Downregulation of VDR in benign paroxysmal positional vertigo patients inhibits otolith-associated protein expression levels

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Abstract. Benign paroxysmal positional vertigo (BPPV) is the most common peripheral vertigo-associated disease. Vitamin D (VD) helps maintain normal otolith function and may be associated with BPPV. VD exerts its biological functions primarily via the VD receptor (VDR). The present study demonstrated that serum VD levels were significantly decreased in patients with BPPV compared with in controls. VDR, otolith-associated protein otoconin-90 (OC90) and NADPH oxidase 3 (NOX3) expression levels were also significantly decreased in patients with BPPV compared with in controls. Furthermore, a positive correlation was observed between VD levels and VDR expression. Receiver operating characteristic curve analysis identified VDR expression levels as a potential diagnostic marker for BPPV. OC90 and NOX3 expression levels were notably lower in the inner ear tissue of VDR knockout mice compared with in those of wild-type mice. In mice overexpressing VDR, OC90 and NOX3 were also overexpressed. Following intravenous injection of VD in VDR knockout mice, expression levels of OC90 and NOX3 were not significantly different from those in VDR knockout mice injected with saline. This indicated that VDR may be underexpressed in patients with BPPV and was associated with the expression levels of otolith-associated proteins. Moreover, VDR mediated VD activation, leading to otolith protein formation. The present study provided a novel theoretical basis for BPPV onset that may facilitate the development of more effective diagnostic and treatment options.

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Introduction

Benign paroxysmal positional vertigo (BPPV), also known as otolithiasis, is the most common peripheral vertigo-associated disease (1,2). The incidence rate of BPPV is ~2.4% (3) with a male-to-female ratio of ~1: 2, and peak incidence among those aged 50-60 years (4). The primary clinical treatment options for BPPV include manual manipulation based on the semicircular canal and otolith involved. Vertigo symptoms can be relieved using the Epley, Semont and Barbecue maneuvers to reposition the detached otolith from the semicircular canal into the utriculus (5). However, these methods are only symptomatic treatments, and can neither cure BPPV, nor prevent its recurrence (6). Therefore, it is of clinical significance to investigate the pathogenesis of BPPV to provide a theoretical basis for targeted prevention and treatment.

BPPV is classified as either primary or secondary based on its pathogenesis. Secondary BPPV is defined as BPPV secondary to ear surgery, trauma, use of ototoxic drugs such as aminoglycoside, Meniere's disease, vestibular neuronitis and other etiologies (7). Underlying mechanisms of primary BPPV include aging, ear changes such as hair cell loss or injury, metabolic disorders affecting the endolymph calcium ion, decreased otolith protein secretion and greater fragility of anchorin filaments, which results in otolith detachment and dislocation (8,9). At present, certain scholars consider osteoporosis to be a predisposing factor for BPPV (10). Other factors, such as vitamin D (VD) deficiency, hypertension, diabetes, hyperlipemia, atherosclerosis and cerebrovascular disease, may also contribute to BPPV (11,12).

VD is a class of lipid-soluble secosteroid associated with human health, and is important in maintaining calcium homeostasis in humans (13,14). VD helps maintain normal otolith function by regulating calcium ion homeostasis in the vestibular lymph (7). Büki *et al* (15) suggested that VD deficiency is associated with BPPV and this hypothesis has been supported by other research. For example, serum 25-hydroxy VD levels <10 ng/ml are associated with high recurrence rates of BPPV (16). VD primarily exists in the human body as calcitriol [$1,25(OH)_2D_3$] and exerts its biological function via

the VD receptor (VDR); VDR is a member of the ligand-activated transcription factor steroid/thyroid hormone receptor superfamily and is found in numerous types of cell, such as immune, neural and epithelial cells (17). Following interaction with activated VD [1,25$(OH)_2D_3$], VDR has been shown to induce multiple antitumor gene regulation and cell signaling pathways, including those involved in proliferation suppression, stimulation of cell apoptosis and autophagy, angiogenesis inhibition and immune system regulation (18,19). To the best of our knowledge, however, there is little information regarding the role of VDR in BPPV.

The present study analyzed serum VD levels in patients with BPPV, then investigated the potential role of VDR in BPPV. The results demonstrated that VDR expression was directly associated with the expression of otolith-associated proteins *in vivo*. The association between VDR levels and the occurrence of BPPV suggested that VDR expression levels may be an important diagnostic marker. VDR may also be a potential target for the prevention and control of BPPV.

Materials and methods

Patients. Patients with BPPV treated at Affiliated Hospital of Inner Mongolia Medical University (Hohhot, China) were enrolled from April 2017 to December 2018. Participants in the control group had no prior history of vertigo onset. For inclusion in the experimental group, patients were required to have met the diagnostic criteria for BPPV (20). Predisposing factors (position or head position change), vertigo characteristics upon onset and positive physical examination (Dix-Hallpike or Roll test) were used to confirm diagnosis. In total, 48 patients were enrolled in the experimental group in strict accordance with the inclusion and exclusion criteria, and 48 controls were recruited. The patients comprised 30 females and 18 males (ratio, 1.67:1.00) aged 50-80 years (mean age, 64.65±1.24 years). The majority (86%) of patients were evaluated within 1 week of symptom onset. The controls comprised 28 females and 20 males (ratio, 1.40:1.00) aged 50-80 years (mean age, 63.25±1.33 years).

