

#### 40 A NOVEL MUTATION OF 25-HYDROXY VITAMIN D<sub>3</sub> 1 $\alpha$ -HYDROXYLASE GENE IN A JAPANESE FAMILY WITH VITAMIN D DEPENDENT RICKETS TYPE1

Hara M, Motomura K, Shimizu T, Kinoshita E, Oka S\*

Department of Pediatrics, Nagasaki University Graduate School of Biomedical Sciences \*Sasebo Kyosai Hospital

**[Introduction]** Vitamin D dependent rickets type1 (VDDR1) characterized by the early onset of rickets with hypocalcemia is a rare autosomal recessive disorder caused by the mutation of 1 $\alpha$ -hydroxylase gene (*CYP27B1*). We here report a novel mutation of *CYP27B1* identified in a Japanese family.

**[Patients] Proband:** Patient is a 2 years and 5 months girl. She was pointed out growth retardation and motor development delay on public physical examination at age 1 year 6 months. Her height was -2.81 SD and was unable to stand-alone. She had hypocalcemia (7.7 mg/dl) and a high serum ALP level (4,584 IU/l). Bone X-P showed typical rachitic changes. Hormonal examination revealed an extremely low levels of serum 1 $\alpha$  25(OH)2D (<4 pg/ml) and high levels of 25(OH)D (51.7 ng/ml) and intact PTH (520 pg/ml). She was diagnosed as having VDDR1 from the clinical features and endocrinological findings. Gene analysis of *CYP27B1* using PCR and direct sequencing showed a compound heterozygous mutation for 1022delC in paternal origin and 2620delC in maternal origin. She did not first responded to oral 1 $\alpha$  OHD3 of 0.01-0.05  $\mu$ g/kg/day, but has been successfully treated with 0.2  $\mu$ g/kg/day. **Younger sister:** The baby is a now five month's girl. She was diagnosed as having VDDR1 from genetic analysis in neonatal period. At age 2 months, she had normal levels of serum Ca, P and ALP, but hormonal examination showed low level of serum 1 $\alpha$  25(OH)2D (5 pg/ml) and high level of intact PTH (136 pg/ml). She has been successfully treated with oral 1 $\alpha$  OHD3 of 0.05  $\mu$ g/kg/day since then.

**[Discussion]** Both mutations identified in this family induce frame-shift and would produce truncated protein lacking sterol-binding domain and heme-binding domain. The truncated protein is predicted to have no enzymatic activity, which is consist with severe clinical phenotype of proband. However, younger sister with identical mutation responds well to relatively low dosage of oral 1 $\alpha$  OHD3 by early treatment., suggesting that genetic analysis would be useful for early diagnosis and treatment.

#### 42 Molecular investigation of ACTH receptor gene in three patients with familial glucocorticoid deficiency

Imamine H, Sugiyama Y, Ohro Y, Mizuno H

Department of Pediatrics, Neonatology and Congenital Disorders, Nagoya City University Graduate School of Medical Sciences

Familial glucocorticoid deficiency (FGD) may have heterogeneous molecular etiology. However, tall stature and advanced bone age have previously been described in some children with FGD, and the mechanisms for this remain unknown.

We investigated clinical findings in three FGD patients as well as the results of molecular investigations of the ACTH receptor gene. A homozygous mutation in the coding region of the ACTH receptor gene was detected in two patients with normal stature, who were siblings. This mutation was a cytosine to thymidine change at the first position of codon 137, resulting in substitution of an arginine residue with tryptophan (R137W).

No abnormalities in the coding or promoter regions of the ACTH receptor gene were detected in the patient with tall stature and advanced bone age. She was Tanner stage II in breast development and I in pubic hair. Her plasma estradiol was elevated at 21.3 pg/ml, and in the GnRH test, responses to LH and FSH were suppressed throughout the test period. In addition to oral dexamethasone, increased doses of hydrocortisone decrease estradiol levels, and thus the rate of bone aging was normalized. We therefore suspected that elevated plasma estradiol is related to FGD and was a causative factor in the advanced bone age and tall stature symptoms observed in this patient.

