A novel *PRKAR1A* gene mutation with mild brachydactyly

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COI

We have nothing to disclose.

Take home message

PRKAR1A gene mutation carriers may show mild brachydactyly.

Background

Acrodysostosis is a rare skeletal dysplasia with severe brachydactyly, facial dysostosis and nasal hypoplasia. In 2012, *PRKAR1A* gene was identified as one of the responsible genes of Acrodysostosis with hormonal resistance (ADOHR). Reported phenotypes of *PRKAR1A* gene mutations are severe brachydactyly and mild hormone resistance.

Objective

To report a novel heterozygous mutation of *PRKAR1A* gene in an ADOHR patient with mild brachydactyly.

Patient

【Patient】5-year-old boy

He was a second child of nonconsanguineous healthy Japanese parents. He was normally delivered at 39 weeks of gestation after an uncomplicated pregnancy. Birth weight and height were 2520g (-1.67SD) and 49.5cm (0.38SD), respectively. At the age of five, he was referred to us for the evaluation of the short stature. His growth motor and pshychological development was normal for his age. His height, weight were 100.7cm (-2.3SD), 15.7kg (-1.3SD), respectively. He had mild round face and nasal hypoplasia, which was similar to his father's facial appearance. Also he presented mild brachydactyly and undescended testis of the right side. Testes size were both 2ml.

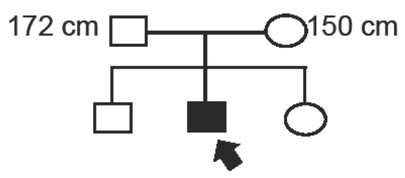


Fig. 1 Pedigree

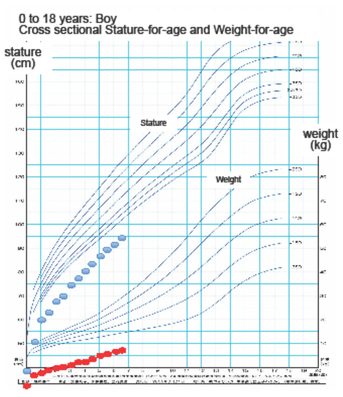


Fig. 2 Growth Chart

【Radiograph of the left hand (Fig.3)】

Brachymesophalangy with cone shaped epiphyses and mild metacarpal shortening

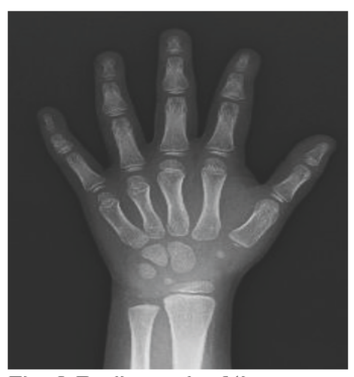
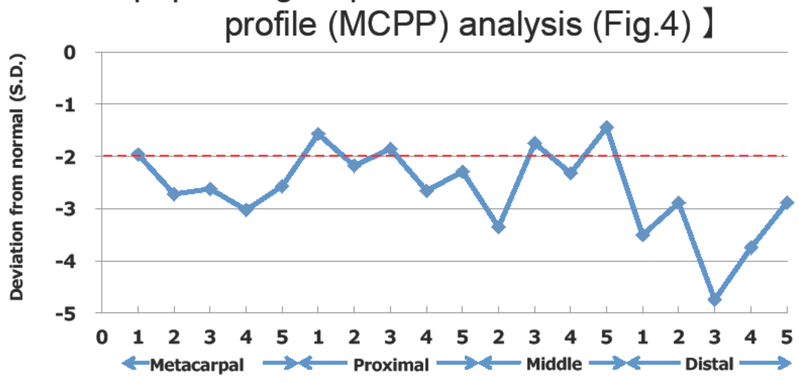