Primary exclusion criteria for both the experimental and control groups included: i) Head trauma or ear surgery history; ii) prior medication such as calcium, VD, hormone and associated drug substitution therapy; iii) ear disorders, such as vestibular neuronitis, sudden deafness, otitis media or Meniere's disease; iv) other central nervous system diseases such as transient ischemic attack of posterior circulation, cerebral infarction, cerebral hemorrhage or migraine; v) relevant endocrine diseases such as hypothalamic or pituitary space-occupying lesions; vi) severe renal insufficiency; vii) long-term anxiety or aversion to noisy environments; and viii) unwillingness to cooperate. Written informed consent was obtained from all participants for the use of their blood samples. The present study was approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University.

Bone densitometry. Before treatment, the bone mineral density of patients was measured via dual-energy x-ray absorptiometry using a Hologic Discovery dual-energy x-ray absorptiometer (Hologic, Inc.). Bone quality change was determined by T value: T≥-1.0, normal bone mineral density; -2.5<T<-1.0, osteopenia; and T≤-2.5, osteoporosis.

Detection of plasma 1,25$(OH)_2D_3$. The subjects were food-fasted for 12 h and liquid-fasted for 8 h before the test. Plasma 1,25$(OH)_2D_3$ levels were measured using an electrochemiluminescence kit (Elecsys Vitamin D total) according to the manufacturer's directions (Roche Diagnostics). This assay has a sensitivity of 0.83 ng/ml and a measurement range of 0.83-322.50 ng/ml.

RNA extraction and reverse transcription-quantitative (RT-q) PCR. A total of 5 ml peripheral venous blood was collected from all subjects and anticoagulated using citric acid. Lymphocyte separation medium (cat. no. Corning LSM 25-072-CV; Corning, Inc.) was used to separate lymphocytes from peripheral blood, and total RNA was extracted using TRIzol® reagent (cat. no. 15596026, Invitrogen; Thermo Fisher Scientific, Inc.). Total mRNA extraction (PolyATtract® mRNA Isolation Systems; cat. no. Z5210), first chain synthesis and PCR fluorescent quantitation kits (GoTaq® qPCR Master Mix; cat. no. A6001) were purchased from Promega Corporation and used according to the manufacturer's protocols. RT-qPCR was performed using the following thermocycling conditions: Initial denaturation at 95˚C for 30 sec, followed by 40 cycles of 5 sec at 95˚C, 10 sec at 60˚C and 30 sec at 72˚C. The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative gene expression levels (21). The housekeeping gene β-actin was used as an internal reference. The relative amount of the target gene was obtained by dividing the mean copy number of the target gene by the mean copy number of reference gene. The primer sequences were as follows: Otoconin-90 (OC90) forward, 5'-AGTGGTTTGGATGGTGCCAA-3' and reverse, 5'-GCA CCATCATTTCCACGAGC-3'; VDR forward, 5'-CAGGCT ATCATTACGGAGTC-3' and reverse, 5'-CTGGCATTTGTT TCTGTTCT-3'; NAPDH oxidase 3 (NOX3) forward, 5'-TTT TGGGTTCAACACTGGCT-3' and reverse, 5'-GTCTAATTG CCTCCTCCACG-3'; and β-actin forward, 5'-TAGTTGCGT TACACCCTTTCTTG-3' and reverse, 5'-TGCTGTCACCTT CACCGTTC-3'.

ELISA. Protein expression levels of OC90 and NOX3 were detected in each serum sample using ELISA kits (cat. nos. CSB-EL016255HU and CSB-E17448h-1, respectively; both Cusabio Technology LLC.) according to the manufacturer's instructions. The calibration curve was plotted according to the concentration and optical density value of the standard to calculate the concentration of test sample.

Mouse model. A total of 12 VDR gene knockout and 12 knock-in female C57BL/6JGpt mice (age, 6-8 weeks; weight, ~20 g) were purchased from GemPharmatech Co., Ltd. All mice were raised in specific pathogen free conditions with 12 h light/dark cycle and free access to food and water at 21-24˚C and 40-67% humidity. After 3 days, one group of knockout (n=6) and knock-in mice (n=6) was sacrificed to harvest inner ear tissue for subsequent experiments. Another group of knockout mice (n=6) was injected with 1,25$(OH)_2D_3$ 100 ng/day via the caudal vein daily for 1 week. A total of 12 control wild-type C57BL/6JGpt mice (GemPharmatech

Table I. Clinical characteristics of patients and controls.

Characteristic	Control (n=48)	Patients with BPPV (n=48)	P-value
Age, years	63.25±1.33	64.65±1.24	0.440
Male	20 (41.2%)	18 (37.5%)	0.830
Body mass index	25.44±0.12	25.17±0.13	0.120
Bone mineral density			<0.050
Normal	25 (52.0%)	8 (16.7%)	
Osteopenia	15 (31.3%)	10 (20.8%)	
Osteoporosis	8 (16.7%)	30 (62.5%)	<0.001[a]
Diabetes	6 (12.5%)	7 (14.6%)	>0.050
Hypertension	10 (20.8%)	11 (22.9%)	>0.050

Continuous data are presented as the mean ± SD. P-values were calculated using independent t-tests or χ^2 tests. [a]vs. Normal. BPPV, benign paroxysmal positional vertigo.

