資料３－１． The 9th Biennial Scientific Meeting of the Asia Pacific Paediatric Endocrine Society / The 50th Annual Meeting of the Japanese Society for Pediatric Endocrinology 抄録

Detection of somatic activating GNAS mutations in girls with isolated autonomous ovarian cyst by next generation sequencing.

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Background: GnRH-independent precocious puberty (PP) due to autonomous ovarian cyst is one of the features of McCune-Albright syndrome (MAS) caused by somatic activating *GNAS* mutations. In a previous report, somatic activating *GNAS* mutations were found in 13 (33.3%) of 39 ovarian samples from girls with isolated autonomous ovarian cyst (J Clin Enocrinol Metab 2004; 89: 2107). In the same report, a series of nested PCR and restriction enzyme digestion detected somatic activating *GNAS* mutations in only 3 (7.7%) in 39 peripheral blood leucocytes (PBL) samples. We reported that next generation sequencing (NGS) detected somatic activating *GNAS* mutations sensitively from PBL samples in MAS (PLoS One 2013; 8: e60525).
Objective: To determine if we could detect somatic activating *GNAS* mutations in girls with isolated autonomous ovarian cyst by NGS using PBL samples.
Method: The study included 7 prepubertal girls with GnRH-independent PP due to isolated autonomous ovarian cyst. We excluded cases with fibrous dysplasia (FD) and/or cafe´-au-lait skin spots. We performed both NGS and combinatory method of peptide nucleic acids (PNA) probe with NGS (PNA-NGS) using PBL samples from all patients.
Results: We detected somatic activating *GNAS* mutations in one (14.3%) by NGS and 5 (71.4%) by PNA-NGS of PBL samples (Table.1).
Conclusion: The combinatory method of PNA-NGS can detect somatic activating *GNAS* mutations sensitively from PBL samples in girls with isolated autonomous ovarian cyst. Our data suggest that somatic activating *GNAS* mutation is the major cause of isolated autonomous ovarian cyst.

Table 1. Characteristics of 7 girls with isolated autonomous ovarian cyst.

Case	First symptom		First evaluation			MAS feature		Ovarian cyst	Last evaluation	Mutation detection method	
	Age (yrs)	Type	Age (yrs)	Bone age (yrs)	Tanner stage	FD	Skin Lesion		Age (yrs)	NGS	PNA-NGS
1	4.8	B	5.3	5.4	B2P1	Absent*	Absent	Present	12.0	Negative	Negative
2	0.3	B, M	1.9	1.9	B3P1	Absent*	Absent	Present	4.8	Negative	Negative
3	3.0	B, M	7.5	10.5	B2P1	Absent*	Absent	Present	10.2	Negative	R201C
4	1.5	B	4.6	4.6	B2P1	Absent*	Absent	Present	7.3	Negative	R201H
5	1.0	B	2.7	3.0	B3P1	Absent**	Absent	Present	5.3	Negative	R201H
6	3.3	B	3.3	3.1	B2P1	Absent**	Absent	Present	5.5	Negative	R201C
7	1.4	B, M	1.5	1.5	B2P1	Absent**	Absent	Present	4.3	R201H	R201H

Abbreviations: B breast, P pubic hair, M vaginal bleeding.
*FD was ruled out by skeletal radiographic examination.
**FD was ruled out by absence of history both of fracture and skeletal deformity.