Fig. 3 Radiograph of the left hand

【Metacarpophalangeal pattern profile (MCPP) analysis (Fig.4) 】

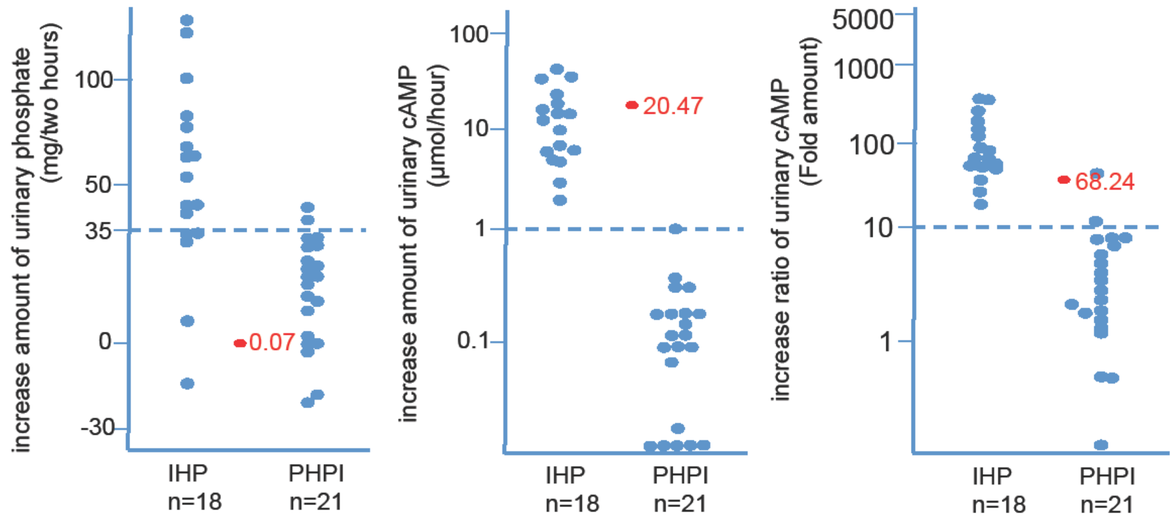


【Blood test】

Ca	9.7	mg/dl	iPTH	95	pg/ml		
P	4.8	mg/dl	IGF1	95	ng/ml	LH	<0.10 mIU/ml
ALP	658	U/l	TSH	8.64	μIU/ml	FSH	0.83 mIU/ml
Mg	1.9	mg/dl	FT3	3.95	pg/ml	Testosterone	
25OHD	42	ng/ml	FT4	1.25	ng/dl		<0.03 ng/ml

【Urine test】 U-Ca/Cr 0.05 %TRP 88.2%

【Ellsworth-Howard test】 ●Patient



IHP: Idiopathic hypoparathyroidism PHP1: Pseudohypoparathyroidism type 1

[Ogata, *et al. Nihon Naibunpi Gakkai Zasshi.* 1984]

Fig. 5 Urinary P and cAMP response to PTH infusion of the patient and reported cases.

➡Urinary cAMP responded to PTH while urinary P excretion did not increase

Methods

This study protocol was approved by the Institutional Ethical Review Board of the Tokyo Metropolitan Children's Medical Center and Keio University.

1, Mutational Analyses

Genomic DNA of the patient and his parents were extracted from the peripheral leukocytes or saliva by using standard techniques. We sequenced three genes of *GNAS*, *PRKAR1A* and *PDE4D* using the next generation sequence strategy. A *PRKAR1A* mutation indicated by the screening analysis was confirmed by Sanger sequencing.

2, Functional Assay

We generated PRKAR1A expression vectors containing wild type and mutant type. To compare the novel identified mutant type with previously reported one, we also generated R368X PRKAR1A expression vector, which is well characterized. Using HEK293 cells, we performed Forskolin induced CRE-Luciferase activity to analyse the Protein Kinase A activity.