Co., Ltd.) were injected with an equivalent dose of normal saline daily for 1 week. The present study was approved by the Animal Ethics Committee of Affiliated Hospital of Inner Mongolia Medical University (approval no. QZ2017023). All experiments were conducted in accordance with the Code for the Care and Use of Animals for Scientific Purposes (22) and the principles of replacing, refining, and reducing.

Western blotting. Bilateral temporal bones of mice were separated, and the muscle and other adherent soft tissues were removed manually. Inner ear samples were ground in liquid nitrogen and placed in PBS buffer (cat. no. C0221A; Beyotime Institute of Biotechnology) containing 1% protease inhibitor (cat. no. 78438; Invitrogen; Thermo Fisher Scientific, Inc.), then placed on ice for 10 min. The solution was centrifuged at 4˚C at 12,000 x g for 10 min, and the supernatant was reserved. Following quantification using the BCA Protein Assay kit (cat. no. P0012S, Beyotime Institute of Biotechnology), and electrophoresis (40 μg protein/lane separated via 10% SDS-PAGE), proteins were transferred onto a PVDF membrane. Then, the membrane was blocked for 1 h with 5% skimmed milk at 25˚C. After washing, the membrane was incubated at 4˚C overnight with the following prediluted primary antibodies: Anti-transferrin (1:1,000; 77 kDa; cat. no. ab109503; Abcam), anti-GAPDH (1:2,000; 36 kDa; cat. no. ab181602; Abcam), anti-VDR (1:1,000; 48 kDa; ab109234; Abcam), anti-NOX3 (1:1,000; 65 kDa; cat. no. ab254572; Abcam) and anti-OC90 (1:1,000; 51 kDa; cat. no. PA5-71564; Invitrogen; Thermo Fisher Scientific, Inc.). The membrane was then incubated with horseradish peroxidase-conjugated secondary antibody (1:1,000; cat. no. A0208; Beyotime Institute of Biotechnology) without agitation for 60 min at 20-25˚C. Signals were visualized using ECL reagents (EMD Millipore) and detected using an Amersham™ Imager 680 (Cytiva). ImageJ software (1.8.0; National Institutes of Health) was used for densitometry.

Statistical analysis. Data are presented as the mean ± SD of three independent repeats. SPSS 19.0 software (IBM Corp.) was used for statistical analysis. Normally distributed data were analyzed via independent sample t-tests; non-parametric Wilcoxon rank-sum tests were used to analyze data with non-normal distributions. Receiver operating characteristic (ROC) curve analysis was performed to reveal the potential diagnostic value of VDR expression levels for BPPV. Differences among multiple groups were detected via ANOVA (one-way) with post hoc Tukey's test. χ^2 test was used for categorical data. Pearson's correlation analysis was used to assess the correlation between VDR mRNA and 1,25(OH)2D3 levels. P<0.05 was considered to indicate a statistically significant difference.

Table II. Involvement of different sites in patients with benign paroxysmal positional vertigo.

Site	Number of cases (%)
Posterior	25 (52.1)
Horizontal	16 (33.3)
Anterior canal	7 (14.6)
Canalolithiasis	38 (79.2)
Cupulolithiasis	10 (20.8)

Results

Study subjects. Data was collected from patients with BPPV diagnosed in Affiliated Hospital of Inner Mongolia Medical University. There was no difference in the age, sex ratio, BMI, or diabetes and hypertension rate between the patient and control groups (Table I). However, cases of decreased bone density (particularly osteoporosis) in patients with BPPV were significantly higher than in the control group (Table I). As shown in Table II, BPPV most commonly involved the posterior (n=25, 52.1%), horizontal (n=16, 33.3%) and anterior canals (n=7, 14.6%). The proportion of patients with canalolithiasis (n=38) was 79.2% and that of cupulolithiasis (n=10) was 20.8%.

Plasma expression levels of 1,25(OH)$_2$D$_3$ and otolith-associated protein in patients with BPPV. The level of 1,25(OH)$_2$D$_3$ was measured in patients with BPPV; results indicated that

