

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

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## Take Home Messages

- 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..
- 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.

Greece 2/30=6.7% (Tatsi *et al.* J Clin Endocrinol Metab. 2013.)

One *SHH*, and one *TGIF1* mutation were found in 30 pituitary stalk interruption syndrome patients

Brazil 6/115=5.2% (Paulo SS *et al.* Clin Endocrinol (Oxf). 2015)

One *SHH*, and five *GLI2* mutations were found in 115 CH patients.

Japan ?

## 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+2a or 1+2b

- Anterior pituitary hypoplasia as detected by brain MRI.
- 2a. 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.

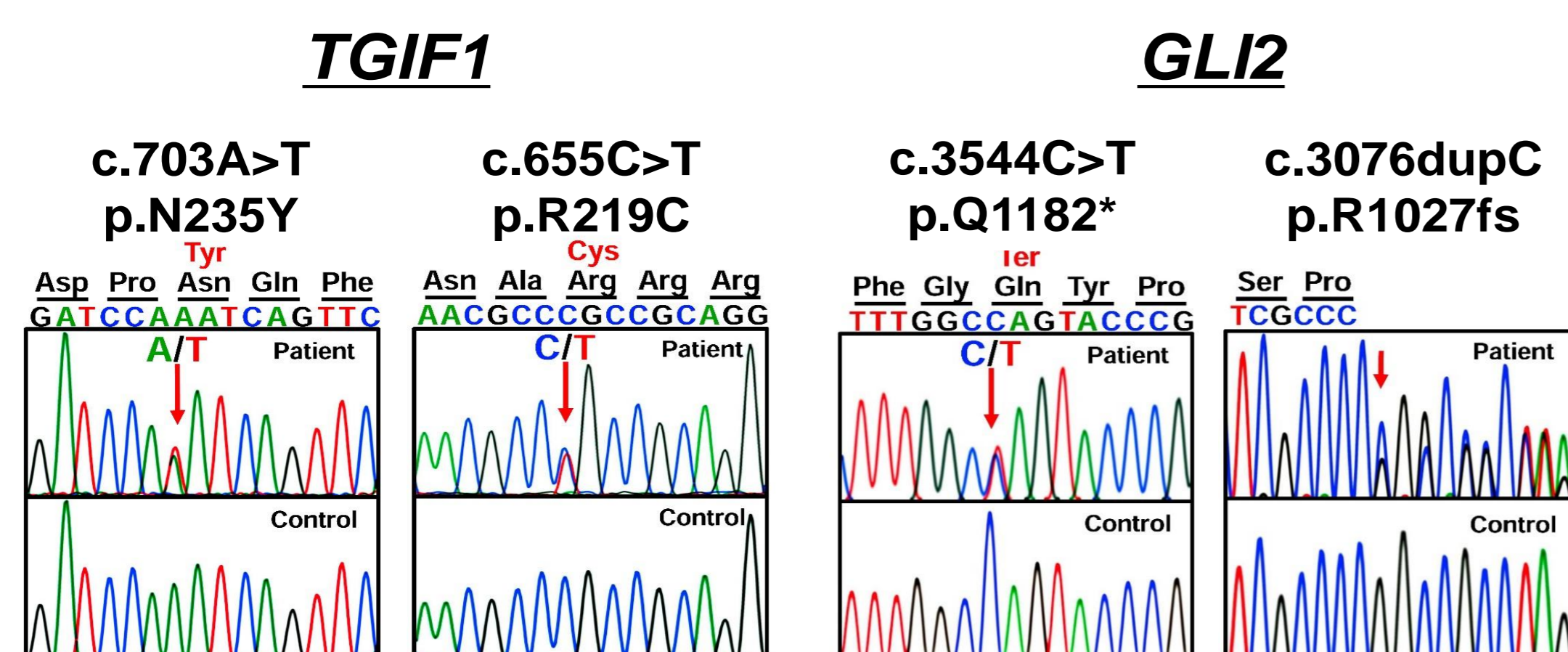
	Midline defect		
	-	+	
		SOD	CP
Isolated GHD	21	4	3
MPHD	67	17	8
Total	88	21	11

MPHD: multiple pituitary hormone deficiency  
SOD: Septo-optic dysplasia  
CP: Cleft palate

Mutations in *POU1F1*, *PROP1*, *LHX3*, *LHX4*, *HESX1*, *SOX2*, *SOX3*, and *OTX2* have been excluded by PCR-direct sequence. We sequenced all coding exons and flanking introns of 7 genes by PCR-direct sequencing or next generation sequencing methods.

## Results①

Two *TGIF1* and two *GLI2* mutations were identified.



