Novel Homozygous *KREMEN1* Mutation causes Ectodermal Dysplasia

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Supplementary Materials and Methods

1. Splicing assay

A genomic DNA fragment encompassing exon 1 of *KREMEN1* was amplified with primers (689 bp, sense: 5'-CAGGTGAGGCGAGAGATGG-3', antisense: 5'-AGGGTCTTGGAAGACTCAAGG-3') and cloned into the TopBlunt vector (Enzynomics, Daejeon, Korea). Conventional PCR mutagenesis was performed with a wild-type clone (sense: 5'-CCGGACCCGGAGAGTGTGAG-3', antisense: 5'-CTCACACTCTCCGGGTCCGG-3'). Wild-type and mutant fragments were subcloned into the pSPL3 vector using restriction enzymes EcoRI and XhoI. Subcloned vector sequences were confirmed by Sanger sequencing of the plasmids. COS-7 cells were transiently transfected with the wild-type and mutant pSPL3 vectors and total RNA was harvested. RT-PCR was performed with primers (normal splicing product 251 bp, sense: 5'-TGCCCCCTTTACCCCG-3', antisense: 5'-TGCACCTGAGGAGTGAATTG-3')

2. Mutation effect

Splicing assay showed two amplification bands with a normal splicing product (251 bp) and a vector sequence (405 bp) in the wild-type vector. However, only a single band with a vector sequence was observed in the mutant vector. The mutation destroyed the splicing donor site of exon 1 and the result would be a retention of intron 1. The retention would introduce a premature stop codon (TAG in red color) located in the intron 1 sequence and this premature stop codon [p.(Glu33Glyfs*316)] would trigger a nonsense-mediated mRNA decay.

Intron 1 retention

ATGGCGCCGCCAGCCGCCCGCCTCGCCCTGCTCTCCGCCGCGGCGCTCACGCTGGCGGCCCGGCCCGCGCCTAGC
CCCGGCCTCGGCCCCGGACCCGGTGAGTGTGAGCGACCCCCCGCCGCCCGCCCTGAGCGGAGCCCACTCGAGGGG
CGACAAGGGCCGGCCGGCCTGAGAGCCCCCTCCCTCCCGCTTCAGGACCTTCCGGGCCCCTTCCCCTCGCCCCTA
GGCGACGCCCCTCAGGCCGGGATGGTCCCTTCCTGGGACCCGGGCTACCCCCAGGCCCGTCATCGACGCCCCCGG

GCCCGGTACTGTCCCCCGGCTGCAGGACCCGGTGCTCCTCAGCGACGCCCCTCAG CCCAGGAGGCCCTCTCCGCC
TTGAGTCTTCCAAGACCCTCTGCGACGCCCCCCGGGCTGGGACATCTTCTCTGTCTCGGGATCTGGGACCCGCTG
CCCGAGTCCCTCAGCGACCCCAACCAGGCCGGGACGCCCCCTCTCCCCGGTACCTCCTGGGATCCCGTCCCAAGT
CCCCAGCGACTTCCCCCGGGCCGGGACGTCCTCTGCTCCCCGGTACCTCCTAGGATCCCGTCCCAAAATCCCCAG
CGACTCCCCACGGGCCAGGAGGCCCCCTGCTCCCCGGTACCTCCTGGGATCCCGTCCCCAAGTCCCCAGCGACCC
CTCCCGGGCCGGGACGACCCCTGCTCCCCAGTACCTCCTGGGATCCCGCCCCAAGTCCTTCATCGACGCACCTTG
CACCGGGACGACTCCCCCGCTACAAGAGGCTATACGCCCCTCTCCGAGACCTCCAGCGACATCCCTCCCCTGGGC
CAAGGTCCCCTCCCTGAGCCTCACTGCGACGCCCCCGCGTCC CCCAGTCCTCTCCTCCCGCCTACACCGGTGGAA
CCCGGCGCCTCCCCGCGCAGAGCAGAGCGGAGGCGGGAGGAGCCGGCGCTCAGCCCCTTTTCCCGAGTCCTCGGC
TGCACCCGCTTGGCGGACATTATAACTTCTGCCTCGCGAGGAACGGGATGGACTTGTTCGCCCTGC **TAG**AGGCAG
GTTAGGGTCCTGGGACGACCTTGTACCCAGACGGACGGGACGTGCCCTCTCTCTCCGCTGGGCCGCTTTGAAC TT
CCCTATGACTCAGGTGATGGCGCAGAAGGGGGAGAGAAAAAAGGAAGCAGTGATGGGAAACTTCTCCCCAACTGA
GTTTAGGGTGCTCTTCCTGAGGGTGCAACGCCGAGCTCCGTGTTTTGGGTCAGCCCACACCTTAGACAGGTCACT
CACTACCCAGGCCAAGGCCAAGGCCAGGTCTTCCCGAGGTGAGGCCCTGGACCAGGATGAAGCTTGGCTTTTGCT
TAACTTCCACACGCAACCTTGTAGCCGAATCCTTTCTAAGTGGAAGAGAAGGCAAGAGGGCGTTGCATTTC

This novel mutation, p.(Glu33Glyfs*316), is predicted to encode a putative protein of 347 aa of which 315 are novel amino acids differ from the native protein.

Table S1. List of the reported mutations in *KREMEN1*.