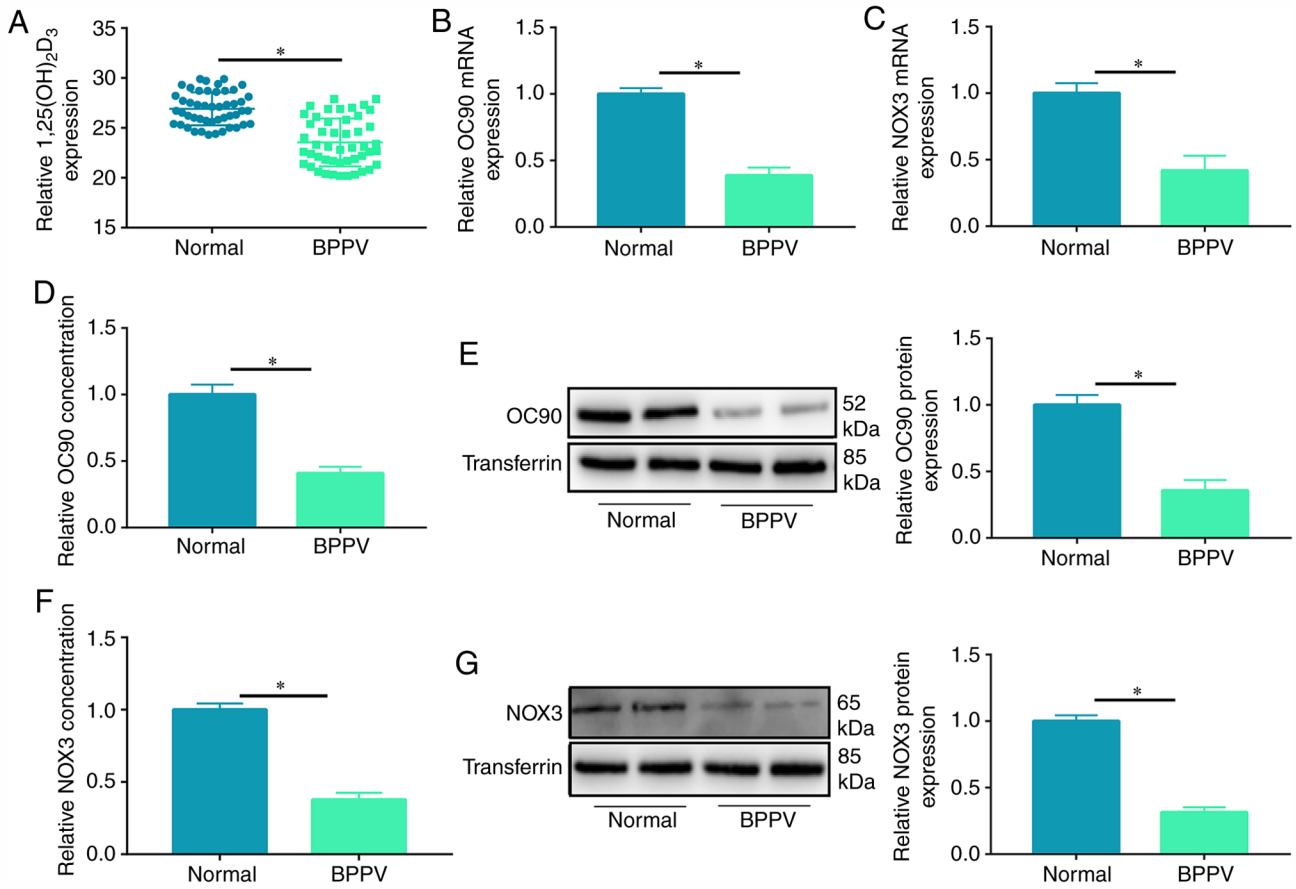

Figure 1. Plasma expression of 1,25(OH)2D3 and otolith-associated proteins in patients with BPPV. (A) Expression levels of $1,25(OH)_2D_3$ were significantly lower in the plasma of patients with BPPV relative to the controls. mRNA levels of (B) OC90 and (C) NOX3 in the plasma of patients with BPPV were notably reduced. (D) ELISA results showed lower OC90 concentrations in the plasma of patients with BPPV compared with in controls. (E) Western blotting results indicated lower protein levels of OC90 in patients with BPPV compared with in controls. (F) Concentration and (G) protein levels of NOX3 were significantly decreased in the plasma of patients with BPPV than in controls. Data are presented as the mean ± SD. *P<0.05. BPPV, benign paroxysmal positional vertigo; OC90, otoconin-90; NOX3, NAPDH oxidase 3.