## Results② Clinical phenotypes of the mutation carriers

### Pedigree 1:(*TGIF* p.N235Y)

The proband was a 13-year-old Japanese male. The patient exhibited GH, TSH, and ACTH deficiencies with micro penis, and cleft of soft palate. Brain MRI showed anterior pituitary hypoplasia with an ectopic posterior pituitary gland. No HPE brain defects were present. Parental gene analysis was refused.

### Pedigree 2:(*TGIF* p.R219C)

The proband 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\*)

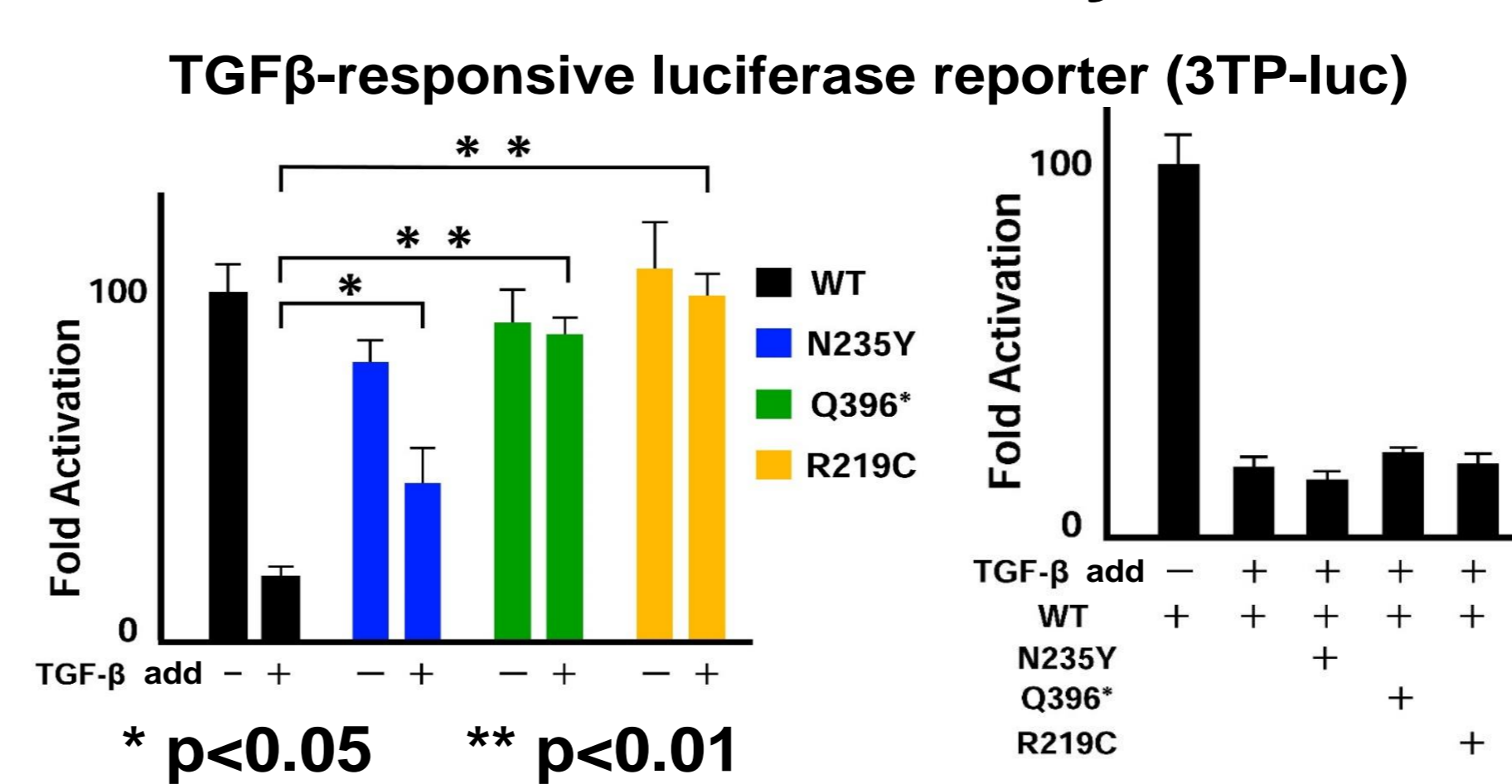
The proband was a 10-year-old Japanese male. The patient exhibited GH, TSH, and ACTH deficiencies with micro penis, and cleft lip and palate. Brain MRI showed severe anterior pituitary hypoplasia with an ectopic posterior pituitary gland. No HPE brain defects were present. Parental gene analysis was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

