

- **Osteomalacia related to
vitamin D deficiency
in a
Patient
with CVID**

Case Report

23 year-old woman diagnosed as having CVID in 1999 was admitted to the hospital by reason of evaluation of her general status in November of 2005.

She did not have a complaint..

Medical History

- Many hospitalizations caused by several recurrent airway tract infections and diarrheae since the age of 11 years.
- She was given a diagnosis of **bronchiectasia** in **1999**.

- **Significantly low immunoglobulin levels**
- (IgG 225 mg/dl (700-1400))
- IgM < 24 mg/dl (70-40)
- IgA < 16 mg/dl (40-230)

- Defective responses to vaccination with *Haemophilus influenzae* and tetanus toxoid,

- Elimination of the causes related to secondary immunodeficiencies prompted the **diagnosis of CVID** and she was started on IVIG 400 mg/kg/month in 1999.

Medical history (2)

- **Lobectomy** operation was performed in 2000.
- She was also diagnosed with **diabetes mellitus** and has been undergoing insulin therapy since 2000.
- Also diagnosed as having **polyneuropathy and sensorineural hearing loss in 2003.**
- There was no history of consanguinity.
- 2-3 times/year hospitalization frequency while under therapy

Examination On Admission

- Unremarkable apart from reduced sounds in the left lower part of the lung.
- Blood pressure 110/80 mmHg
- Temperature 36,5 centigrade
- Pulse 90 beats/minute
- 52 kg weight, BMI of **19,33**

■ Laboratory On Admission

Quantitative immunoglobulin (Ig) evaluation (Dade-Behring, nephelometry) revealed:

IgG **660 mg/dl (700-1400)**

IgA and IgM undetectable.

Evaluation of Lymphocyte Subsets by Flow-cytometry

Lymphocyte Subsets	Patient value (%)	Normal (%)
CD 3	86	60-85
CD 19	5	7-23
CD 4	30	29-59
CD 8	48	19-48
CD 3-/16+56+	3,5	6-29

- The biochemical analyses revealed isolated high alkaline phosphatase (472 U/L, N. 90-260) levels.
- **Persistent finding since 1999 but no further evaluation had been performed..**
- Other biochemical parameters including sedimentation rate (13 mm/h) and C-reactive protein(0,3 mg/L, N=0-5 mg/L) were all normal **except for:**

■ Albumin (g/dl)	4,1	3,5-5
■ Globulin (g/dl)	2,2	2,5-3,5
■ Vitamin B 12 (pg/ml)	243	197-866
■ Folat (ng/ml)	8,16	3-16
■ Vitamin A (mg/L)	1,7	0,3-1,1
■ Beta-Carotene (micg/dl)	11,2	10-80
■ Vitamin E (mg/L)	8,4	6-18
■ Iron (micg/dl)	49	50-140
■ Ferritin (mg/ml)	21,65	13-150
■ Hct (%)	34,9	35-45
■ HgbA1C (%)	6,0	< 6,5

■ Autoantibody profile including:

