Bone 93 (2016) 97–103

Contents lists available at ScienceDirect

Bone

journal homepage: www.elsevier.com/locate/bone

Full Length Article

# Osteomalacia induced by long-term low-dose adefovir dipivoxil: Clinical characteristics and genetic predictors

Zhe Wei, Jin-wei He, Wen-zhen Fu, Zhen-lin Zhang *

*Metabolic Bone Disease and Genetic Research Unit, Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai 200233, China*

ARTICLE INFO

*Article history:*
Received 24 January 2016
Revised 31 August 2016
Accepted 20 September 2016
Available online 21 September 2016

*Keywords:*
Adefovir dipivoxil
Hypophosphatemic osteomalacia
Clinical characteristic
*SLC22A6*
*ABCC2*
Risk genotype

ABSTRACT

*Context:* Adefovir dipivoxil (ADV) was an important cause of adult-onset hypophosphatemic osteomalacia. However, its clinical characteristics and mechanisms have not been well defined.
*Objective:* The objective of the study was to summarize the clinical characteristics of ADV-induced osteomalacia and to explore the association between ADV-associated tubulopathy and polymorphisms in genes encoding drug transporters.
*Design, setting, patients, and main outcome measure:* Seventy-six affected patients were clinically studied. The *SLC22A6* and *ABCC2* genes were screened and compared with healthy people from the HapMap.
*Results:* Hypophosphatemia, high serum alkaline phosphatase (ALP) levels, hypouricemia, nondiabetic glycosuria, proteinuria, metabolic acidosis and high bone turnover markers were the main metabolic characteristics. Fractures and pseudofractures occurred in 39 patients. Stopping ADV administration, supplementing calcitriol and calcium was effective during the follow-up period. Single SNP analysis revealed a higher percentage of the G/A genotype at c.2934 in exon 22 of the *ABCC2* gene (rs3740070) in patients than in healthy people (12% [7 of 58 patients] vs. 0% [0 of 45 patients]; $P = 0.017$), while there was no subject with homozygosity for the A allele at c.2934.
*Conclusions:* ADV can be nephrotoxic at a conventional dosage. The G/A genotype at c.2934 of the *ABCC2* gene may be a predictor of patients at greater risk for developing ADV-associated tubulopathy. Larger case-control studies are needed to further verify this finding.



## 1. Introduction

The rapid development of antiviral drugs has considerably reduced the mortality of chronic hepatitis B patients. Adefovir dipivoxil (ADV), a type of nucleotide analogue developed in 2002, is extensively used for chronic hepatitis B therapy. ADV has high antiviral activity [1,2] and efficacy. However, ADV may be associated with potentially serious nephrotoxicity, which could induce secondary hypophosphatemic osteomalacia. Preliminary studies revealed that significant nephrotoxicity occurs at a high dosage (>30 mg once daily) [3,4]. To date, clinical studies have rarely reported severe nephrotoxicity in patients treated with ADV at a low dosage (10 mg once daily). Thus, the nephrotoxicity associated with ADV may be dose-limiting. Nevertheless, the long-term clinical application of ADV at a conventional dosage has been found to be responsible for proximal renal tubular dysfunction and secondary hypophosphatemic osteomalacia [5–10]. Here, we report our findings from the study of 76 patients who developed hypophosphatemic osteomalacia secondary to ADV use.

The mechanism by which ADV causes renal tubular dysfunction is not well understood, although transporter proteins in the renal tubule may be involved. The nucleoside analogue enters the epithelial cells of the proximal tubule through the basolateral membrane and utilizes organic anion transporters (OATs), mainly OAT1 (encoded by the solute carrier gene *SLC22A6*) [11–13]. The drug is then secreted into the urine through the luminal membrane utilizing multidrug-resistance proteins (MRPs), primarily MRP2 (encoded by adenosine triphosphate-binding cassette sub-family C gene *ABCC2*) [12–14]. For many antiviral drugs, the efflux at the luminal membrane is rate limiting and occasionally results in intracellular accumulation [15]. Therefore, transporter expression may modulate the extent of tubular damage. To further explore the mechanism, we investigated the association between polymorphisms in the *ABCC2* and *SLC22A6* genes and the development of tubular dysfunction in chronic hepatitis B patients treated with ADV.

## 2. Materials and methods

### 2.1. Study subjects

From June 2008 through June 2015, 76 patients were diagnosed with ADV-induced hypophosphatemic osteomalacia. These patients were

* Corresponding author at: Metabolic Bone Diseases and Genetic Research Unit, Department of Osteoporosis and Bone Diseases, Shanghai Jiao Tong University Affiliated Sixth People's Hospital, 600 Yi-shan Road, Shanghai 200233, China.
*E-mail address:* zzl2002@medmail.com.cn (Z. Zhang).

