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**Single Case**

# The First Korean Case of *SLC12A3* Aberrant Skipping of Two Exons Detected by RNA Splicing Analysis

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**Keywords**
Gitelman syndrome · *SLC12A3* · Aberrant skipping · RNA splicing analysis

**Abstract**
Gitelman syndrome is a salt-losing tubular disorder that is transmitted as an autosomal recessive trait. Variants in the *SLC12A3* gene are found in the majority of Gitelman syndrome patients. A 26-year-old woman visited the genetic counseling clinic. Her fiancé was a known Gitelman syndrome patient who was previously diagnosed with 2 pathogenic variants in *SLC12A3*. In advance of marriage and future family planning, she wanted to perform genetic testing of *SLC12A3*. A silent exonic variant c.1050G>A was found, and multiple splice site in silico algorithms predicted this variant to have potential alteration of splicing. This variant was classified as "variant of uncertain significance," and RNA splicing analysis was additionally performed. RNA splicing analysis showed aberrant splicing of exon 7–8 skipping. The result points out the potential pathogenicity of this variant, which should be considered a candidate of variant reclassification in the future. We highly recommend the performance of additional RNA splicing analysis, especially for silent variants predicted to have potential alteration of splicing.



## Introduction

Gitelman syndrome is a salt-losing tubular disorder characterized by hypokalemic metabolic alkalosis and hypomagnesemia. It is transmitted as an autosomal recessive trait, and variants in the *SLC12A3* gene are found in the majority of patients [1]. Here, we report the first Korean case of *SLC12A3* aberrant skipping of 2 exons detected by RNA splicing analysis.

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As it was not a part of research subjects, approval from the Institutional Review Board was exempt. Informed consent for genetic testing was obtained from the patient.

## Case Report/Case Presentation

In May 2019, a 26-year-old woman visited the genetic counseling clinic. Her fiancé was a known Gitelman syndrome patient who was previously diagnosed with 2 pathogenic variants in *SLC12A3*. In advance of marriage and future family planning, she wanted to perform genetic testing of *SLC12A3*. She was healthy with no specific past medical history or family history.

Results showed a heterozygous change of guanine to adenine at nucleotide 1050, located at exon 8 (NM_000339.2:c.1050G>A). Although this was a silent variant that does not alter the amino acid sequence of the encoded protein (p.Ser350 = ), multiple splice site in silico algorithms including Human Splicing Finder [2] and MaxEntScan [3] predicted this variant to have potential alteration of splicing. To be more specific, in silico algorithms predicted that the variant may create a new cryptic acceptor site within the exon (TCTTCCCCTCGGCC>TCT TCCCCTCAGCC), which will eventually decrease the length of exon 8 by 87 base pairs. The population frequency of this variant was 0.005% in total and 0.04% in East Asians. Although the East Asian population showed higher frequency than other populations, the frequency was still very low and not enough to consider this variant to be benign. This variant was initially classified as "variant of uncertain significance" based on the 2015 American College of Medical Genetics and Genomics and the Association for Molecular Pathology guidelines [4].

Additional RNA splicing analysis was performed to gain more significance of the detected variant. RNA was extracted from the patient's peripheral blood using TRIzol RNA extraction reagent (Invitrogen, Carlsbad, CA, USA) and was reverse transcribed with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). *SLC12A3* cDNA was amplified by PCR using 2 primer sets designed by the authors: (1) e4/5F + e10R, forward 5′-AATGGCAAGGTCAAGTCAGGT-3′ and reverse 5′-AGTTGATGAGGCCGTAGTGG-3′ and (2) e5/6F + e10R, forward 5′-GACCTGCTCCAGGAGTATGG-3′ and reverse 5′-AGTTGATGAGGCC-GTAGTGG-3′.

Along with normal splicing, 2 types of aberrant splicing products were observed (shown in Fig. 1a). RT-PCR performed with primers e4/5F + e10R showed 2 cDNA products for both the patient and control (shown in Fig. 1b). The large-sized fragment (745 bp) corresponded to the correctly spliced mRNA and the small-sized fragment (502 bp) corresponded to the skipping of exons 7–8. The small-sized fragment was more apparently observed for the patient. RT-PCR performed with primers e5/6F + e10R showed 3 cDNA products for both the patient and control (shown in Fig. 1b). The large-sized fragment (605 bp) corresponded to the correctly spliced mRNA, the middle-sized fragment (493 bp) corresponded to the skipping of exon 7, and the small-sized fragment (362 bp) corresponded to the skipping of exons 7–8. Once again, the small-sized fragment was more apparently observed for the patient. Sequencing of the cDNA products confirmed that while normal control showed skipping of exons 7–8 in low level, the patient sample showed skipping in high level (shown in Fig. 1c). Comprehensively, RNA splicing analysis results showed aberrant splicing of exon 7–8 skipping.