Antinuclear

Antimitochondrial

Anti-smooth muscle

Liver-kidney microsomal

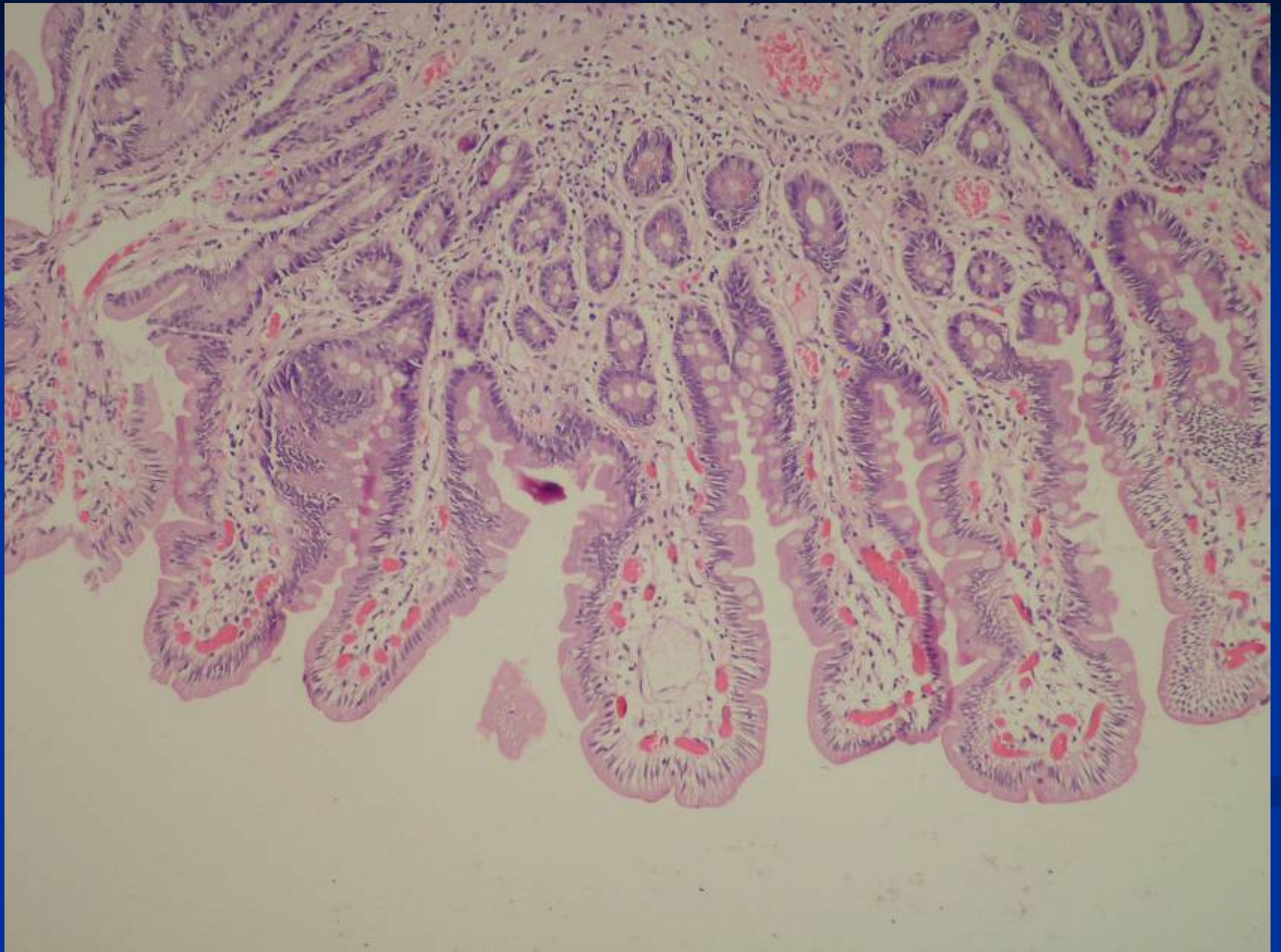
Gastric parietal cell

Anti-neutrophil cytoplasmic antibodies

Rheumatoid factor (RF)

Anti-thyroid antibodies were **negative**.

- The high resolution computed tomography (CT) of thorax and CT of the abdomen did not reveal granulomatous disease (liver size normal).
- Endoscopy and biopsy revealed normal duodenal mucosa, there were **no intraepithelial lymphocytes**.



Is there a bone metabolism abnormality ??

- **Osteocalcin** was found to be **increased (35,5- N: 3,1-13,7 ng/ml)**
- **Deoxypyridinoline** was **normal (6,3 nM/mM N:3,0-7,4)**
- **Parathyroid hormone (PTH)** was **normal (49 pg/mL N:15-65)**
- **Urinary calcium** was low (**25 mg/24 h N:100-300**)
- **Calcium** of 8,8 mg/dl (N:8,2-10,4)
- **Phosphate** 3,3 mg/dl (N:2,3-4,5)
- **Magnesium** 2,2 mg/dl (N:1,5-2,6)

Radiology

- The X-ray examination of the cranium and extremities revealed increased radiolucency without specific abnormalities suggestive of Paget's disease.
- The bone density evaluation showed osteoporosis.
- The T score for the left hip was -3.2 and for the lumbar spine was -3.00 .
- The bone radionuclide scan was found to be normal.

