Carrier Frequency and Prevalence of Citrin Deficiency in East Asians and Koreans Based on Comprehensive Analysis of Pathogenic *SLC25A13* Variants

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Supplemental methods

Validation of the IVS16ins3kb variant in *SLC25A13* via PCR amplification and Sanger sequencing

DNA primer sequences for long-range PCR were adapted from Tabata, *et al.* [1] (Supplemental Data Table S1). *SLC25A13* exons 16–18 were amplified using 200 ng of genomic DNA in a 50-μL reaction mixture including 2 μL of 10% dimethyl sulfoxide and the long-range PCR primers, using the Expand High Fidelity PCR System (Roche Diagnostics, Mannheim, Germany). PCRs were run in a Thermal Cycler 9700 instrument (Applied Biosystems, Foster City, CA, USA) under the following conditions: 94°C for 2 mins; 10 cycles at 94°C for 10 secs, 56°C for 30 secs, and 68°C for 10 mins; 25 cycles at 94°C for 15 secs, 56°C for 30 secs, and 68°C for 10 mins and 20 secs; and a final extension step at 68°C for 7 mins. The PCR products were quality-assessed using agarose gel electrophoresis. To detect the chimeric motif sequence, DNA fragments containing the variant allele were extracted, purified, and subjected to nested PCR. We designed primer pairs for nested PCR (Supplemental Data Table S1). PCRs were run in 20 μL reactions, including Platinum II Hot Start PCR 2X master mix (Invitrogen, California, USA) in the Thermal Cycler 9700 under the following conditions: 94°C for 20 secs; 32 cycles at 94°C for 20 secs, 65°C for 20 secs, and 68°C for 20 secs; and a final extension step at 68°C for 2 mins. After treating the amplification product with shrimp alkaline phosphatase and exonuclease I (USB Corporation, Cleveland, OH, USA), sequencing was performed using the BigDye Terminator v.3.1 Kit (Applied Biosystems). The product was purified and analyzed using an ABI 3730xl DNA Analyzer (Applied Biosystems).

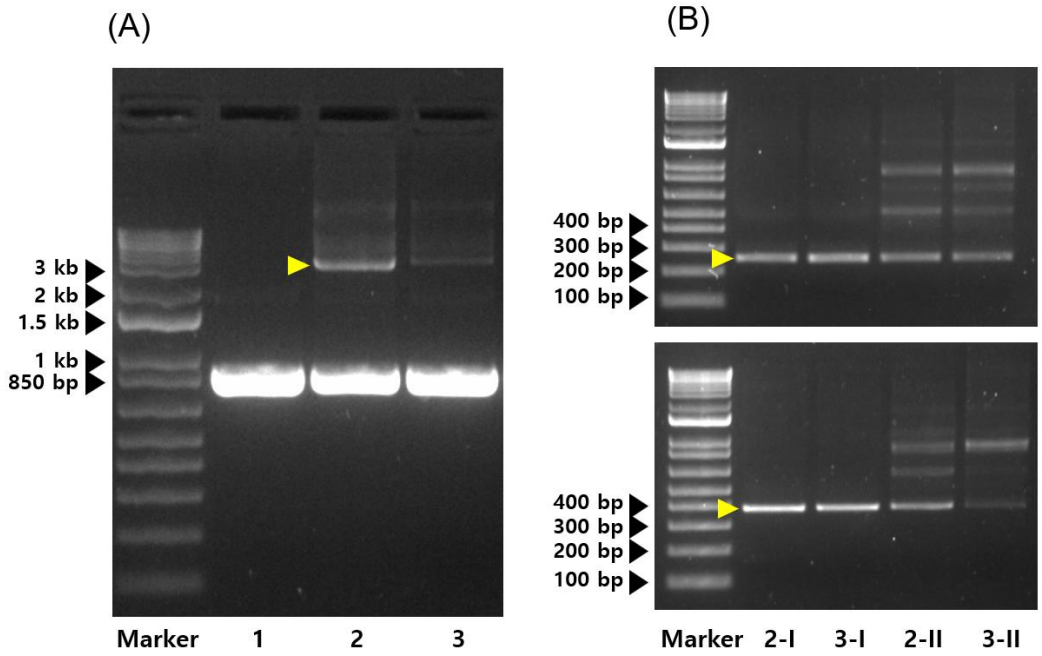
Supplemental Data Table S1. Primers used to amplify the chimeric *SLC25A13-NUS1* sequence

PCR	Name	Sequence (5′→3′)	Direction	Product size
Long-range	E16F	GTATGCCTGCAGCATCTTTAG	F	881 bp
PCR*	E18R	TGCTTCATTCCCAGGAGGGA	R	3.5 kb[†]
Nested	NUS1_1	CTTTCCACTGCCAACACCTC	F	246 bp
PCR set I	SLC25A13_I17	TTCCCTACGACAACAGAGCA	R	
Nested	NUS1_2	TACTCTTGTGGCCCTCAGAC	F	373 bp
PCR set II	SLC25A13_I17	TTCCCTACGACAACAGAGCA	R	

*Primer sequences for long-range PCR were adapted from Tabata, *et al.* [1].