Location	cDNA	Protein	Phenotype except oligodontia	Hereditary mode	Ethnicity	Reference
Intron 1	c.97+2T>A	p.(Glu33Glyfs*316)	Curly fluffy hair, thin eyebrows and eyelashes, mildly dry skin and perioral pigmentation	homozygous	Turkish	This report
Exon 2	c.146C>G	p.(Thr49Arg)	sparse hair, dry skin, sparse eyebrows and eyelashes, protruded lips, and heat intolerance	homozygous	Turkish	Dinckan et al., 2018
Exon 3	c.331T>A	p.(Cys111Ser)	Sparse hair, thin eyebrows and eyelashes, dry skin and perioral hyperpigmentation	maternal	Thai	Intarak et al., 2018
Exon 5	c.626T>C	p.(Phe209Ser)	abnormal hair distribution of the scalp, low hairline with forehead fuzziness, broad and low nose bridge, columella extending with age, thick lips, slight ocular hypertelorism, and downward slanting of the palpebral fissures	homozygous	Palestinian	Issa et al., 2016
Exon 6	c.774_779del	p.(Phe258_Pro259del)	sparse hair, dry skin, sparse eyebrows and eyelashes, protruded lips, and heat intolerance	homozygous	Turkish	Dinckan et al., 2018
Exon 7	c.1036_1046del	p.(Ala346Cysfs*27)		paternal	Thai	Intarak et al., 2018

Sequences based on the reference sequence for mRNA (NM_032045.5) and protein (NP_114434.3), where the A of the ATG translation initiation codon is nucleotide 1.

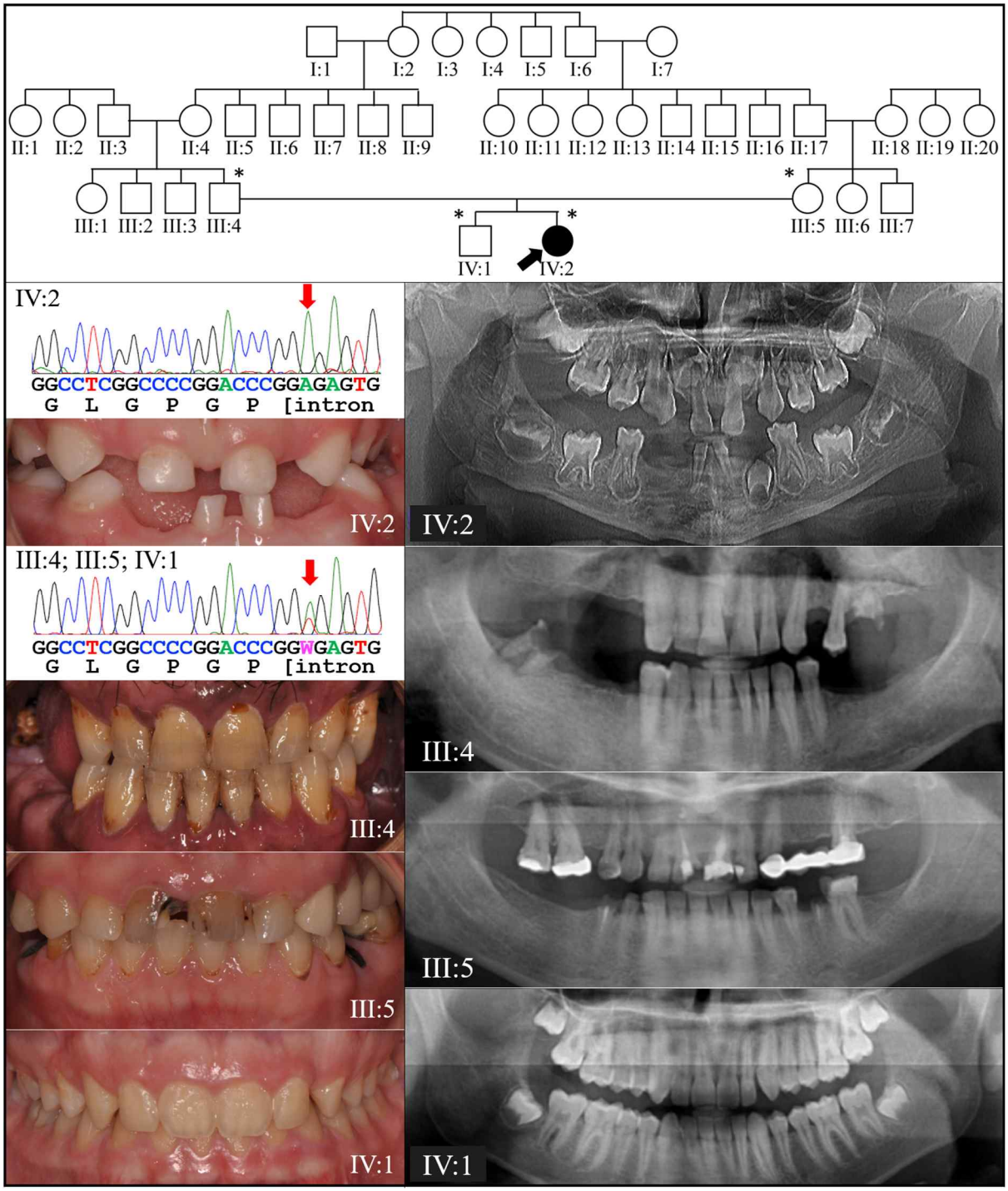

Figure S1 Pedigree, clinical photos, sequencing chromatograms, and panoramic radiographs. Participating individuals are indicated by asterisk above the symbol. The proband is indicated by an arrow. Mutated nucleotide is indicated by red arrows in the sequencing chromatograms. Panoramic radiograph of each individual is shown on the right side. Father (III:4) and mother (III:5) lost many teeth, but there was no congenitally missing tooth. They reported that the teeth were extracted due to dental caries. Complete dentition without any missing tooth can be seen in the brother of the proband (IV:1).