- The patient's status was considered **preosteomalacic phase.**

- **25 OH vit D** and **1,25 (OH) 2 vit D** levels were found to be low.

25 OH vit D **5** ng/ml (N: 7,6-75)

1,25 (OH) 2 vit D **< 8** pg/ml (N:29,6-65,1)

- **Vitamin A** **1,7** (N: 0,3-1,1 mg/L)

- **Vitamin E** **8,4** (N: 6-18 mg/L)

Active vitamin D (rocaltrol 0,25 mcg bid and calcium (1 gr/day) therapy was started.

- Rehospitalized 6 months later, by reason of breakdown of therapy..
- Osteocalcin **normalized** (11 ng/ml)
- PTH **increased** (68 pg/ml)
- ALP **increased** (508 U/L)
- 25 OH vit D **significantly low** (1,2 ng/ml)
- 1,25 OH vit D **significantly low** (1,2 pg/ml)

- Active vitamin D (rocaltrol 0,5 mcg bid and calcium therapy 1 gr/day were commenced.

Six months later...

- ALP was normalized 239 U/L (90-260)
- Urinary calcium increased.....117 mg/24 h)
- PTH was decreased no normal range.....50,7 pg/ml (15-65)
- 25 OH vit D was increased to..... 5 ng/ml (7,6-65)

Still lower than normal.....

Possible Reasons for Low Vit D Levels

- Living to the north of latitude 37
- Dark-skinned people, vegetarians and veiled women
- Anticonvulsant or antituberculous drug use
- Gastrointestinal and pancreatobiliary diseases

Celiac disease

- Vitamin D resistance related to **VDR mutation**
- Vitamin D binding protein deficiency

were **ruled out.**

- Vitamin D receptor expression (VDR) was evaluated and found to be **significantly decreased** in PBMC and hair follicle compared with control group...