http://dx.doi.org/10.1016/j.bone.2016.09.017
8756-3282/

treated in the Department of Osteoporosis and Bone Diseases, Shanghai Sixth People's Hospital Affiliated to Shanghai Jiaotong University, Shanghai, China. All patients were chronically infected with hepatitis B virus (HBV) and were treated with low-dose ADV (10 mg once daily). These patients sought care or were referred to our institution with symptoms of fatigue, bone pain, and/or pathological fractures. No patient had other conditions known to affect bone metabolism or renal function, including hyperthyroidism, diabetes mellitus, primary hyperparathyroidism, pituitary and adrenal diseases, rheumatic diseases, and other kind of metabolic bone diseases. After obtaining a detailed medical and family history, the following tests were conducted or factors were measured to make a definite diagnosis: urine routine tests, arterial blood gas analyses, hepatic and renal function, serum electrolytes (including serum calcium, serum phosphorus and serum potassium), serum alkaline phosphatase (ALP), serum 25-hydroxy vitamin D [25(OH)D], and serum parathyroid hormone (PTH), and bone turnover markers (including serum osteocalcin (OC) in the form of N-terminal midmolecule fragment (N-MID) and beta C-terminal cross-linked telopeptides of type l collagen (β-CTX)). X-ray radiography of the thoracic and lumbar vertebrae, limbs and hips was individually performed. A Lunar Prodigy dual-energy X-ray absorptiometry (DXA) densitometer (Lunar Corporation, Madison, WI, USA) was used to obtain the bone mineral density (BMD) values of the left proximal femur, including the femoral neck, total hip, and anteroposterior lumbar spine 1–4 (L1–4). The instrument was calibrated daily, and the coefficient of variability (CV) values of the DXA measurements (obtained from 15 individuals repeated three times) were 1.39% for the lumbar spine and 2.22% for the femoral neck. Whole-body bone images were obtained by single-photon emission computed tomography (SPECT)/CT on 20 patients. Patients were followed for an average of 9.4 months (1–62 months) after therapy.

### 2.2. Ethics statement

The study protocol was approved by the Committee of the Ethics of Human Research in the Affiliated Sixth People's Hospital of Shanghai Jiaotong University. All patients provided informed consent for inclusion in the study according to the guidelines at our institution, and genetic consent was approved.

### 2.3. SNP detection of the SLC22A6 and ABCC2 genes

DNA was isolated from peripheral blood leukocytes using the QuickGene DNA Whole Blood Kit L (KURABO, Japan) and the Nucleic Acid Isolation System (QuickGene-610L, FUJIFILM, Japan). DNA sequencing of polymerase chain reaction (PCR) amplified *SLC22A6* and *ABCC2* gene fragments covering the entire coding region and sequencing of the intron-exon boundaries were performed using an ABI 3730 automated sequencer and the Big Dye Terminator Sequencing protocol (ABI). The mRNA sequences (NM_004790.4 and NM_000392.3) of the *SLC22A6* and *ABCC2* genes were used as reference sequences. DNA mutation numbering was based on cDNA sequencing using the A of the ATG translation initiation start site as nucleotide +1.

### 2.4. Statistical analyses

Descriptive results of continuous variables were expressed as medians and interquartile ranges. The X2 test was used to compare proportions, and Fisher's corrections were applied when needed. All statistics were performed using SPSS, version 22.0 (SPSS), and differences with $P$ values < 0.05 were considered significant.

### 2.5. Mutation prediction

To assess the damaging effects of missense mutations in the *SLC22A6* and *ABCC2* genes, the online databases, PolyPhen-2 (Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/) [16], SIFT (Sorting Tolerant From Intolerant; http://sift.jcvi.org/) [17], and Mutation Taster (http://www.mutationtaster.org) [18] were used.

## 3. Results

### 3.1. Clinical characteristics

#### 3.1.1. General characteristics

We obtained detailed demographic and clinical information about all 76 patients. Some of the main characteristics are presented in Table 1, including age, gender, chronic hepatitis B duration, ADV treatment duration and course of bone pain. Notably, the median duration of ADV treatment before the onset of secondary hypophosphatemic osteomalacia was 5 years, while the minimum duration was only 1.5 years, and the maximum was 13 years (median and interquartile range are listed in Table 1). All 76 patients were taking ADV at a dosage of 10 mg once daily, while 17 patients were treated with imported ADV, 28 patients with domestically produced ADV, and 31 patients with the combination of both. Some patients had been treated with various courses of antiretroviral therapies before they began ADV treatment, 26 of 76 (36.1%) had received lamivudine, and 7 of 76 (9.7%) had received entecavir. The main reason these patients switched treatments was lack of treatment efficacy.

The patients' clinical manifestations included muscle weakness/fatigue (100%), gradually exacerbated bone pain (100%) (primarily in the heel, then spreading to the lower limbs, lower back and ribs), and difficulty walking (63.9% [46 of 76 patients]). Because of the nonspecific symptoms, several patients were misdiagnosed. Of these patients, 13 were misdiagnosed with osteoporosis, 3 were misdiagnosed with spondyloarthropathy, and 1 was misdiagnosed with multiple myeloma.

#### 3.1.2. Laboratory tests

Urine routine and arterial blood gas tests were carried out. In addition, hepatic and renal function, serum electrolytes and bone turnover markers were measured. The results primarily revealed hypophosphatemia, high ALP, hypouricemia, nondiabetic glycosuria, proteinuria, metabolic acidosis and high bone turnover markers (Table 1). The bone turnover makers are evaluated based on the reference range we previously established [19].

#### 3.1.3. Imaging examination

Radiography tests (X-ray, CT, or MRI) were carried out for 65 patients. Fractures were verified in 39 of 65 (60%) patients. These fractures consisted of vertebral compression fractures (30.8% [20 of 65 patients]), rib fractures (21.5% [14 of 65 patients]), pelvic deformities (18.5% [12 of 65 patients]) and pseudofractures (6.2% [4 of 65 patients]). All 20 patients who underwent a whole body bone scan by SPECT/CT demonstrated high uptake in multiple locations throughout the skeleton, and 4 patients were misdiagnosed as bone metastasis. The results of the BMD examination revealed a significant decrease in bone density (Table 1).

