

# Recent Advances in Vitamin D3 Intervention to Eradicate *Helicobacter pylori* Infection

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**Abstract:** *Helicobacter pylori* (HP) infections affect approximately one-third of children worldwide. In China, the incidence of HP infection in children ranges from approximately 30% to 60%. In addition to damaging the gastrointestinal tract mucosa, HP infection in children can negatively affect their growth and development, hematology, respiratory and hepatobiliary system, skin, nutritional metabolism, and autoimmune system. However, the rate of HP eradication also fell considerably from the previous rate due to the presence of drug-resistant HP strains and the limited types of antibiotics that can be used in young patients. Vitamin D3 (VitD3) is a steroid hormone that can reduce inflammation in the stomach mucosa induced by HP and can alleviate and eradicate HP through a variety of pathways and mechanisms, including immune regulation and the stimulation of antimicrobial peptide (AMP) secretion and  $\text{Ca}^{2+}$  influx, to reestablish lysosomal acidification; thus, these results provide new strategies and ideas for the eradication of drug-resistant HP strains.

**Keywords:** vitamin D3, *Helicobacter pylori*, immune response, AMPs,  $\text{Ca}^{2+}$  channels

## Introduction

*Helicobacter pylori* (HP) is a helical, microaerobic bacterium that colonizes the epithelium and lamina propria of the gastric mucosa. HP is the only microorganism known to survive in the human stomach, and HP survives by producing ammonia from urea to neutralize gastric acid. As a dependent risk factor for gastric and duodenal erosions, as well as for peptic ulcers,<sup>1</sup> HP is thought to induce chronic inflammation, which is characterized by lymphoplasmacytic infiltration, or acute inflammatory responses, which are characterized by neutrophil infiltration.<sup>2</sup> The prevalence of HP infection varies between regions of the world, although it affects roughly one-third of children worldwide.<sup>3</sup> The incidence of HP infection in children varies significantly by area in China, with infection rates ranging from approximately 30% to 60%.<sup>4,5</sup> Ren<sup>4</sup> and Yuan<sup>6</sup> discovered that the prevalence of HP infection increased with age while investigating the epidemiology of HP infection in children in China and worldwide. However, the rate of HP eradication also fell considerably from the previous rate due to the presence of drug-resistant HP strains and the limited types of antibiotics available for use in young patients. In addition to causing damage to the gastrointestinal tract mucosa, HP infection in children can negatively affect growth and development, hematology, respiratory and hepatobiliary health, skin, nutritional metabolism, and the autoimmune system.<sup>7</sup> Recent research has revealed a link between vitamin D3 (VitD3) deficiency and HP infection.<sup>8–10</sup>

1,25-dihydroxyvitamin D3 [1,25(OH)2D3] is a steroid hormone derived from vitamin D. 1,25(OH)2D3 is the pre-dominant metabolite used to determine vitamin D levels in the body, and it is derived from both dietary vitamin D (D2) and cholecalciferol (D3) through photochemical interactions with sunshine or ultraviolet light, the latter of which is its principal source. Vitamin D is converted to 25-hydroxyvitamin D3 [25(OH)D3] and 1,25-(OH)2D3 by the enzymes 25-hydroxylase in the microsomes of hepatocytes and 1- $\alpha$  hydroxylase in the mitochondria of proximal renal tubular epithelial cells. In

2006, Kawaura showed that regular usage of an analog of vitamin D3 called 1 alpha-hydroxyvitamin D3 decreased the incidence of *Helicobacter pylori* infection.<sup>10</sup> El Shahawy<sup>9</sup> reported that patients with HP infection who were vitamin D deficient had lower rates of successful eradication. Supplemental vitamin intake substantially impacts HP infection, either by directly modifying the host's inflammatory pathways or by subtly enhancing the host's immune response.

## VitD3 Inhibits HP-Induced Gastric Mucosal Inflammation by Regulating Immunity

It has been reported that HP infection in adults increases the number of CD4+ T cells and induces activation of CD4+ and CD8+ T cells.<sup>11</sup> Helper T-cell type 1 (Th1), Th2, Th9, Th17, Th22, and T regulatory (Treg) cells can influence the outcome of HP infection either individually or in combination with each other.<sup>12</sup> It has been demonstrated that Th1 cells selectively identify HP antigens. In contrast, the protective response of the immune system to specific HP antigens triggered by Treg and Th2 cells helps reduce tissue damage in the host.<sup>13,14</sup> Th1 and Th17 cells promote the expression of interferon-gamma (IFN- $\gamma$ ) and interleukin-17 (IL-17), respectively, the latter of which increases the risk of hyperacidity, gastric atrophy, and gastric adenocarcinomas.<sup>15,16</sup>