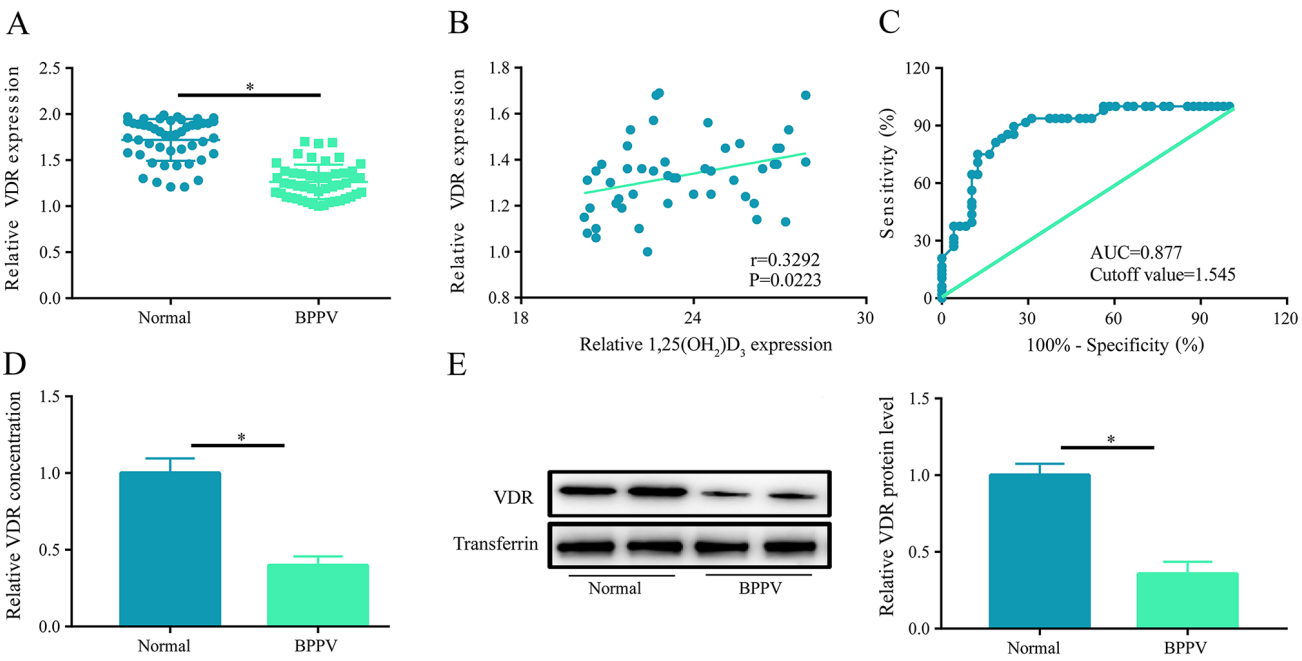

Figure 2. VDR is significantly underexpressed in patients with BPPV. (A) VDR was underexpressed and (B) positively correlated with $1,25(OH)_2D_3$ expression levels in the plasma of patients with BPPV. (C) Receiver operating characteristic curve analysis of VDR. (D) ELISA and (E) western blotting results showed lower protein levels of VDR in patients with BPPV compared with in controls. Data are presented as the mean ± SD. *P<0.05. VDR, vitamin D receptor; BPPV, benign paroxysmal positional vertigo; AUC, area under the curve.