No correlation between gene abnormality and tall stature was observed, and this differed from previous reports. Our results indicate that estrogenic action resulting from elevated ACTH levels is one possible cause of the tall stature and advanced bone age associated with FGD.

#### 41 Pseudohypoparathyroidism type Ib with atypical features: the relationship with DNA methylation pattern in XL $\alpha$ s region of GNAS1 gene

Kazukawa I, Minagawa M, Watanabe T, Kinoshita K, Sato Y, Shimohashi K, Minamitani K\*, Yasuda T\*\*, Kohno Y

Department of Pediatrics, Graduate School of Medicine, Chiba University \*Department of Pediatrics, Chiba Municipal Kaihin Hospital \*\*Department of Pediatrics, Chiba National Hospital

Pseudohypoparathyroidism type Ib (PHP-Ib) is distinguished from type Ia (PHP-Ia) by the absence of Albright's hereditary osteodystrophy (AHO) and multiple-hormone resistance. However, some patients with PHP-Ib have PHP-Ia-like clinical presentation. The etiology of PHP-Ib is reported to be caused by the abnormalities of the DNA methylation in GNAS1 gene. We previously reported that the methylation pattern is different between cases. Therefore we analysed the relationship between the difference in the methylation pattern and the clinical features in PHP-Ib. **[Methods]** Genomic DNA from 10 Japanese patients with PHP-Ib, which were all sporadic cases, with the methylation-sensitive restriction enzymes and subjected to Southern blot analysis. Bisulfite treated genomic DNA was amplified by PCR and sequenced. Intragenic polymorphisms were examined by PCR-RFLP in order to test the possibility of uniparental disomy (UPD). **[Results]** All 10 cases had abnormal methylation pattern (i.e. both alleles were paternal methylation pattern) in NESP55, AS, 1A region. Abnormal methylation patterns in XL $\alpha$ s region were observed in 5 cases. Three cases which had mental retardation at diagnosis (later it improved) had wider spanning abnormal methylation than others. UPD was unlikely in these cases according to the intragenic polymorphisms. **[Discussion]** Juppner et al. reported that PHP-Ib caused by the paternal UPD in 20q showed infantile mental retardation, which was improved with age, and other special features. Atypical PHP-Ib we examined had broad abnormality in methylation and in part similar clinical presentation to 20q UPD. Abnormality in methylation pattern in XL $\alpha$ s region may modify the clinical presentation of PHP-Ib.

#### 43 Common origin of the Q258X mutation in STAR gene responsible for congenital adrenal lipoid hyperplasia in Japanese

Yoshio Makita, Toshio Okamoto, Toshihiro Tajima\*, Kenji Fujieda.

Department of Pediatrics, Asahikawa Medical College.

\*Department of Pediatrics, Hokkaido University, School of Medicine.

The mutation Gln to termination codon at amino acid 258 of steroidogenic acute regulatory protein (STAR) responsible for lipoid congenital adrenal hyperplasia (lipoid CAH) found in several areas of Japan. Whereas, the founder effect of this mutation was unclear. Here, we analyzed STAR gene haplotype, using three single nucleotide polymorphisms and four microsatellite markers inside and neighboring region spanning 100 kb of the gene (loci 1 to 7), in the patients and parents. We found disease-responsible haplotype, spanning 69kb containing 6 markers (loci 2 to 7), is almost the same in Japan, indicating the existence of a common founder. Case-control haplotype analysis, using permutation method, shows disease-responsible haplotype is significant higher in case (Chi-Squ 84.97) than control. And we also found the disease-responsible haplotype is very rare in Japanese control subjects. The hypothesis that a mutant allele from another area could be an origin of the mutation for most of the Japanese lipoid CAH patients may be considered.