[†]Predicted amplicon size when the IVS16ins3kb variant is present.

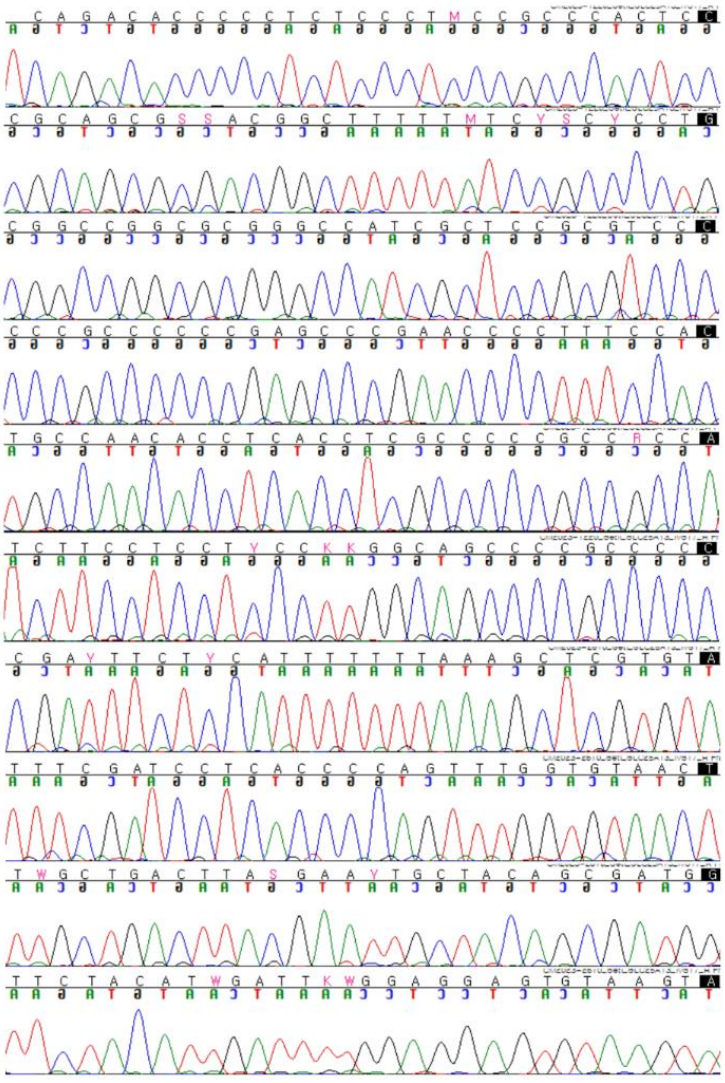
Abbreviations: F, forward; R, reverse.



Supplemental Data Fig. S1. Confirmation of the IVS16ins3kb variant in *SLC25A13* using PCR and gel electrophoresis. (A) Gel electrophoresis images following long-range PCR. The 3.5-kilobase DNA fragment (yellow arrowhead) in lanes 2 and 3 represents the IVS16ins3kb variant, whereas lane 1 contains only the 881 bp wild-type fragment. (B) Gel electrophoresis images following nested PCR. DNA fragments of 246 bp (upper) and 373 bp (lower) resulting from nested PCR with IVS16ins3kb-positive samples (yellow arrowheads) are shown. Samples 2-I and 3-I, which exhibited a single band, were obtained by cutting the 3.5 kb region from the gel after long-range PCR, followed by purification and subsequent nested PCR. Samples 2-II and 3-II, displaying multiple bands, were processed without gel extraction. The samples were derived from the same experiment, and the gels were processed in parallel.

(A)[3′-(*NUS1 sequence, omitted*)TCAGACACCCCCTCTCCCTCCCGCCCACTCC
CGCAGCGCGACGGCTTTTTATCCGCCCCTGCGGCCGGCGCGGGCCATCG
CTCCGCGTCCCCCCGCCCCCCGAGCCCGAACCCCTTTCCACTGCCAACA
CCTCACCTCGCCCCCGCCGCCATCTTCCTCCTCCCTTGGCAGCCCCGCCC
CCC-5′]GATTTCTCCATTTTTTTAAAG
CTCGTGTATTTCGATCCTCACCCCAGTTTGGTGTAACTTTGCTGACTTACG
AATTGCTACAGCGATGGTTCTACATTGATTTTGGAGGAGTGTAAGTA

(B)



Supplemental Data Fig. S2. Sanger sequencing data for the *SLC25A13* IVS16ins3kb variant. (A) In the sequence, each domain is represented by a distinct color. The *NUS1* sequence is shown in gray, intron 16 of *SLC25A13* is highlighted in yellow, and exon 17 of *SLC25A13* is depicted in green. (B) The corresponding sequence chromatogram for the information presented in (A) is shown.

REFERENCE

1. Tabata A, Sheng JS, Ushikai M, Song YZ, Gao HZ, Lu YB, et al. Identification of 13 novel mutations including a retrotransposal insertion in *SLC25A13* gene and frequency of 30 mutations found in patients with citrin deficiency. J Hum Genet 2008;53:534-45.