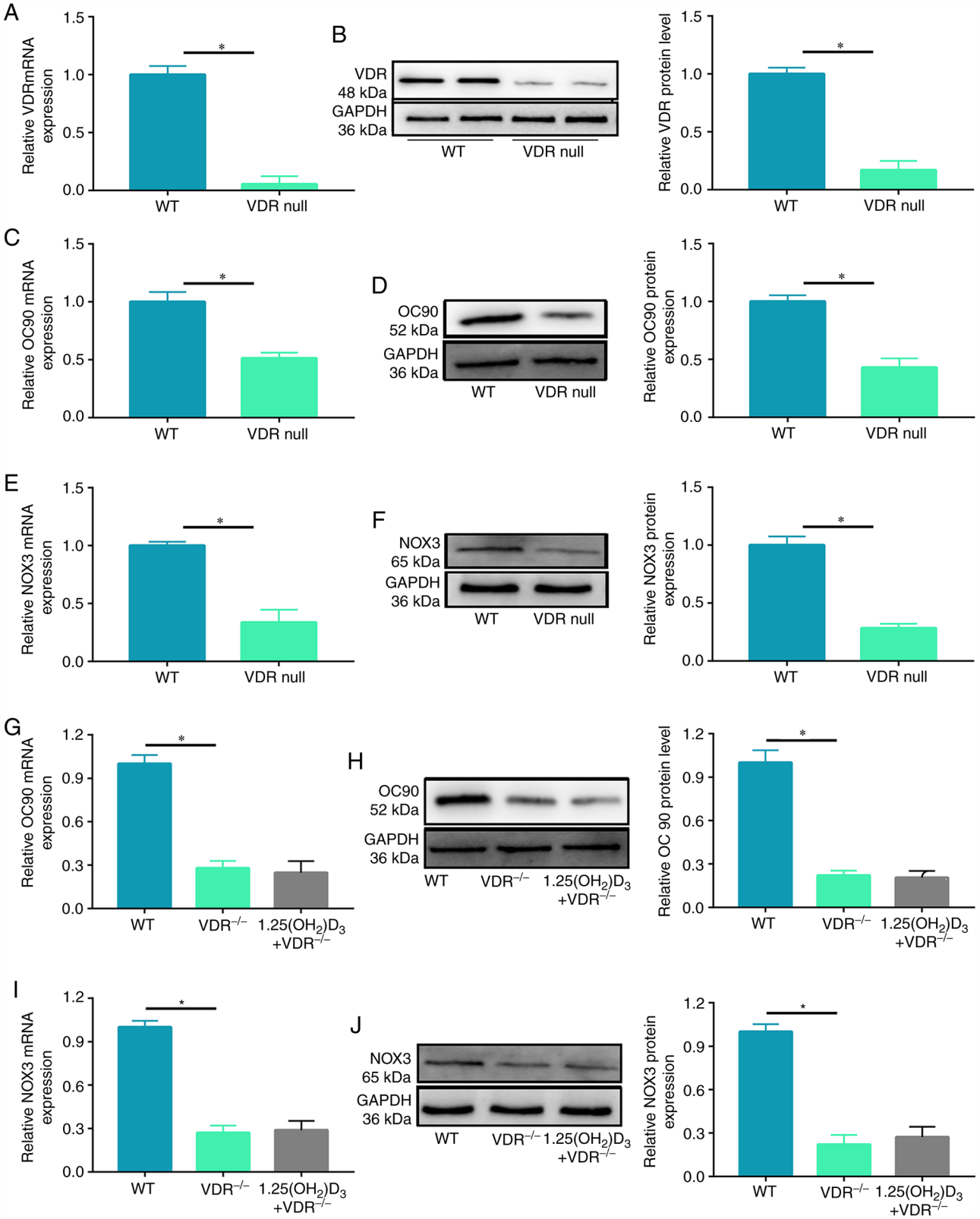

Figure 3. VD affects expression levels of OC90 and NOX3 via VDR. (A) RT-qPCR and (B) WB results revealed decreased mRNA and protein levels of VDR in VDR$^{-/-}$ mice. (C) RT-qPCR and (D) WB results indicated that mRNA and protein levels of OC90 in VDR$^{-/-}$ mice were significantly lower compared with those in WT mice. NOX3 (E) mRNA and (F) protein expression in WT mice was significantly higher compared with in VDR$^{-/-}$ mice. Following injection of $1,25(OH)_2D_3$ into the caudal vein of VDR$^{-/-}$ mice, OC90 (G) mRNA and (H) protein and NOX3 (I) mRNA and (J) protein expression levels did not significantly change relative to VDR mice injected with an equivalent amount of normal saline. Data are presented as the mean ± SD. *P<0.05. VD, vitamin D; OC090, otoconin-90; NOX3, NAPDH oxidase 3; VDR, vitamin D receptor; RT-qPCR, reverse transcription-quantitative PCR; WB, western blotting; WT, wild-type.

plasma VD levels in patients with BPPV were significantly decreased compared with the control group (Fig. 1A). Serum VD expression levels in patients with canalolithiasis and cupulolithiasis exhibited no significant difference (Fig. S1). As