#### 3.1.4. Treatment

The diagnosis of ADV-associated nephrotoxicity and secondary hypophosphatemic osteomalacia in our patients was established by evidence of generalized dysfunction of the proximal renal tubule causing impaired reabsorption of glucose, protein, and phosphate. This impaired reabsorption resulted in increased excretion of glucose and protein into the urine, hypophosphatemia and metabolic acidosis. ADV administration was therefore ceased immediately after diagnosis, and entecavir was administered as the hepatitis B antiviral therapy for some of the patients. Calcium carbonate (1.5 g/d) and calcitriol (0.5 μg/d) were also administered. Sodium bicarbonate (1.5 g/d) was used for patients with severe metabolic acidosis. Notably, elemental phosphate supplementation was not used for any patients.

**Table 1**
Characteristics of the study population.

| Characteristics | Number of available data | Values | Abnormal cases (frequency) | Normal range |
|---|---|---|---|---|
| Age, years | 76 | 52.5 (46–62) | | |
| Gender, male/female | 76 | 48/28 | | |
| Duration of chronic hepatitis B, years | 61 | 10 (7–17) | | |
| Duration of adefovir treatment, years | 72 | 5 (5–7) | | |
| Course of bone pain, months | 68 | 12 (10–24) | | |
| Blood gas analysis | 60 | | | |
| Metabolic acidosis | | | 28 (45.9) | |
| Blood parameters | | | | |
| ALP (U/L) | 73 | 248 (194–317) | 71 (97.3) | 15–112 |
| Cr (μmol/L) | 68 | 95 (81–112) | 12 (18.2) | 53–115 |
| UA (μmol/L) | 53 | 120 (95–140) | 50 (94.3) | 210–430 |
| Ca (mmol/L) | 69 | 2.28 (2.21–2.39) | 3 (4.3) | 2.08–2.60 |
| P (mmol/L) | 73 | 0.54 (0.46–0.65) | 70 (95.9) | 0.80–1.60 |
| PTH (pg/mL) | 70 | 26.65 (18.31–39.26) | 14 (20.0) | 15–65 |
| OC (ng/mL) | 51 | 29.46 (24.98–38.33) | 45 (88.2) | Female: 4.91–22.31<br>Male: 5.58–28.62 |
| β-CTX (ng/L) | 68 | 1125 (596–1650) | 51 (75.0) | Female: 112–497<br>Male: 100–612 |
| 25(OH)D (ng/mL) | 65 | 17.51 (12.34–23.60) | 39 (60.0) | >30 |
| Hypokalemia (mmol/L) | 75 | | 12 (16.0) | |
| Urine parameters | 62 | | | |
| Nondiabetic glucosuria | | | 40 (64.5) | |
| Proteinuria | | | 52 (82.5) | |
| BMD | 60 | | | |
| L1–4 (Z-score) | | −2.3 [(−3.1)–(−1.5)] | | |
| Neck (Z-score) | | −1.9 [(−2.5)–(−1.3)] | | |
| Total hip (Z-score) | | −2.2 [(−3.0)–(−1.7)] | | |

Note. Data are median (interquartile range) for quantitative variables and no. (%) of patients for qualitative variables. *ALP*, alkaline phosphatase; *Cr*, creatinine; *UA*, uric acid; Ca, calcium; *P*, phosphorus; *PTH*, parathyroid hormone; *OC*, osteocalcin; *β-CTX*, beta C-terminal cross-linked telopeptides of type I collagen; *25(OH)D*, 25-hydroxy vitamin D.

#### 3.1.5. Follow-up

The average follow-up period was 9.4 months (1–62 months). During the follow-up, symptomatic relief was achieved within 3 to 6 months. In most patients, the serum phosphorus levels rapidly returned to the normal range after ADV use was discontinued, and the abnormalities did not recur when antiviral therapy with entecavir replaced ADV. Serum phosphorus levels became normal in 54.3% patients (19 of 35) 3 months after the withdrawal of ADV and in 90.5% (19 of 21 patients) after 6 months. Serum ALP and β-CTX concentrations gradually decreased, glucosuria and proteinuria gradually disappeared after therapy. 15 patients underwent another BMD test approximately 1 year after therapy, and the results revealed improvements of 6.2–51.3% (25.4 ± 13.1) in the lumbar spine and 2.8–60.0% (25.8 ± 16.4) in the femoral neck compared to baseline before therapy. The changes in biochemical parameters are shown in Table 2.

### 3.2. Genetic variations in the SLC22A6 and ABCC2 genes

The sequencing results of the *SLC22A6* gene among 55 samples revealed 11 SNP sites and 1 mutation site, while sequencing of the *ABCC2* gene among 58 samples revealed 23 SNP sites (involving 9 novel or extremely low frequency SNP sites) and 5 mutation sites.

#### 3.2.1. Association between single nucleotide polymorphisms in SLC22A6 and ABCC2 genes and the risk of ADV-associated tubulopathy

We selected 4 SNP sites on the *SLC22A6* gene and 12 SNP sites on the *ABCC2* gene on the basis of a minor-allele frequency > 5% and compared the genotypes and alleles to the healthy CHB population from the HapMap (http://hapmap.ncbi.nlm.nih.gov). The distribution of genotypes and alleles at these SNP sites was provided in Table 3. Single SNP analysis revealed a higher percentage of genotype G/A at c.2934 in exon 22 of the *ABCC2* gene (rs3740070) in patients than in the healthy CHB population from HapMap (12% [7 of 58 patients] vs. 0% [0 of 45 patients]; $P = 0.017$), while there was no subject with homozygosity for the A allele at c.2934. No significant differences of SNPs on the *SLC22A6* gene and other SNPs on the *ABCC2* gene were observed.