### Pedigree 4:(*GLI2* p.Q1182\*)

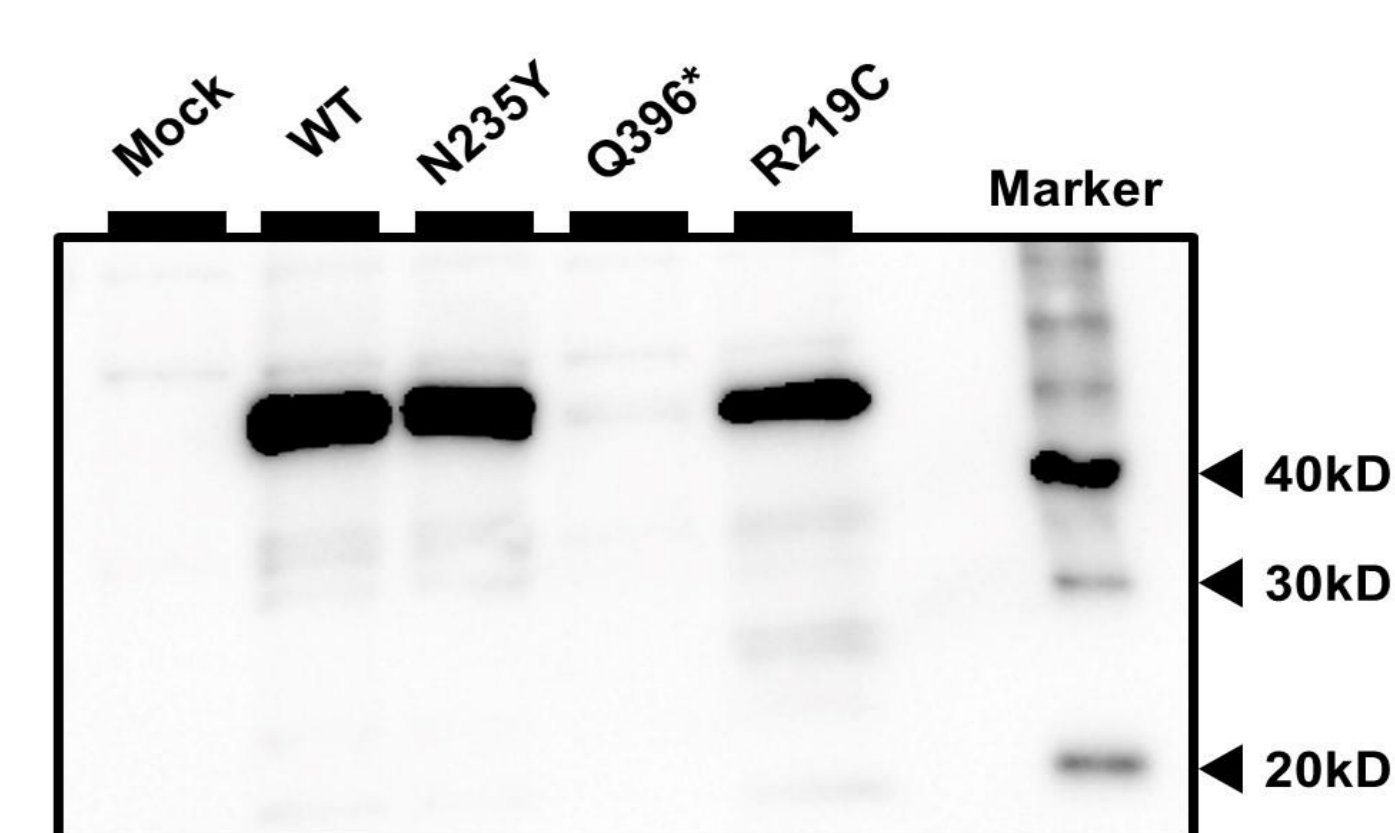
The proband was a 18-year-old Japanese male. The patient exhibited GH, TSH, LH/FSH, and ACTH deficiencies. Brain MRI showed anterior pituitary hypoplasia with an ectopic posterior pituitary gland. No HPE brain defects were present. Asymptomatic Father carried the same mutation.