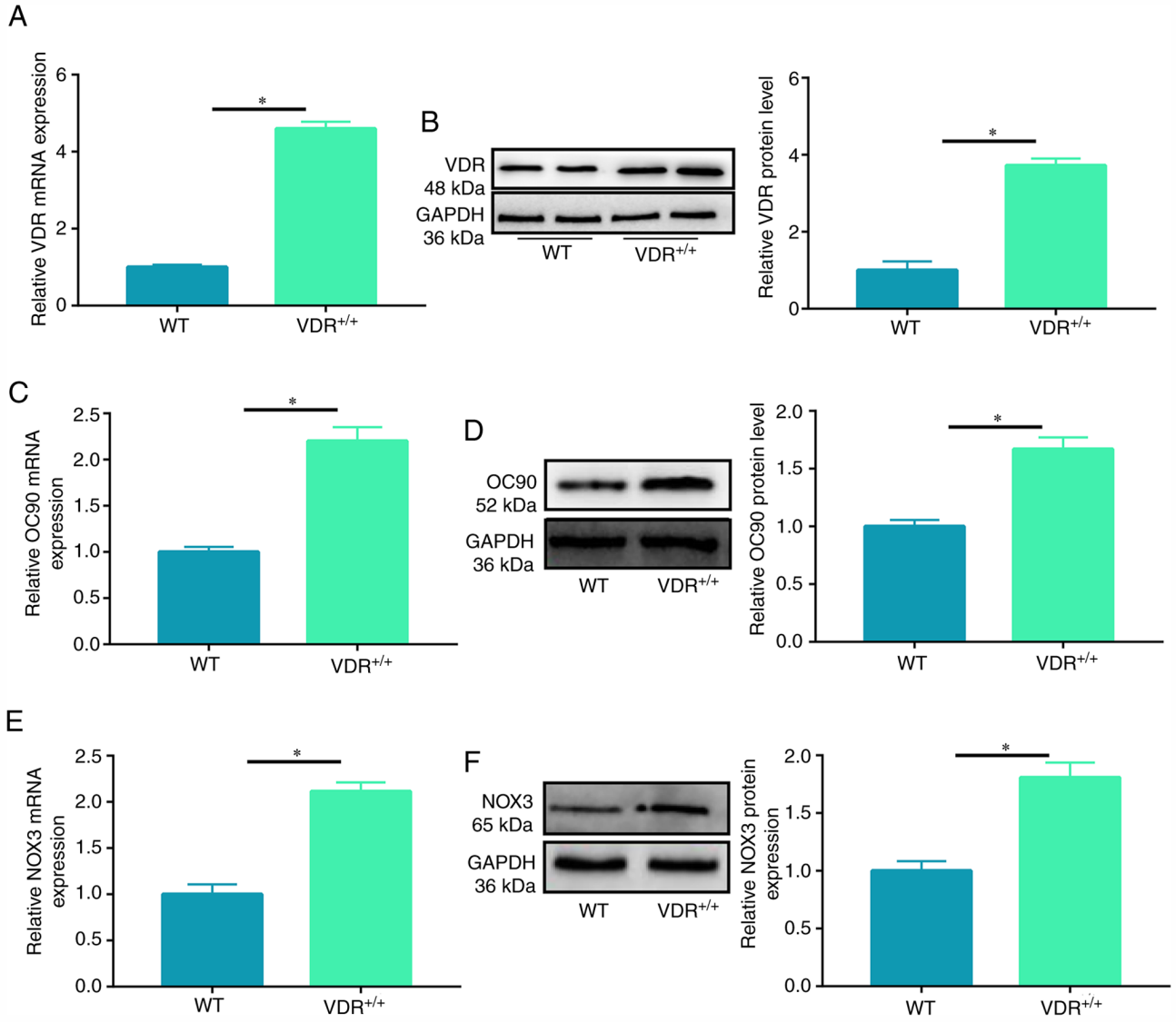

Figure 4. Overexpression of VDR increases expression of OC90 and NOX3. Reverse transcription-quantitative PCR and western blotting indicated significantly higher (A) mRNA and (B) protein levels of VDR in overexpression mice compared with WT mice. VDR overexpression mice exhibited significantly higher OC90 (C) mRNA and (D) protein expression levels compared with WT mice. NOX3 (E) mRNA and (F) protein expression levels were significantly higher in VDR overexpression mice compared with in WT mice. Data are presented as the mean ± SD. *P<0.05. VDR, vitamin D receptor; OC90, otoconin-90; NOX3, NAPDH oxidase 3; WT, wild-type.

demonstrated via RT-qPCR, serum mRNA expression levels of otolith-associated proteins OC90 and NOX3 were notably reduced in patients with BPPV compared with in controls (Fig. 1B and C). ELISA indicated that protein levels of OC90 were lower in the serum of patients with BPPV compared with in the control group; this was supported by western blot analysis (Fig. 1D and E). Finally, NOX3 protein levels were also significantly lower in the serum of patients with BPPV compared with in the controls, as revealed via both ELISA and western blotting (Fig. 1F and G). The expression levels of $1,25(OH)_2D_3$ and otolith-associated proteins were lower in patients with BPPV than in controls. This suggested that the expression levels of VD regulated OC90 and NOX3 expression, and were associated with the occurrence and development of BPPV.

VDR is significantly underexpressed in patients with BPPV. As VD exerts its biological role primarily via interacting with VDR, a potential association between VDR expression levels and BPPV was investigated. RT-qPCR was performed to analyze VDR expression levels in the serum of patients with BPPV: Compared with the control, VDR expression was notably decreased (Fig. 2A), and its expression was positively correlated with that of $1,25(OH)_2D_3$ (r=0.3292; P=0.0223; Fig. 2B). Furthermore, ROC curve analysis reported an area under the curve of 0.877 and cutoff value of 1.545, suggesting that VDR was a candidate diagnostic marker of BPPV (Fig. 2C). The mRNA and protein expression levels of VDR were also detected and shown to be significantly lower in patients with BPPV (Fig. 2D and E).

VD affects expression of OC90 and NOX3 via VDR. In order to verify the effects of VDR on the expression levels of OC90, $VDR^{-/-}$ mice were purchased, and inner ear tissue was harvested for the detection of OC90 expression levels. VDR expression levels were first verified in knockout mice: mRNA

and protein levels of VDR were significantly decreased in $VDR^{-/-}$ mice compared with wild-type mice (Fig. 3A and B). Subsequent RT-qPCR experiments using inner ear tissue found that mRNA levels of OC90 were significantly lower in $VDR^{-/-}$ mice compared with those in wild-type mice (Fig. 3C); western blot analysis showed the same trend for OC90 protein expression (Fig. 3D). Similarly, NOX3 mRNA and protein expression levels were significantly downregulated in $VDR^{-/-}$ mice compared with in wild-type mice (Fig. 3E and F). This suggested that there was an association between VDR and both OC90 and NOX3 expression levels. In order to investigate whether VD acted via VDR to regulate expression levels of OC90 and NOX3, activated VD or saline was injected into the caudal vein of $VDR^{-/-}$ mice (wild-type injected with saline, $VDR^{-/-}$ injected with saline and $VDR^{-/-}$ injected with activated VD), and inner ear tissue was collected from each group 1 week later. Injection of VD did not reverse the inhibiting effect of the $VDR^{-/-}$ on otolith protein formation (Fig. 3G-J). This indicated that VD required VDR to exert its biological function.

Overexpression of VDR increases expression of OC90 and NOX3. VDR overexpression mice were constructed to further verify the role of VDR in regulating OC90 and NOX3 expression. The model was successful, indicated by the detection of VDR mRNA and protein (Fig. 4A and B). RT-qPCR and western blotting were used to detect mRNA and protein levels of OC90 and NOX3 in inner ear tissue. mRNA and protein expression levels of both OC90 and NOX3 were significantly increased in VDR overexpression mice compared with in wild-type mice (Fig. 4C-F). Thus, VDR was important for expression of the otolith-associated proteins OC90 and NOX3.

Discussion

It has previously been proposed that BPPV is caused by numerous factors, such as aging, genetic mutation, head trauma and ototoxic drugs, causing otolith particles on macula utriculi and macula sacculi to detach and move into the semicircular canal (9,23). In the present study, anterior canal BPPV accounted for 14.6% or cases, while anterior canal BPPV was rare, as previously reported (24). This may be because patients of only one hospital were included and anterior canal BPPV may have been underdiagnosed. In addition, the number of patients in the present study was not large enough, so anterior canal BPPV morbidity in the present study may not be consistent with that in the population. Therefore, more clinical samples from multiple centers are needed.