#### 3.2.2. Mutation analysis

The mutational analysis revealed 1 missense mutation of the SLC22A6 gene in 1 of 55 patients, p.Pro416His (c.1247C > A) in exon 7. Four missense mutations of the *ABCC2* gene, p.Thr486Ile (c.1457C > T, rs17222589) in exon 10, p.Ser789Phe (c.2366C > T) in exon 18, p.Asn807Ser (c.2420A > G, rs113300169) in exon 18 and p.His1414Tyr (c.4240C > T) in exon 30, were identified separately in 1 of 58 patients. Among them, the P416H (SLC22A6), S789F (ABCC2) and H1414Y

**Table 2**
Changes in biochemical parameters (baseline and post-treatment).

| Characteristics | Baseline (0 month) | 3 months (n = 53) | 6 months (n = 28) | 12 months (n = 23) | 18 months (n = 11) | Normal range |
|---|---|---|---|---|---|---|
| P (mmol/L) | 0.54 (0.46–0.65) | 0.78 (0.68–0.92) | 0.84 (0.72–0.98) | 0.85 (0.76–1.01) | 0.94 (0.84–1.08) | 0.80–1.60 |
| ALP (U/L) | 248 (194–317) | 249 (176–357) | 249 (190–366) | 182 (134–240) | 135 (120–218) | 15–112 |
| UA (μmol/L) | 120 (95–140) | 159 (122–188) | 186 (142–198) | 173 (154–203) | 182 (176–223) | 210–430 |
| OC (ng/mL) | 29.46 (24.98–38.33) | 57.14 (40.22–82.10) | 59.32 (46.23–107.50) | 49.97 (35.12–74.88) | 46.78 (41.77–59.59) | Female: 4.91–22.31<br>Male: 5.58–28.62 |
| β-CTX (ng/L) | 1125 (596–1650) | 2015 (1298–2565) | 1950 (1280–2930) | 1430 (922–2318) | 1384 (919–1715) | Female: 112–497<br>Male: 100–612 |
| Nondiabetic glucosuria | 40 (64.5) | 8 (29.6) | 5 (22.7) | 4 (23.5) | 1 (14.3) | |
| Proteinuria | 52 (82.5) | 16 (59.3) | 11 (50.0) | 7 (41.2) | 3 (42.9) | |

Note. Data are median (interquartile range) for quantitative variables and no. (%) of patients for qualitative variables. *P*, phosphorus; *ALP*, alkaline phosphatase; *UA*, uric acid; *OC*, osteocalcin; *β-CTX*, beta C-terminal cross-linked telopeptides of type I collagen.

**Table 3**
Genotypes and allelic frequencies at *SLC22A6* and *ABCC2* in patients and healthy people from HapMap.

| Gene (protein), based on db cDNA, SNP identification, and genotype or allele | Patients ($n = 58$) | CHB (HapMap) | *P*-value |
|---|---|---|---|
| *SLC22A6* (hOAT1) | | | |
| c.-127G > A, rs4149170 | | | |
| G/G | 29 | 23 | |
| G/A | 22 | 18 | 1.000 |
| A/A | 4 | 3 | |
| G | 80 | 64 | |
| A | 30 | 24 | 1.000 |
| c.-20A > G, rs4149171 | | | |
| A/A | 28 | NA | |
| A/G | 24 | | |
| G/G | 3 | | |
| A | 80 | | |
| G | 30 | | |
| c.921 + 33C > T, rs2276300 | | | |
| C/C | 34 | 27 | |
| C/T | 19 | 16 | 1.000 |
| T/T | 2 | 2 | |
| C | 87 | 70 | |
| T | 23 | 20 | 0.822 |
| c.1362-176 T > C, rs3017670 | | | |
| C/C | 36 | 34 | |
| T/C | 19 | 9 | 0.119 |
| T/T | 0 | 1 | |
| C | 91 | 77 | |
| T | 19 | 11 | 0.352 |
| *ABCC2* (MRP2) | | | |
| c.-24C > T, rs717620 | | | |
| C/C | 38 | 26 | |
| C/T | 19 | 18 | 0.768 |
| T/T | 1 | 1 | |
| C | 95 | 70 | |
| T | 21 | 20 | 0.463 |
| c.868-218G > T, rs2756109 | | | |
| G/G | 25 | 18 | |
| G/T | 27 | 23 | 0.895 |
| T/T | 6 | 4 | |
| G | 77 | 59 | |
| T | 39 | 31 | 0.901 |
| c.1249G > A, rs2273697 (p.Val417Ile) | | | |
| G/G | 43 | 38 | |
| G/A | 15 | 7 | 0.206 |
| A/A | 0 | 0 | |
| G | 101 | 83 | |
| A | 15 | 7 | 0.235 |
| c.1967 + 169C > T, rs3740074 | | | |
| T/T | 35 | 23 | |
| C/T | 21 | 20 | 0.644 |
| C/C | 2 | 2 | |
| T | 91 | 66 | |
| C | 25 | 24 | 0.392 |
| c.2095-105T > C, rs3740073 | | | |
| C/C | 34 | 23 | |
| T/C | 22 | 19 | 0.671 |
| T/T | 2 | 3 | |
| C | 90 | 65 | |
| T | 26 | 25 | 0.376 |
| c.2620 + 148_2620 + 149delGT, rs3047477 | | | |
| N2/N2 | 23 | NA | |
| N2/− | 20 | | |
| −/− | 15 | | |
| N2 | 66 | | |
| – | 50 | | |
| c.2934G > A, rs3740070 | | | |
| G/G | 51 | 45 | |
| G/A | 7 | 0 | **0.017** |
| A/A | 0 | 0 | |
| G | 109 | 90 | |
| A | 7 | 0 | **0.019** |
| c.3258 + 56T > C, rs4148396 | | | |
| C/C | 34 | 20 | |
| C/T | 22 | 23 | 0.360 |
| T/T | 2 | 2 | |
| C | 90 | 63 | |
| T | 26 | 27 | 0.217 |
| c.3414 + 108C > T, rs3740068 | | | |