## Results③ Functional assays for mutant *TGIF1*

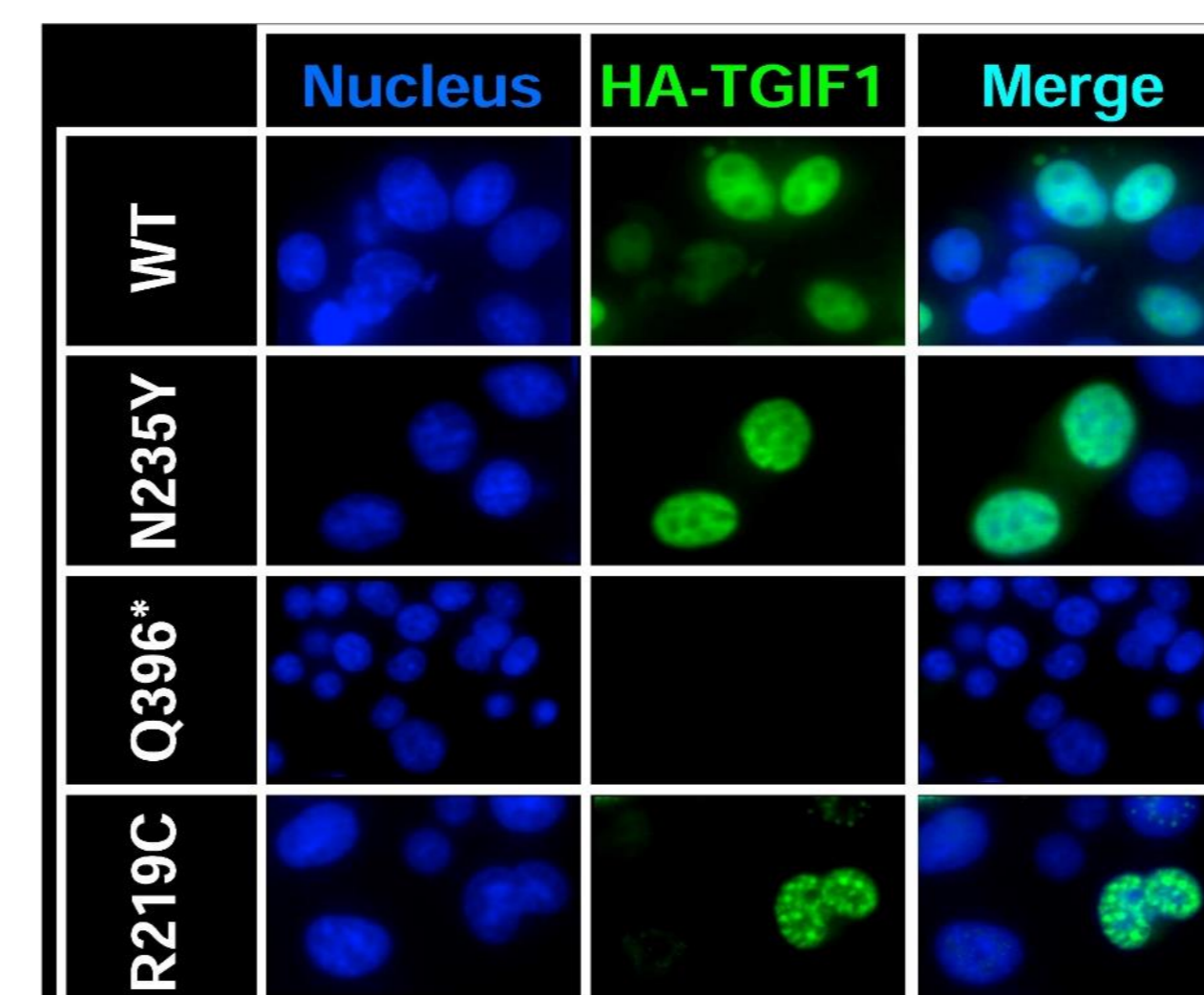
### A. Transactivation assays



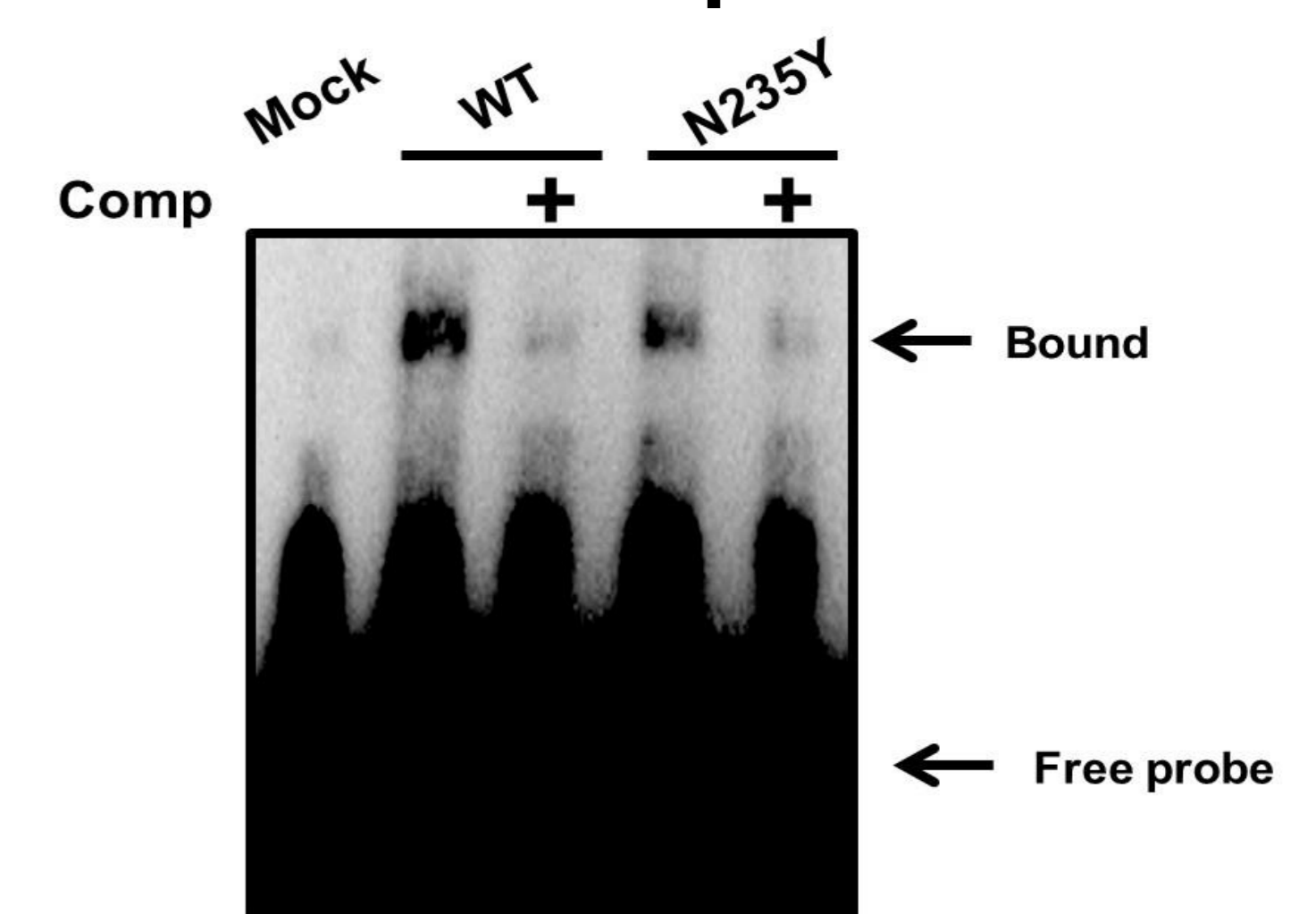
### B. Western blotting



### C. Subcellular localization



### D. 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 *TGIF1* mutant, which is related to CH.

## Discussion

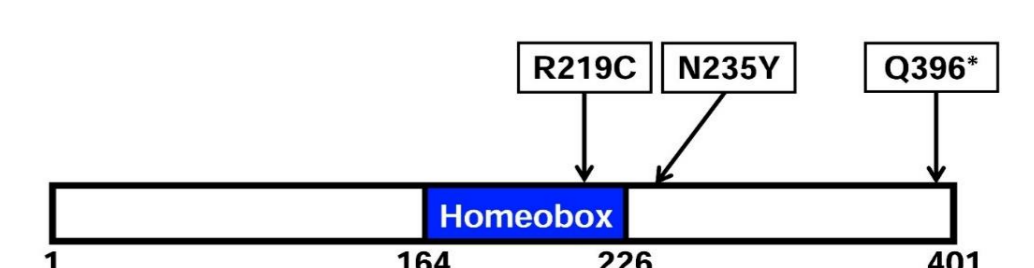
The frequency of SHH signaling or HPE related gene mutations in patients with CH was 3.3 % (4/120) in Japan.

	Midline defect		
	-	+	
		SOD	CP
Isolated GHD	21	4	3
MPHD	67	17	8
Total	88	21	11

TGIF1 1 case  
GLI2 1 case  
2/8 = 25%!

MPHD with Cleft palate cases could be a good candidate for SHH signaling or HPE related gene analysis.

Mutant	Hormone deficiency	MRI findings	Extra pituitary anomaly
N235Y	GH, TSH, LH/FSH?, ACTH	APH, eutopic PP	-
R219C	GH, TSH, LH/FSH?	APH, eutopic PP	cleft of soft palate
Q396*	GH, TSH, LH/FSH	APH, ectopic PP	Single central incisor



Genotype phenotype correlation is not clear.