Otoliths are inlaid in the otolithic membrane covering the macula surface and consist of an organic matrix composed primarily of otolith proteins and deposited calcium carbonate. These specific proteins mediate the special functions of the inner ear. In cases of dysfunction or injury, they are released into the circulatory system, and can be detected in the serum (25,26). For example, otolith protein otolin-1 is specifically expressed in the inner ear (27), but can be detected in serum when it moves into systemic circulation via the blood-labyrinth barrier (28). OC90 is the most common otolith protein in humans, mice and other mammals: It accounts for >90% of the otolith organic matrix, and is considered to be a necessary factor for otolith growth and maintenance (29). Although the organic matrix accounts for a smaller percentage of the otolith, it is now considered to be vital in the development of the otolith (25).

OC90 is generated by non-sensory cells outside the macula and transferred to the macula sacculi and macula utriculi post-synthesis, where it recruits calcium deposits to promote the formation of the original otolith body (30,31). In addition, OC90 may also regulate otolith growth and promote the transformation of otoliths from their original form to their mature hexagonal form (27). Zhao *et al* (31) demonstrated that OC90 gene knockout mice do not effectively recruit Ca^{2+} to the macula from the bloodstream during development. In these mice, significantly fewer otoliths, with a notably larger volume and loose arrangement, were observed compared with wild-type mice. The knockout mice also exhibited etiological posture disorders (26). Multiple non-intra-otolith proteins participate in the regulation of the growth and development of otoliths: One protein that regulates otolith growth is NOX3 (32-34). NOX3 controls otolith growth by regulating of the secretion or function of otolith proteins, as well as the spatial and temporal distribution of calcium and other ions. Therefore, OC90 and NOX3 were selected as detection indices to assess the effects of VD and VDR on BPPV onset in the present study.

VD is known for its role in the homeostasis of calcium and phosphorus (35). VDR is present in cells throughout the body, and is involved in cell proliferation and differentiation as well as immunomodulation (36). The functions of VD are mediated by nuclear VDR. Studies have shown that increased VDR expression levels are associated with decreased mortality rates and improved prognosis in numerous types of cancer, including breast and prostate cancer (37,38). Wen *et al* (39) reported that VDR expression levels are significantly decreased in gastric cancer tissue but are high in well- and moderately differentiated tissue and small tumors. This indicates that VDR may be a prognostic factor of gastric cancer.

To the best of our knowledge, there are few studies on the role of VDR in BPPV. The present study investigated the potential role of VDR in the occurrence and development of BPPV. It was first verified that patients with BPPV had lower serum levels of VD. Additionally, patients with BPPV had notably lower plasma expressions levels of OC90 and NOX3, indicating an association between VD levels and BPPV occurrence. VDR expression levels were significantly lower in the serum of patients with BPPV than in that of the controls. VDR expression levels were positively associated with serum VD levels, indicating that VD deficiency and VDR expression may serve a role in BPPV. However, the present study was relatively small, and the association between VDR and VD in patients with BPPV should be further verified using a larger sample group. ROC curve analysis showed that VDR may be used as a diagnostic marker of BPPV, providing a new theoretical foundation for clinical, auxiliary or early diagnosis of BPPV.

A previous study detected VDRs in the nuclei of the epithelium lining the crista ampullaris, membranous semicircular canal and surrounding osteocytes in mice (40). Furthermore, VDR mutant mice showed decreased balance function using the accelerating rotarod, tilting platform, rotating tube and swim tests, which suggested that decreased VD causes vestibular dysfunction (40). The present study investigated the formation

of inner ear-associated protein using VDR gene knockout and knock-in mice. The results revealed that expression levels of the inner ear-associated proteins OC90 and NOX3 were significantly lower in VDR knockout mice than in wild-type mice. Conversely, expression levels of OC90 and NOX3 in the inner ear tissue of VDR overexpression mice were notably higher than those in wild-type mice. This suggested that VDR was vital for the expression of otolith proteins. Following injection of VD into the caudal vein of VDR knockout mice, expression levels the OC90 and NOX3 in the inner ear tissue were not notably increased. This indicated that VDR was crucial for the expression of inner ear-associated proteins.

Taken together, the results of the present study indicated that VDR expression levels are significantly decreased in patients with BPPV. *In vivo* experiments demonstrated that VDR was associated with the expression of inner ear-associated proteins OC90 and NOX3, and that VDR expression may have diagnostic potential, with the protein potentially serving an important role in BPPV pathogenesis. This not only enriches knowledge of BPPV, but may also provide a new theoretical basis for clinical diagnosis and treatment of BPPV. However, the present study has certain limitations. It is unknown whether the downregulation of VDR in patients with BPPV is a universal phenomenon. Additional samples and multicenter cooperation will be needed for further validation. In addition, only two inner ear-associated proteins, OC90 and NOX3, were investigated. Further investigation is required to determine how VDR affects the expression of other inner ear-associated proteins; additionally, the underlying mechanisms concerning how VDR modulates the synthesis of inner ear-associated proteins remains unclear.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SZ and JX performed the experiments and generated data. SZ and PL made substantial contributions to the conception and design of the study. YG, BW and LX conducted data analysis and interpretation. All authors contributed to the drafting and revision of the manuscript and agreed to be accountable for all aspects of the research. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present animal study was approved by The Affiliated Hospital of Inner Mongolia Medical University (approval no. QZ2017023). Human experiments were approved by the Ethics Committee of the Affiliated Hospital of Inner Mongolia Medical University. Written informed consent was provided by all subjects.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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