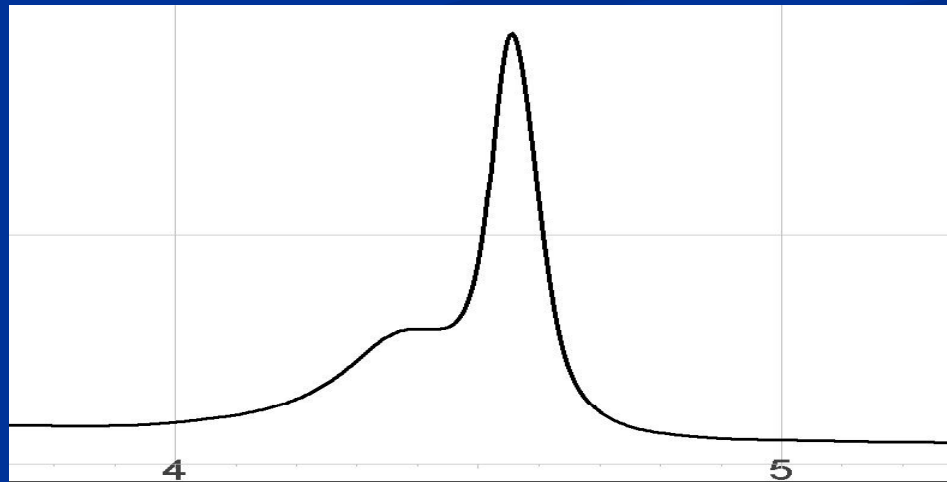
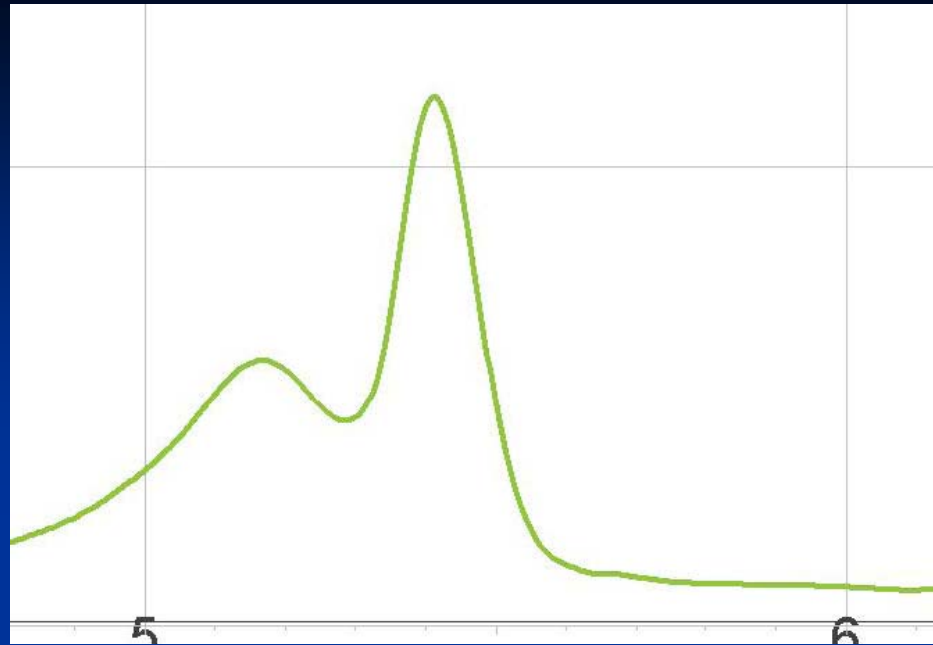
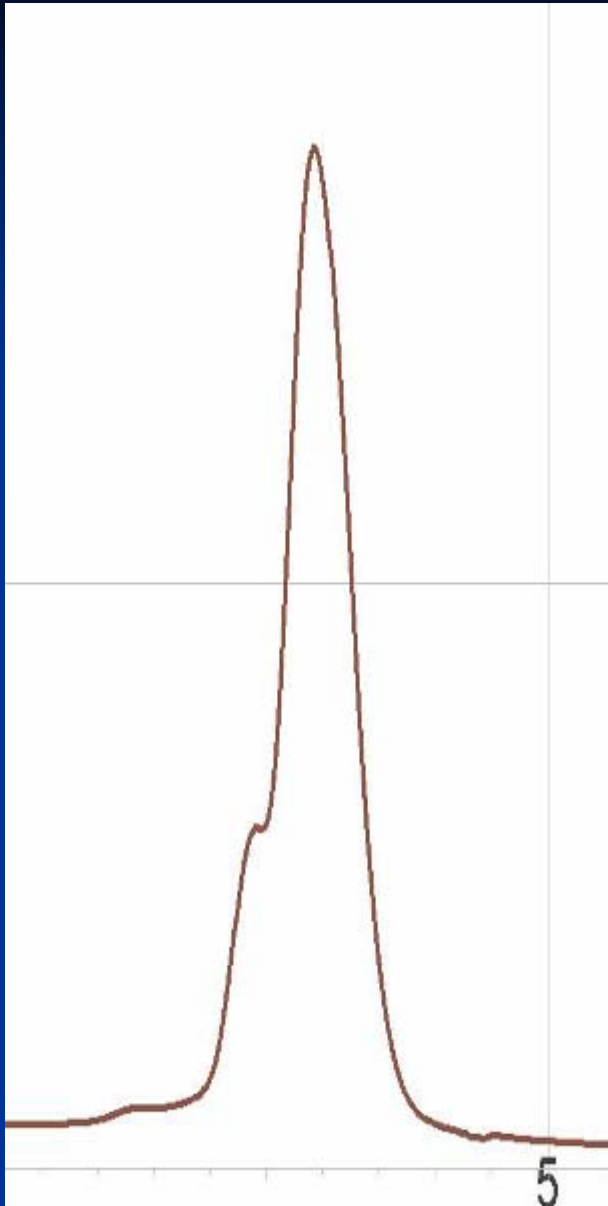
VDR mRNA EXPRESSION of THE CASE and THE CONTROL

	Control		Case	
	PBMC	HF	PBMC	HF
Expression	0,1424	37,9825	0,0143	0,6907
SD	0,0130	18,9454		
95% CI	0,0127	18,5661		

VDR Gene Polymorphism

She was found to be **heterozygous** regarding **Apa, Bsm and Taq genes** by High-Performance Liquid Chromatography (DHPLC)..

