcomputing.com/assp/).
[e]Human splicing finder (http://umd.be/Redirect.html).
[f]Splice site prediction by neural network (https://www.fruitfly.org/seq_tools/splice.html).
[g]https://spliceailookup.broadinstitute.org/.
[h]Splice Vault (https://kidsneuro.shinyapps.io/splicevault/) predicted skipped exon and its frequency. The skipped exon was determined based on the samples observed in 300K-RNA (hg38) analysis. The frequency was calculated by dividing the number of observed missplicing events by the total counts.

TABLE 2 Clinical data of the patient.

	Patient	Reference range
Blood hormone data		
Testosterone (ng/mL)	3.8	2.5–8.4
LH (mIU/mL)	3.9	1.7–8.6
FSH (mIU/mL)	13.8	1.5–12.4
Prolactin (ng/mL)	3.6	3.0–14.7
Estradiol (pg/mL)	20.5	14.6–48.8
Testicular volume (mL)	10 (Left)/10 (Right)	>12
Johnsen's score[a]		
Before FSH treatment	5.8 (Left)/5.5 (Right)	8–10
After FSH treatment	5.8 (Left)/6.0 (Right)	8–10

Note: Abnormal values are underlined.
Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone.
[a]A quantitative evaluation of spermatogenesis based on the results of testicular biopsy. The scores range from 1 to 10. Higher scores indicate better spermatogenesis.

3 | DISCUSSION

We identified compound heterozygous variants of *KCTD19* in a Japanese man with isolated NOA. The c.300+2T>A and c.2667C>T variants affected consensus sequences of splice-donor sites and were predicted to result in the skipping of one or more exons. We assume that c.300+2T>A variant induces inframe deletion of 99 amino acids or nonsense-mediated decay[11] and the c.2667C>T variant causes inframe deletion of 34 amino acids or deletion of 85 amino acids and addition of 22 aberrant amino acids (Figure 1). Thus, the patient is likely to lack functional KCTD19 protein. Consistent with this, his clinical features, that is, severe hypospermatogenesis or spermatocyte maturation arrest without other clinical abnormalities, are similar to the phenotype of *Kctd19* knockout mice.[4] These data, together with the normal fertility of the patient's father, indicate that loss-of-function variants of *KCTD19* result in isolated NOA as an autosomal recessive disorder. In this context, previous studies have shown that the penetrance of *KCTD19*-associated spermatogenic failure is high; there are no reports of men with biallelic *KCTD19* variants and normal fertility.[6–8] Since we identified damaging *KCTD19* variants in 1 of 97 patients with etiology-unknown NOA, such variants appear to account for only a small percentage of the genetic causes of isolated NOA. Yet, considering that some patients with homozygous missense variants of *KCTD19* were reported to have oligozoospermia/oligoasthenozoospermia,[7] *KCTD19* abnormalities are likely to be associated with various types of spermatogenic failure. Further studies are needed to clarify whether the phenotypic severity of *KCTD19* abnormalities is correlated with the residual activity of the mutant proteins.

Our patient exhibited no hormonal abnormalities except for a slightly elevated FSH level that indicates mildly decreased inhibin

secretion from the testes.[12] Testicular histology demonstrated borderline findings between severe hypospermatogenesis and spermatocyte maturation arrest. These results are similar to those of previously reported patients with homozygous *KCTD19* variants.[6–8] In this context, Zhou et al.[13] have shown that spermatocyte maturation arrest of *Kctd19* knockout mice is associated with disrupted bivalent chromosome individualization at the meiotic metaphase I. Since the murine KCTD19 protein is assumed to form a transcriptional repressor complex with ZFP541, DNTTIP1, and HDAC1/2,[14] spermatogenic failure in patients with *KCTD19* abnormalities may also be associated with dysfunction of this complex.

Six-month FSH treatment did not ameliorate the results of MD-TESE in our patient. Since Shiraishi et al. reported that human chorionic gonadotropin (hCG) treatment with or without FSH injection successfully induced sperm production in 21% of men with NOA,[15] the effectiveness of hCG treatment for patients with *KCTD19* abnormalities needs to be examined in future studies. Thus far, there are no reports of successful medical treatment for infertility in men with biallelic *KCTD19* variants. Wang et al. reported that intracytoplasmic sperm injection failed to induce pregnancy in any of three cases with *KCTD19*-associated oligozoospermia.[7] Hence, *KCTD19* abnormalities may be associated with poor outcomes of assisted reproductive technology.

In conclusion, this study provides evidence that biallelic *KCTD19* variants represent rare genetic causes of isolated NOA. Future studies are necessary to clarify phenotypic variations and long-term prognosis of patients with *KCTD19* abnormalities.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was approved by the Institutional Ethics Committee of the National Research Institute for Child Health and Development.

HUMAN RIGHTS STATEMENT AND INFORMED CONSENT

Written informed consent was obtained from the patient.

ORCID

Yuki Muranishi https://orcid.org/0009-0001-7989-6748
Akiyoshi Osaka https://orcid.org/0000-0002-5159-8028
Hiroshi Okada https://orcid.org/0000-0002-9950-0245
Maki Fukami https://orcid.org/0000-0001-9971-4035

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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