**Table 3** (*continued*)

| Gene (protein), based on db cDNA, SNP identification, and genotype or allele | Patients ($n = 58$) | CHB (HapMap) | *P*-value |
|---|---|---|---|
| C/C | 49 | 42 | |
| C/T | 9 | 3 | 0.221 |
| T/T | 0 | 0 | |
| C | 107 | 87 | |
| T | 9 | 3 | 0.179 |
| c.3741 + 154T > C, rs3758395 | | | |
| T/T | 33 | 23 | |
| T/C | 22 | 17 | 0.539 |
| C/C | 3 | 5 | |
| T | 88 | 63 | |
| C | 28 | 27 | 0.346 |
| c.3843 + 124C > G, rs3740067 | | | |
| C/C | 35 | 20 | |
| C/G | 21 | 18 | 0.640 |
| G/G | 2 | 1 | |
| C | 91 | 58 | |
| G | 25 | 20 | 0.508 |
| c.3976C > T, rs3740066 | | | |
| C/C | 35 | 23 | |
| C/T | 21 | 20 | 0.639 |
| T/T | 2 | 2 | |
| C | 91 | 66 | |
| T | 25 | 24 | 0.392 |

Note. Data are no. of patients for genotypes and no. of alleles for alleles (total number is twice the number of patients). Statistically significant *P* values ($P < 0.05$) are shown in boldface. hOAT1, human organic anion transporter 1; MRP2, multidrug-resistance protein 2; SNP, single-nucleotide polymorphism. NA, not available.

(ABCC2) mutations were predicted to have a pathogenic effect according to Polyphen-2, SIFT and Mutation Taster analyses (Table 4). Furthermore, the homology analysis among different animal species indicated that the positions of these 3 mutations were highly conserved (Fig. 1). In addition, we identified a splice site mutation, c.2620 + 3A > G in intron 19 of the ABCC2 gene.

## 4. Discussion

Nephrotoxic effects due to antiviral therapies have previously been described (e.g., tenofovir [20,21], indinavir [22,23] and foscarnet [24] in HIV-infected patients). Hypophosphatemic osteomalacia after ADV (10 mg daily) administration has also been previously reported in the form of case reports [5,8,10,25,26]. However, nephrotoxicity and subsequent hypophosphatemic osteomalacia induced by ADV at 10 mg daily dose is a significant phenomenon, more patients than we previously knew have suffered from it. Here, we present the clinical features of 76 patients with ADV-associated nephrotoxicity and severe secondary hypophosphatemic osteomalacia.

The clinical manifestations of ADV-induced hypophosphatemic osteomalacia are usually not specific and frequently manifest as muscle weakness and bone pain. Pain often begins in the weight-bearing sites, such as the lower limbs, and then gradually spreads to the entire body. ADV-induced nephrotoxicity may occur at the therapeutic dose after long-term application, mainly after >5 years. The biochemical manifestations of osteomalacia induced by ADV are similar to those of tumor-induced osteomalacia (TIO), with high ALP, hypophosphatemia and high bone turnover markers. However, hypouricemia, metabolic acidosis, nondiabetic glucosuria and proteinuria, which indicate Fanconi syndrome and nephrotoxicity, are specific to ADV (as well as some other antiviral drugs) induced hypophosphatemic osteomalacia. These characteristics are almost absent in patients with TIO. Furthermore, to exclude the possibility of TIO, PET-CT was performed on some of the patients. The main treatments were withdrawing ADV, adjusting the acid-base balance with sodium bicarbonate, and administering calcitriol and calcium. Notably, elemental phosphate supplementation was not necessary for therapy. During the follow-up, the patients experienced significant improvements in their clinical symptoms (reduction in bone pain and gradual restoration of biochemical indicators).

Owing to the potentially severe nephrotoxicity of ADV and the known susceptibility of certain people, identifying the possible risk factors is of great significance. In this study, we report the results of an investigation of the pharmacogenetic determinants of ADV-associated tubulopathy in chronic hepatitis B-infected patients. The most important finding was the association between ADV-associated tubulopathy and the c.2934G > A substitution of the *ABCC2* gene (rs3740070).

The underlying mechanisms by which the c.2934G > A substitution in the *ABCC2* gene influences the risk of nephrotoxicity due to ADV in these patients remain to be determined. This substitution, located at exon 22 of the *ABCC2* gene, is a synonymous variation because both the wild-type (TCG) and mutant (TCA) alleles encode a serine residue. However, the hypothesis that there is linkage disequilibrium between this polymorphism and other SNPs in genes coding for as-yet-unidentified proteins that influence tubular function should be considered, and

