SUPPLEMENTAL METHODS

Genetic testing

Genomic DNA from the patient and her family was extracted from peripheral blood using the Gentra Puregene Blood kit (Qiagen, Hilden, Germany) as previously described [1]. Polymerase chain reaction and Sanger sequencing of protein kinase A regulatory subunit 1 alpha (*PRKAR1A*) (NM_002734.5) were performed using the extracted DNA with primers for all exons (Supplemental Table S1). To confirm the identity of the variant, exon 2 and adjacent intronic regions were amplified and sequenced using DNA samples from family members. Direct sequencing and *in silico* prediction of the variant were performed as previously mentioned [2].

Total RNA was extracted from fresh frozen pituitary adenoma tissue using the QIAamp RNA Blood Mini kit (Qiagen), and complementary DNA was reverse-transcribed. The coding sequence of *PRKAR1A* was amplified and sequenced using a forward primer (5′-GTCAGTAGCCGAACGCTGAT-3′) and reverse primer (5′-TTAACCACTGGGTTGGGTGG-3′).

Functional analysis

Human cDNA coding for *PRKAR1A* was cloned into the pCMV6-HA plasmid as a template using the TOPcloner™ TA kit (Enzynomics, Daejeon, Korea) following the manufacturer's instructions. Mutant constructs were generated by site-directed mutagenesis using the EZchange™ Site-directed Mutagenesis kit (Enzynomics). Two mutated constructs (c.491_492del and c.176A>T) were generated by modifying the primers (Supplemental Table S2). Plasmids encoding wild-type *PRKACA* were cotransfected.

COS-7, an African green monkey kidney fibroblast-like cell line suitable for transfection with vectors requiring the expression of SV40 T antigen, was maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37°C in a humid atmosphere of 95% air and 5% CO_2. The cells were transfected with different constructs using Lipofectamine 3000 (Invitrogen, Waltham, MA, USA) following the manufacturer's instructions. The cells were incubated at 37°C in a CO_2 incubator for 18 hours post-transfection.

The activity of purified active PKA was assayed in cell lysates using a PKA Kinase Activity kit (Enzo Life Sciences, New York, NY, USA) according to the manufacturer's instructions. The samples were incubated for 60 minutes at 30°C. Cyclic adenosine monophosphate levels were measured using a colorimetric assay kit (R&D Systems, Minneapolis, MN, USA), following the manufacturer's instructions.

Repeated-measures analysis of variance (ANOVA) and the Bonferroni multiple comparison test for *post hoc* analysis (Friedman test and Dunn multiple comparison test for *post hoc* analyses were used to analyze non-normally distributed continuous variables) were used for statistical analysis.

Ethical statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. This study was reviewed and approved by the Institute Review Board of Seoul National University Hospital (IRB No. 2204-048-1314). Informed consent for genetic testing was obtained from all the participants included in the study.

SUPPLEMENTAL REFERENCES

1. Kim B, Kim MJ, Hur K, Jo SJ, Ko JM, Park SS, et al. Clinical and genetic profiling of nevoid basal cell carcinoma syndrome in Korean patients by whole-exome sequencing. Sci Rep 2021;11:1163.
2. Lee S, Kim EJ, Cho SI, Park H, Seo SH, Seong MW, et al. Spectrum of MNX1 pathogenic variants and associated clinical features in Korean patients with currarino syndrome. Ann Lab Med 2018;38:242-8.