Results

【1、Gene analysis: *PRKAR1A* gene】

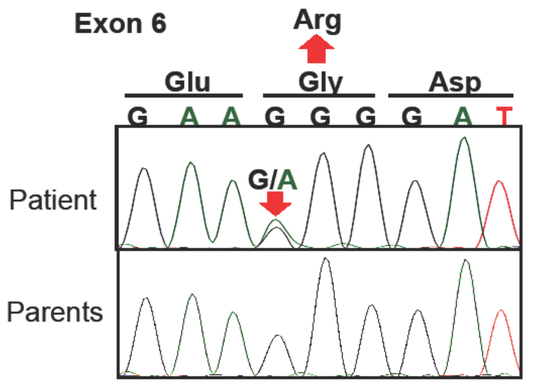


Fig.6 Partial sequences of the PCR products

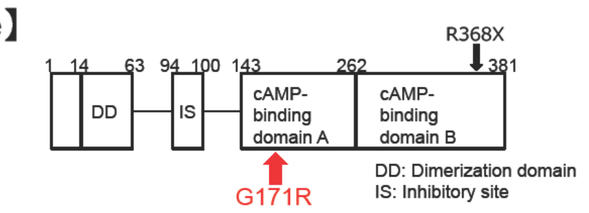


Fig.7 *PRKAR1A* functional domains and the novel mutation of the patient.

PRKAR1A

Human	QGDEGDNFYVIDQ
Mouse	QGDEGDNFYVIDQ
Rat	QGDEGDNFYVIDQ
Chicken	QGDEGDNFYVVDQ
Xenopus	QGDEGDNFYVVDQ
Zebrafish	QGDEGDNFYVIDQ

Fig.8 Homology study

【2、Crystal structure modeling】

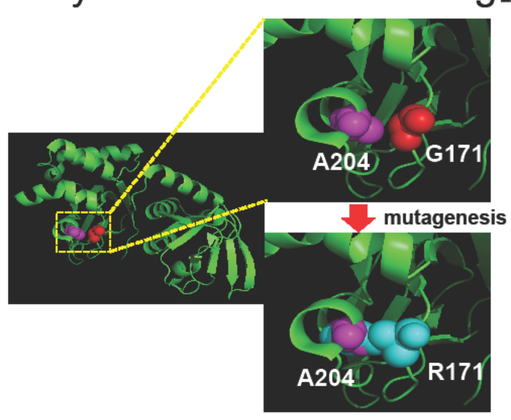


Fig.9 Crystal structure modeling of WT and G171R

【3、Functional analysis】

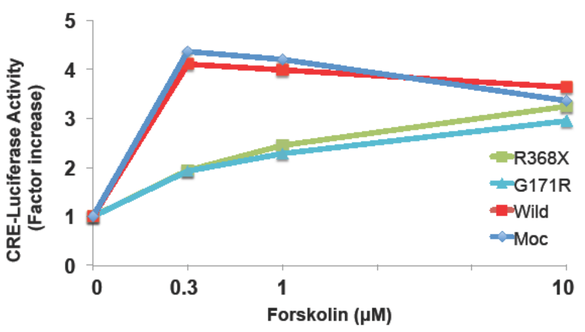


Fig.10 PKA transcriptional activity in cells stimulated with forskolin

1, Mutational Analysis

We identified a novel heterozygous mutation of c.511G>A, p.G171R in *PRKAR1A* gene (Fig. 6). G171 was located in the cAMP binding domain A (Fig. 7). Homology study revealed that the glycine residue at codon 171 is highly conserved throughout the species (Fig. 8). Familial analysis revealed that his parents did not carry the mutation.

According to the crystal structure modeling, the glycine to arginine substitution was predicted to make an abnormal residue-residue binding to Ala204 (Fig. 9).

2, Functional Analysis

In HEK293 cells, G171R showed reduced activity of protein kinase A (PKA) stimulated with forskolin compared to wild type. The reduced PKA activity of G171R was equivalent to that of R368X (Fig. 10) .

Discussion

- The facial appearance and mild brachydactyly with hormone resistance indicated PHP rather than ADOHR. But the response to PTH infusion indicated abnormality of downstream of the GNAS.
- MCPP analysis revealed that the severity of brachydactyly of the patient were similar to that of PHP, which reported to be milder than Achrodysostosis [de Sanctis et al., 2004].
- At present, he does not develop hypocalcemia or hypothyroid. It is considered that hormone resistance of this patient is mild as it has been reported [Linglart et al., 2011].
- Only one patient was reported to carry the missense mutation of cAMP binding domain A [Nagasaki et al., 2012]. The reported patient presented typical ADOHR phenotypes including severe brachydactyly. The phenotype of mild brachydactyly may not associate with reduced function of this domain.

Conclusion

PRKAR1A gene mutation carriers may show mild brachydactyly.

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