**Table 4**
Mutational analysis of 5 missense mutations.

| Gene | Nucleotide change | Predicted amino acid change | Frequency in patients | Polyphen-2 score | Prediction | SIFT score | Prediction | Mutation taster prob | Prediction |
|---|---|---|---|---|---|---|---|---|---|
| SLC22A6 | c.1247C > A | p.Pro416His | 1/55 | 0.997 | Probably damaging | 0.00 | Affect protein function | 1.0000 | Disease causing |
| ABCC2 | c.1457C > T | p.Thr486Ile | 1/58 | 0.063 | Benign | 0.19 | Tolerated | 1.0000 | Polymorphism |
| ABCC2 | c.2366C > T | p.Ser789Phe | 1/58 | 0.999 | Probably damaging | 0.00 | Affect protein function | 1.0000 | Disease causing |
| ABCC2 | c.2420A > G | p.Asn807Ser | 1/58 | 0.026 | Benign | 0.06 | Tolerated | 0.8932 | Disease causing |
| ABCC2 | c.4240C > T | p.His1414Tyr | 1/58 | 0.999 | Probably damaging | 0.00 | Affect protein function | 1.0000 | Disease causing |

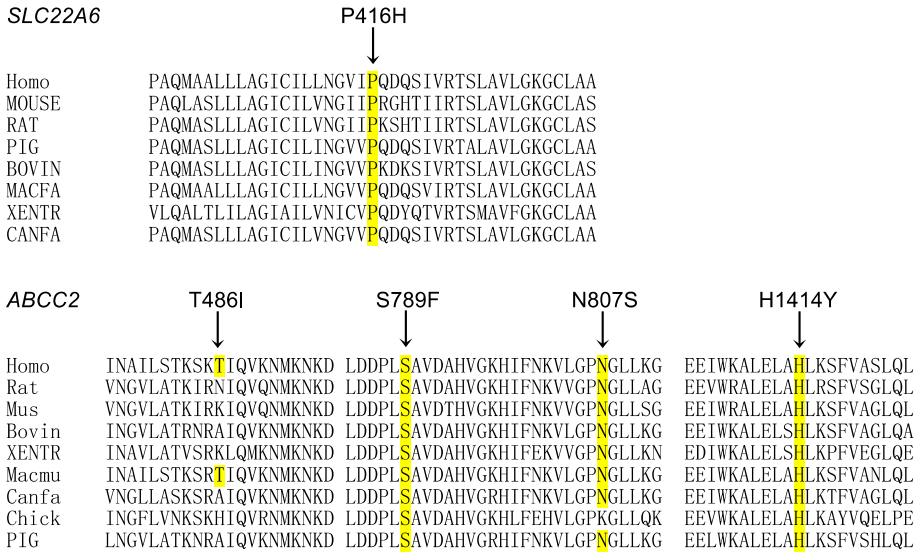


**Fig. 1.** Homology analysis of the mutation sites in different species.

the c.2934G > A substitution may be a sequence polymorphic marker. Further study is needed to verify this idea.

Six mutation sites were identified in affected subjects. Three missense mutations (P416H of the SLC22A6 gene and S789F and H1414Y of the ABCC2 gene) were predicted to have a pathogenic effect based on the mutational analysis. However, these mutations occurred at an extremely low frequency in a single patient indicating that it is likely not a common pathogenic determinant of ADV-associated tubulopathy. Nevertheless, we should consider the possibility that these mutations may play a role in the pathogenesis of some patients, and this possibility remains to be confirmed by increasing the sample size and further functional analysis.

Previous studies revealed that *ABCC2* genetic variants were associated with drug-induced nephrotoxicity [27–30]. In a study by Izzedine et al. [29], 4 SNPs in *ABCC2* were associated with tenofovir-induced renal proximal tubulopathy. However, 2 missense polymorphisms, c.3563T > A (Val1188Glu) and c.4544G > A (Cys1515Tyr), were not observed among our study subjects. The 2 synonymous polymorphisms, c.24C > T and c.1249G > A, which were also observed among our study subjects, were not associated with ADV-associated tubulopathy. However, the mechanisms by which these *ABCC2* SNPs affect MRP2 function have not been clarified.

In another study [13], Servais and colleagues studied adefovir clearance in rat after inhibition of transporters by probenecid and in mutant transport-deficient (TR−) rats, in which MRP2 is lacking. They found that the renal clearance of adefovir was significantly lower in probenecid treated TR− rats than in normal control rats, probenecid treated normal rats and TR-control rats. MRP2 mutation alone did not affect adefovir clearance. Thus, MRP2 does play a role in the tubular secretion of adefovir, but it is not the only participant. Additional transporters, which can be inhibited by probenecid, also take part in the renal clearance of adefovir. Further studies are needed to discover other transporters and genetic markers that may be associated with ADV-associated renal toxicity.

To the best of our knowledge, there are no previous reports of ADV-induced hypophosphatemic osteomalacia with such a large sample size. Our experience in managing these 76 patients with ADV-induced osteomalacia may facilitate early diagnosis and treatment, and exploration of the pathogenesis may provide a basis for individualized ADV medication. However, several limitations of our study must be acknowledged. Firstly, we lacked strictly control groups, such as chronic hepatitis B patients treated with ADV for >5 years and not suffering from ADV-induced osteomalacia (matched by age, race and sex). Case-control studies could further verify our findings and may identify new SNPs, mutations and haplotypes at genes associated with ADV-associated tubulopathy. Secondly, measure of urinary phosphate is absent in our study. Therefore, we can only presume phosphaturia based on other evidences of tubulopathy, such as nondiabetic glucosuria and proteinuria. Thirdly, we only assessed two transporters. Other transporters that were not considered may also play an important role in the elimination of ADV throughout the renal tubule, as discussed above. Finally, the validation of our results presented here certainly requires sufficiently powered independent clinical studies and functional analyses. Functional data are not yet available.