## Discussion/Conclusion

A previous report has also shown that aberrant splicing variants of exon 7 skipping and exon 7–8 skipping were to be found in normal control at low levels [5]. In this report, the novel intron 7 and exon 8 boundary variants, which lead to high-level skipping of exons 7–8,

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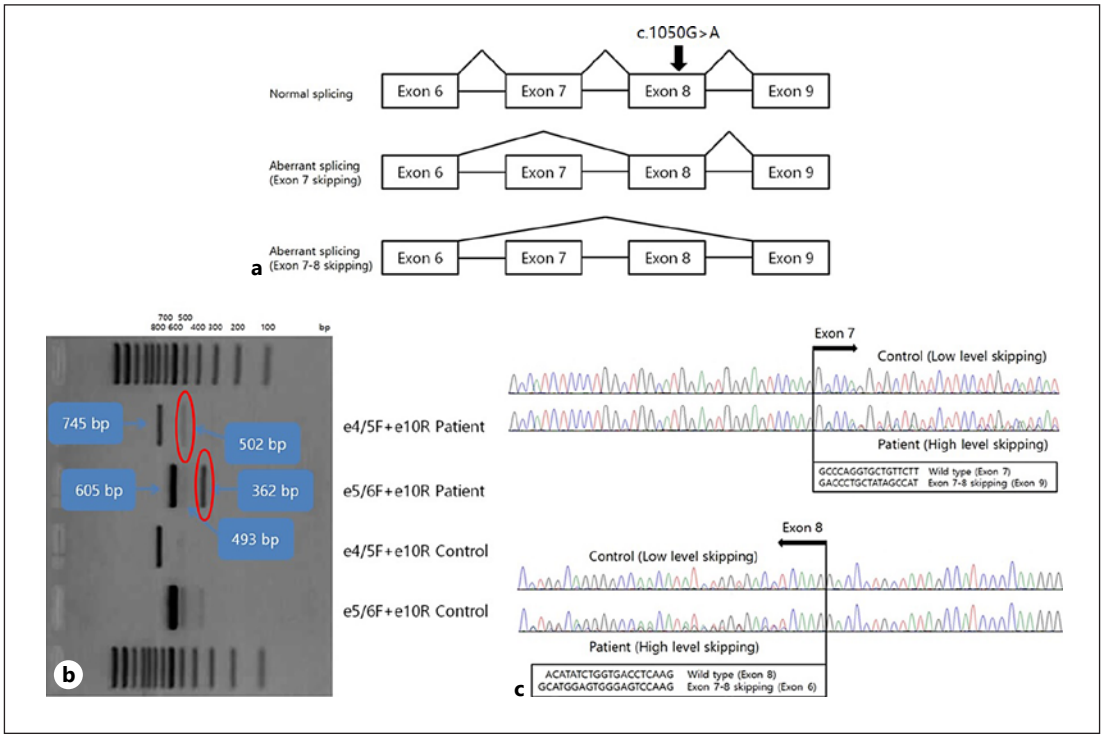


**Fig. 1. a** Schematic overview of normal and aberrant splicing harboring c.1050G>A in *SLC12A3*. **b** Electrophoresis for amplification of *SLC12A3* cDNA. High-level skipping of exons 7–8 circled in red. **c** Sequencing of *SLC12A3* cDNA shows different levels of exon 7–8 skipping.

were described to likely contribute to the pathogenesis of the patient. Exons 7–8 encode regions between the membrane-spanning domains 5 and 6 of the thiazide-sensitive NaCl cotransporter (NCC), which is one of the highly conserved protein superfamily of cotransporters frequently reported to have correlation with Gitelman syndrome [6, 7]. Skipping of exons 7–8 eliminates 243 base pairs, which is expected to lead to an in-frame deletion within one of the important protein domains of *SLC12A3*.

The silent exonic variant c.1050G>A, which is distant from the 5′ and 3′ splice sites, created neither a cryptic splicing acceptor nor a donor site. It is possible that the variant may have altered the exonic splicing enhancer/silencer element that eventually led to increased skipping of 2 exons. However, the mechanism is not yet fully understood and further studies are required.

The major limitation of this case report is that we were not able to extract from the 2 bands detected in the subject with the variant expected to show exon 7–8 skipping and sequence them, which were unfortunately due to conditional limitations. We are fully aware that this would have been very important to define the significance of the study.

No previous reports are available for the variant c.1050G>A in patients with Gitelman syndrome including in Korea [8]. The RNA splicing analysis result points out the potential pathogenicity of this variant, which should be considered a candidate of variant reclassification in the future. However, in order to reclassify the variant, more evidence to support the pathogenicity is necessary. In the case of our patient, the impact of variant reclassification should be crucial. If the variant was to be assumed "likely pathogenic" or "pathogenic," there will be a 50% risk of her future newborn to be diagnosed with Gitelman syndrome considering the genetic condition of her fiancé. She will also meet the indication to possibly perform

preimplantation genetic testing. If more evidence can be gained to support the interpretation of the variant, not only will it be helpful to patients in similar situations but it may also contribute to the diagnosis of suspected Gitelman syndrome patients with only 1 pathogenic variant and c.1050G>A found in trans. We highly recommend the performance of additional RNA splicing analysis, especially for silent variants predicted to have potential alteration of splicing.

## Statement of Ethics

As it was not a part of research subjects, approval from the Institutional Review Board was exempt. Written informed consent was obtained from the patient for publication of this case report and any accompanying images.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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## Author Contributions

K.K. wrote the manuscript. J.W.K. reviewed the manuscript.

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