She was homozygous (**FF**) for **Fok** gene..

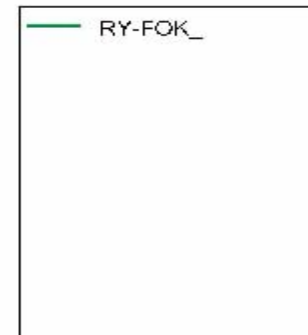
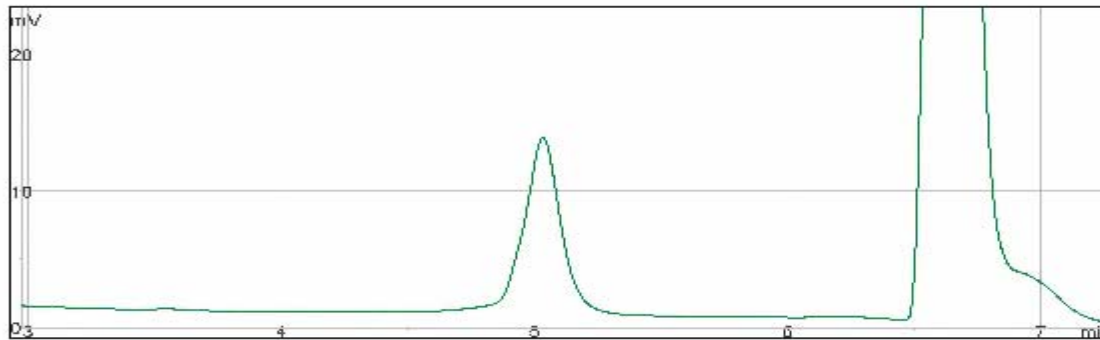




TRANSGENOMIC

CONSOLIDATED ANALYSIS REPORT

User: WADMIN
Date: Mon May 07 14:07:06
Selection: Chromatogram + Peak



- **None of these polymorphisms related to impaired VDR expression and function...**

- We could not investigate *intestinal VDR expression*
- Low intestinal VDR expression is related to resistance to vit D treatment and calcium absorption...

■ *Clinical Consequenses*

- *Vitamin D deficiency might have a role in the pathogenesis of **polyneuropathy** in this patient.*
- *Untreated vit D deficiency might play a role as an **additive risk factor** in terms of **tendency to malignancy** in patients with CVID.*
- *Increased tendency to **autoimmune diseases (type-1 diabetes !)***
- *Increased tendency to **infections (tuberculosis)***
- *Increased tendency to **sepsis***
- *Irreperable **bone loss and fractures***

Isolation of total RNA and cDNA synthesis

- 2 mL of blood and 10 follicles of hair sample of the case were collected. **Fifty microliters** of total RNA was isolated from peripheral blood mononuclear cells (PBMC) and hair follicle by using **High Pure RNA Isolation Kit (Roche, Germany)**. **Reverse transcription** procedure was performed for **cDNA** synthesis by using Transcriptor First Strand cDNA Synthesis Kit according to the manufacturers' instructions.

Relative quantification of VDR

- Real-time quantitative RT-PCR analyses of *VDR* were performed with Lightcycler instrument and software. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH* “housekeeping” gene) was chosen as an internal standard to control for variability in amplification. The sequences of primers and probes used are shown in Table-3. PCR was performed by using TaqMan Master Kit (Roche Diagnostics) according to the instructions of the manufacturer. The *VDR* target probe was labeled at the 5' end with the reporter dye molecule 6-carboxyfluorescein (FAM). The *GAPDH* target probe was labeled with 6-carboxyfluorescein. Both probes were labeled with the quencher fluor 6-carboxytetramethylrhodamine (TAMRA) at the 3' end. To quantify *VDR* mRNA from PBMC and hair follicle, we constructed a calibration curve (Error: 0.100 Efficiency: 1,790) using *GAPDH* mRNA as an endogenous control.