## 5. Conclusion

In conclusion, long-term (an average term of 5 years), low-dose (10 mg/d) ADV therapy was an important cause of adult-onset, hypophosphatemic osteomalacia in Chinese individuals. Clinicians should be aware of this side effect when using ADV for antiviral therapy. For early diagnosis, we suggest clinicians to pay more attention to the serum ALP and serum phosphorus level during the course of ADV treatment. Especially when other indexes of the hepatic function, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and γ-glutamyltranspeptidase (γ-GT), are all within normal levels, only the ALP level above normal, we should be warned of ADV-induced hypophosphatemic osteomalacia. Urine routine and arterial blood gas tests are needed when necessary. According to our results, some people may have a genetic predisposition to developing ADV-associated tubulopathy and secondary hypophosphatemic osteomalacia, which may contribute to inter-individual differences in the renal tolerance to ADV. Furthermore, larger studies are needed to establish this relationship. If these preliminary data are confirmed in prospective studies, then ADV may not be suggested for patients with predisposing genotypes as the antiviral therapy or close monitoring of tubular function must be warranted when they receiving ADV treatment.

## Disclosure statement

The authors have nothing to disclose.

## Conflict of interest

The authors have no conflict of interest.

## Acknowledgments

This study was supported by the National Basic Research Program of China (2014CB942903), the National Natural Science Foundation of China (81370978), the Frontier Technology Joint Research Program of the Shanghai municipal hospitals (SHDC 12013115), the Chongqing Science and Technology Commission (CSTC2013jcyjC00009), the Science and Technology Commission of Shanghai Municipality (14JC1405000). We thank all participants for their cooperation.

