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

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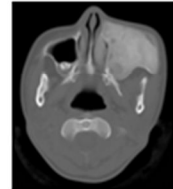
Take Home Message

Somatic activating *GNAS* mutations
is the major cause of
isolated autonomous ovarian cyst

McCune-Albright syndrome (MAS)

- Caused by an somatic activating mutation of *GNAS*
- Characterized clinically by the classic triad
  - Fibrous dysplasia (FD)
  - Café-au-lait skin spots
  - GnRH-independent precocious puberty (PP) due to autonomous ovarian cyst

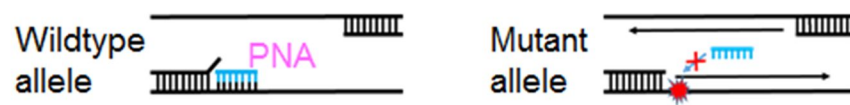# Somatic activating *GNAS* mutations in isolated autonomous ovarian cyst

- Ovarian samples
  direct sequencing
  detected in 13 (33.3%) of 39

- Peripheral blood leucocytes (PBL) samples
  nested PCR and restriction enzyme digestion
  detected in only 3 (7.7%) in 39

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Detection of somatic activating *GNAS* mutations by next generation sequencing (NGS)

- PBL samples in MAS

  NGS with peptide nucleic acids (PNA) probe detected in 12 (75.0%) of 16

Wildtype allele   PNA

Mutant allele

Objective

To determine if we could detect somatic activating *GNAS* mutations in girls with isolated autonomous ovarian cyst by NGS using PBL samples
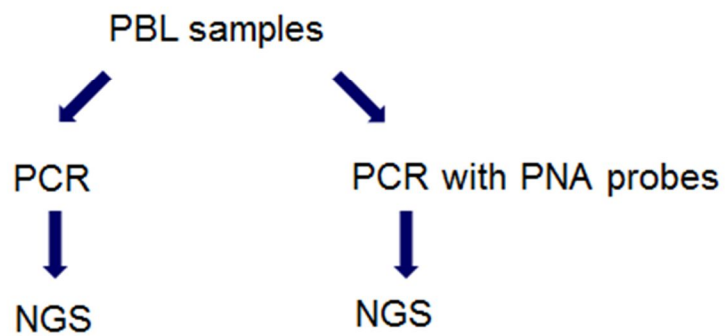
Participants

- Inclusion criteria
  - GnRH-independent PP due to isolated autonomous ovarian cyst
- Exclusion criteria
  - Fibrous dysplasia (FD)
  - Café-au-lait skin spots

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Methods
Mutation detection

PBL samples

PCR   PCR with PNA probes

NGS   NGS

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Results
Characteristics of 8 participants

	First symptom		First evaluation			MAS feature			Last evaluation
Case	Age (years)	Type	Age (years)	Bone age (years)	Tanner stage	FD	Skin lesion	Ovarian cyst	Age (years)
1	4.8	B	5.3	5.4	B2P1	Absent*	Absent	Present	12.0
2	0.3	B, V	1.9	1.9	B3P1	Absent*	Absent	Present	4.8
3	3.0	B, V	7.5	10.5	B2P1	Absent*	Absent	Present	10.2
4	1.5	B	4.6	4.6	B2P1	Absent*	Absent	Present	7.3
5	1.0	B	2.7	3.0	B3P1	No history**	Absent	Present	5.3
6	3.3	B	3.3	3.1	B2P1	No history**	Absent	Present	5.5
7	1.4	B, V	1.5	1.5	B2P1	No history**	Absent	Present	4.3
8	5.3	V	5.5	4.6	B1P1	No history**	Absent	Present	6.4

Abbreviations: B breast, P pubic hair, V vaginal bleeding.
*No lesion on skeletal radiographic examination.
**No history of fracture nor skeletal deformity.

Results
Detection of somatic activating *GNAS* mutations

	Mutation detection method	
Case	NGS	NGS with PNA
1	Negative	Negative
2	Negative	Negative
3	Negative	R201C
4	Negative	R201H
5	Negative	R201H
6	Negative	R201C
7	R201H	R201H
8	Negative	Negative

< Detection probabilities >

- NGS
  one (12.5%) in 8

- PNA-NGS
  5 (62.5%) in 8

Discussion

- This is the first report of somatic activating *GNAS* mutations analyses using NGS in isolated autonomous ovarian cyst
- We detected somatic activating *GNAS* mutations sensitively from PBL samples by PNA-NGS

⇒ Somatic activating *GNAS* mutations is the major cause of isolated autonomous ovarian cyst

Discussion

- Somatic activating *GNAS* mutations analysis by PNA-NGS using PBL sample is less invasive than biopsy

⇒ PNA-NGS using PBL sample may be useful for differentiation of GnRH-independent PP

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