Mutation screening of the Sonic Hedgehog signaling-related genes in 120 Japanese patients with congenital hypopituitarism

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Disclosure statement: The authors have declared no conflicts of interest.

Take Home Messages
1. The frequency of Sonic Hedgehog (SHH) signaling or Holoprosencephaly (HPE) related gene mutations in patients with congenital hypopituitarism was 3.3% (4/120) in Japan.
2. Multiple pituitary hormone deficiencies with Cleft palate cases could be a good candidate for SHH signaling or HPE related gene analysis.

Backgrounds
✔ The Sonic Hedgehog (SHH) signaling pathway plays a crucial role in development of the forebrain and pituitary.
✔ Mutations in SHH signaling related genes are well known to be the cause of Holoprosencephaly (HPE), which results from developmental field defect or impaired midline cleavage of the embryonic forebrain, and is frequently associated with congenital hypopituitarism (CH).
✔ The prevalence of CH attributable to SHH or HPE-related gene mutations appears to be rare and varies among populations.

Objectives
This study aimed to define the prevalence of CH in terms of seven SHH or HPE-related genes (GLI2, SHH, TGIF1, SIX3, ZIC2, GPR161, and CODM) among Japanese patients.

Materials & Methods
We enrolled 120 Japanese CH patients (HPE is not included). The inclusion criteria were 1) or more.
1. Anterior pituitary hypoplasia as detected by brain MRI.
2. Short stature with severe GH deficiency (GH peak < 3 ng/mL), confirmed by more than two provocation tests.
2b. Inadequate low serum GH at a time of severe hypoglycemia as neonate.

Results
Two TGIF1 and two GLI2 mutations were identified.

Pedigree 1: (TGIF p.R219C)
The propositus was a 13-year-old Japanese male. The patient exhibited GH, and TSH deficiencies with micro penis. Brain MRI showed severe anterior pituitary hypoplasia with an eutopic posterior pituitary gland. No HPE brain defects were present. Parental gene analysis was refused.

Pedigree 2: (TGIF p.R219C)
The propositus was a 10-year-old Japanese male. The patient exhibited GH, and TSH deficiencies with micro penis. Brain MRI showed severe anterior pituitary hypoplasia with an eutopic posterior pituitary gland. No HPE brain defects were present. Parental gene analysis was refused.

Pedigree 3: (GLI2 p.Q1182*)
The propositus was a 10-year-old Japanese male. The patient exhibited GH, TSH, and ACTH deficiencies with micro penis, and cleft lip and palate. Brain MRI showed severe anterior pituitary hypoplasia with an ectopic posterior pituitary gland. No HPE brain defects were present. Parental gene analysis was refused.

Pedigree 4: (GLI2 p.G1182*)
The propositus was a 18-year-old Japanese male. The patient exhibited GH, TSH, LH/FSH, and ACTH deficiencies. Brain MRI showed anterior pituitary hypoplasia with an ectopic posterior pituitary gland. No HPE brain defects were present. Asymptomatic Father carried the same mutation.

Results
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Functional assays for mutant TGIF1

A. Transactivation assays
TGFS-responsive luciferase reporter (3TP- luc)

B. Western blotting

C. EMSA experiments

In vitro experiments showed that N235Y TGIF1 resulted in a decrease of repressing activity, and had no dominant negative effect (FIG.A). Western blotting and subcellular localization revealed no significant difference between wild type and N235Y TGIF1 (FIG.B, C). Electrophoretic mobility shift assays showed that the N235Y TGIF1 bound with slightly low efficiency to the wild type (FIG.D). Q396* is a previously reported TGIF mutant, which is related to CH.

Discussion
The frequency of SHH signaling or HPE related gene mutations in patients with CH was 3.3 % (4/120) in Japan.

Genotype phenotype correlation is not clear.