## References

[1] S.J. Hadziyannis, N.C. Tassopoulos, E.J. Heathcote, T.T. Chang, G. Kitis, M. Rizzetto, P. Marcellin, S.G. Lim, Z. Goodman, M.S. Wulfsohn, S. Xiong, J. Fry, C.L. Brosgart, Adefovir Dipivoxil 438 Study G, Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B, N. Engl. J. Med. 348 (2003) 800–807.
[2] P. Marcellin, T.T. Chang, S.G. Lim, M.J. Tong, W. Sievert, M.L. Shiffman, L. Jeffers, Z. Goodman, M.S. Wulfsohn, S. Xiong, J. Fry, C.L. Brosgart, Adefovir Dipivoxil 437 Study G, Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B, N. Engl. J. Med. 348 (2003) 808–816.
[3] H. Izzedine, J.S. Hulot, V. Launay-Vacher, P. Marcellini, S.J. Hadziyannis, G. Currie, C.L. Brosgart, C. Westland, S. Arterbrun, G. Deray, Adefovir Dipivoxil International 437 Study G, Adefovir Dipivoxil International 438 Study G, Renal safety of adefovir dipivoxil in patients with chronic hepatitis B: two double-blind, randomized, placebo-controlled studies, Kidney Int. 66 (2004) 1153–1158.
[4] E.J. Fisher, K. Chaloner, D.L. Cohn, L.B. Grant, B. Alston, C.L. Brosgart, B. Schmetter, W.M. El-Sadr, J. Sampson, Terry Beirn Community Programs for Clinical Research on A, The safety and efficacy of adefovir dipivoxil in patients with advanced HIV disease: a randomized, placebo-controlled trial, AIDS 15 (2001) 1695–1700.
[5] T. Wong, C.M. Girgis, M.C. Ngu, R.C. Chen, L. Emmett, K.A. Archer, M.J. Seibel, Hypophosphatemic osteomalacia after low-dose adefovir dipivoxil therapy for hepatitis B, J. Clin. Endocrinol. Metab. 95 (2010) 479–480.
[6] H. Kim du, D.H. Sung, Y.K. Min, Hypophosphatemic osteomalacia induced by low-dose adefovir therapy: focus on manifestations in the skeletal system and literature review, J. Bone Miner. Metab. 31 (2013) 240–246.
[7] H.J. Jeong, J.M. Lee, T.H. Lee, J.Y. Lee, H.B. Kim, M.H. Heo, G. Choi, J.N. Chae, J.M. Kim, S.H. Kim, K.Y. Kwon, Two cases of hypophosphatemic osteomalacia after long-term low dose adefovir therapy in chronic hepatitis B and literature review, J. Bone Metab. 21 (2014) 76–83.
[8] S.T. Law, K.K. Li, Y.Y. Ho, Acquired Fanconi syndrome associated with prolonged adefovir dipivoxil therapy in a chronic hepatitis B patient, Am. J. Ther. 20 (2013) e713–e716.
[9] H. Shimohata, S. Sakai, Y. Ogawa, K. Hirayama, M. Kobayashi, Osteomalacia due to Fanconi's syndrome and renal failure caused by long-term low-dose adefovir dipivoxil, Clin. Exp. Nephrol. 17 (2013) 147–148.
[10] C. Wu, H. Zhang, Y. Qian, L. Wang, X. Gu, Z. Dai, Hypophosphatemic osteomalacia and renal Fanconi syndrome induced by low-dose adefovir dipivoxil: a case report and literature review suggesting ethnic predisposition, J. Clin. Pharm. Ther. 38 (2013) 321–326.
[11] T. Cihlar, E.S. Ho, D.C. Lin, A.S. Mulato, Human renal organic anion transporter 1 (hOAT1) and its role in the nephrotoxicity of antiviral nucleotide analogs, Nucleosides Nucleotides Nucleic Acids 20 (2001) 641–648.
[12] A. Servais, P. Lechat, N. Zahr, S. Urien, G. Aymard, M.C. Jaudon, G. Deray, B.C. Isnard, Tubular transporters OAT1 and MRP2 and clearance of adefovir, Nephrol. Ther. 1 (2005) 296–300.
[13] A. Servais, P. Lechat, N. Zahr, S. Urien, G. Aymard, M.C. Jaudon, G. Deray, B.C. Isnard, Tubular transporters and clearance of adefovir, Eur. J. Pharmacol. 540 (2006) 168–174.
[14] D.S. Miller, Nucleoside phosphonate interactions with multiple organic anion transporters in renal proximal tubule, J. Pharmacol. Exp. Ther. 299 (2001) 567–574.
[15] H. Izzedine, V. Launay-Vacher, G. Deray, Antiviral drug-induced nephrotoxicity, Am. J. Kidney Dis. 45 (2005) 804–817.
[16] I.A. Adzhubei, S. Schmidt, L. Peshkin, V.E. Ramensky, A. Gerasimova, P. Bork, A.S. Kondrashov, S.R. Sunyaev, A method and server for predicting damaging missense mutations, Nat. Methods. 7 (2010) 248–249.
[17] P. Kumar, S. Henikoff, P.C. Ng, Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm, Nat. Protoc. 4 (2009) 1073–1081.
[18] J.M. Schwarz, D.N. Cooper, M. Schuelke, D. Seelow, MutationTaster2: mutation prediction for the deep-sequencing age, Nat. Methods. 11 (2014) 361–362.
[19] W.W. Hu, Z. Zhang, J.W. He, W.Z. Fu, C. Wang, H. Zhang, H. Yue, J.M. Gu, Z.L. Zhang, Establishing reference intervals for bone turnover markers in the healthy shanghai population and the relationship with bone mineral density in postmenopausal women, Int. J. Endocrinol. 2013 (2013) 513925.
[20] A. Barrios, T. Garcia-Benayas, J. Gonzalez-Lahoz, V. Soriano, Tenofovir-related nephrotoxicity in HIV-infected patients, AIDS 18 (2004) 960–963.
[21] A. Karras, M. Lafaurie, A. Furco, A. Bourgarit, D. Droz, D. Sereni, C. Legendre, F. Martinez, J.M. Molina, Tenofovir-related nephrotoxicity in human immunodeficiency virus-infected patients: three cases of renal failure, Fanconi syndrome, and nephrogenic diabetes insipidus, Clin. Infect. Dis. 36 (2003) 1070–1073.
[22] M. Daudon, L. Estepa, J.P. Viard, D. Joly, P. Jungers, Urinary stones in HIV-1-positive patients treated with indinavir, Lancet 349 (1997) 1294–1295.
[23] K.T. Tashima, J.D. Horowitz, S. Rosen, Indinavir nephropathy, N. Engl. J. Med. 336 (1997) 138–140.
[24] J.F. Navarro, C. Quereda, C. Quereda, N. Gallego, A. Antela, C. Mora, J. Ortuno, Nephrogenic diabetes insipidus and renal tubular acidosis secondary to foscarnet therapy, Am. J. Kidney Dis. 27 (1996) 431–434.
[25] L.J. Xu, Y. Jiang, R.X. Liao, H.B. Zhang, J.F. Mao, Y. Chi, M. Li, O. Wang, X.Q. Liu, Z.Y. Liu, X.P. Xing, W. Yu, W.B. Xia, Low-dose adefovir dipivoxil may induce Fanconi syndrome: clinical characteristics and long-term follow-up for Chinese patients, Antivir. Ther. 20 (2015) 603–611.
[26] B.F. Wang, Y. Wang, B.Y. Wang, F.R. Sun, D. Zhang, Y.S. Chen, Osteomalacia and Fanconi's syndrome caused by long-term low-dose adefovir dipivoxil, J. Clin. Pharm. Ther. 40 (2015) 345–348.
[27] S. Rodriguez-Novoa, P. Labarga, V. Soriano, D. Egan, M. Albalater, J. Morello, L. Cuenca, G. Gonzalez-Pardo, S. Khoo, D. Back, A. Owen, Predictors of kidney tubular dysfunction in HIV-infected patients treated with tenofovir: a pharmacogenetic study, Clin. Infect. Dis. 48 (2009) e108–e116.
[28] L. Wojnowski, B. Kulle, M. Schirmer, G. Schluter, A. Schmidt, A. Rosenberger, S. Vonhof, H. Bickeboller, M.R. Toliat, E.K. Suk, M. Tzvetkov, A. Kruger, S. Seifert, M. Kloess, H. Hahn, M. Loeffler, P. Nurnberg, M. Pfreundschuh, L. Trumper, J. Brockmoller, G. Hasenfuss, NAD(P)H oxidase and multidrug resistance protein genetic polymorphisms are associated with doxorubicin-induced cardiotoxicity, Circulation 112 (2005) 3754–3762.
[29] H. Izzedine, J.S. Hulot, E. Villard, C. Goyenvalle, S. Dominguez, J. Ghosn, M.A. Valantin, P. Lechat, A.G. Deray, Association between ABCC2 gene haplotypes and tenofovir-induced proximal tubulopathy, J. Infect. Dis. 194 (2006) 1481–1491.
[30] J.J. Kiser, M.L. Carten, C.L. Aquilante, P.L. Anderson, P. Wolfe, T.M. King, T. Delahunty, L.R. Bushman, C.V. Fletcher, The effect of lopinavir/ritonavir on the renal clearance of tenofovir in HIV-infected patients, Clin. Pharmacol. Ther. 83 (2008) 265–272.