By modulating T-cell proliferation and cytokine production, vitamin D3 may have an impact on the adaptive immune system. By regulating the expression of costimulatory molecules for antigen presentation, such as CD86, CD80, and CD40, as well as major histocompatibility class II (MHCII), VitD3 decreases the antigen-presenting capacity of dendritic cells (DCs)<sup>17</sup> and affects cytokine production to control the differentiation of naive CD4+ T cells into various helper subsets, such as Th1 and Th2 cells.<sup>18</sup> VitD3 also suppresses IFN- $\gamma$  expression by binding the VDR/RXR (vitamin D receptor/retinoid X receptor) complex to the silencer promoter region of the gene.<sup>19</sup> VitD3 stimulation of human T cells activates VDR signaling, which is connected to the suppression of IFN- $\gamma$  and IL-10 production, as well as the regulation of the C-C motif chemokine receptor 10 (CCR10) homing receptor.<sup>20</sup> However, VitD3 seems to have the opposite effect on IL-10, suggesting that this hormone induces IL-10 in human B cells.<sup>18</sup> Furthermore, VitD3 restricts Th17 cell development while promoting Treg cell development,<sup>21</sup> and Treg cells both reduce the Th1 cell response and modify the Th2 cell response.<sup>12</sup> Furthermore, VitD3 impacts T-cell proliferation, cell cycle progression, and the ability of T cells to produce cytokines, as well as inhibiting the expression of IL-2, a key growth factor for T-lymphocytes. VitD3 inhibited the release of T-cell cytokines and the progression of the cell cycle from G1a to G1b in vitro. VitD3 influences the transition of new T cells toward a Th2-type response by boosting the production of Th2 cytokines (IL-4, IL-5, and IL-10) and decreasing the synthesis of Th1 cytokines (IL-2, IL-12, and IFN- $\gamma$ ).<sup>22,23</sup> Vitamin D signaling, on the other hand, suppresses Th17 cytokine production<sup>24</sup> and promotes CD4+CD25+Foxp3+ regulatory T cells.<sup>25</sup>

More Treg cells were detected in the gastric mucosa of HP-infected children than in that of infected adults, which was accompanied by less severe gastric inflammation; moreover, the decrease in neutrophil aggregation may be due to inhibition of the Th17 and Th1 responses.<sup>26</sup> Nevertheless, the activation and expansion of Treg cells in the gastric mucosa, as well as the decreased Th17 cell-associated response in children, may be crucial for reducing inflammation and increasing bacterial density, as well as representing a mechanism of bacterial persistence.<sup>27</sup>

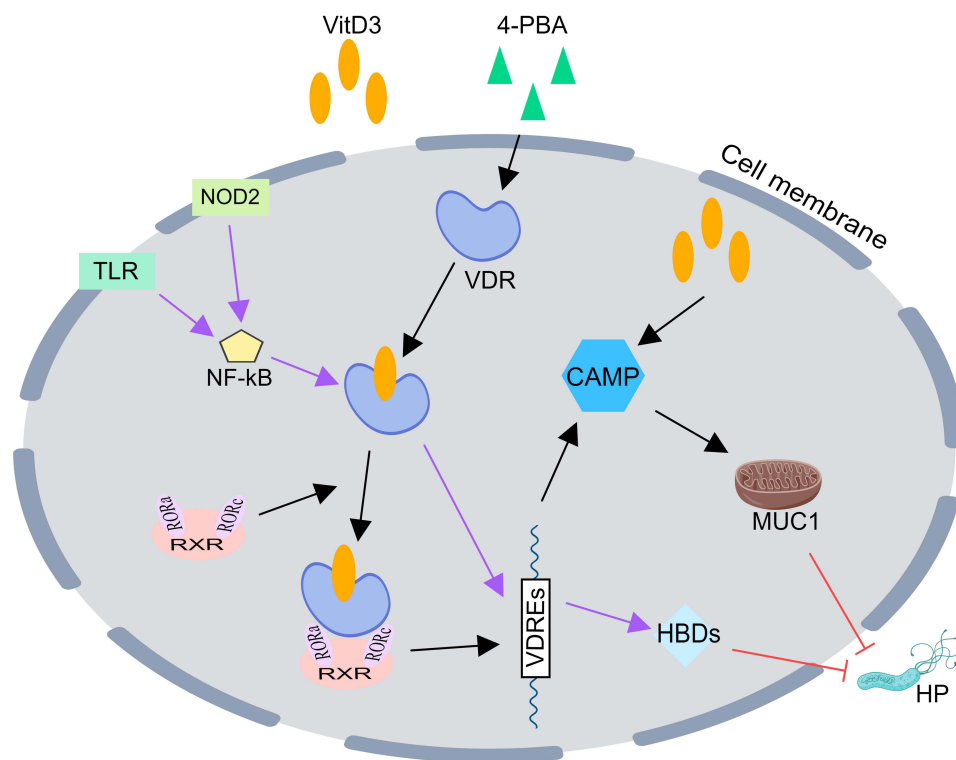
## VitD<sub>3</sub> Inhibits HP by Promoting the Secretion of Antimicrobial Peptides (AMPs)

Fleming and Ridley isolated AMPs from human tears in 1922 and categorized them based on their secondary structures, such as cathelicidins ( $\alpha$ -defensins), defensins ( $\beta$ -defensins), and bactenecins ( $\theta$ -defensins); however, no human  $\theta$ -defensins have been isolated. AMPs are a large class of innate immune system effector molecules with a wide range of biological activities, including immunomodulatory and antimicrobial functions against bacterial, fungal, yeast, and viral pathogens. However, recent studies have identified AMPs as core regulators of autophagy/hemophagy, cytokines, chemokines, and reactive oxygen species (ROS) production, as well as IFN signaling, as they serve as signaling nodes that modulate immune pathways.<sup>28,29</sup>

## VitD<sub>3</sub> Inhibits HP by Promoting the Secretion of Cathelicidin

Human cathelicidin antimicrobial peptides (CAMPs), such as LL-37, are alpha-helical peptides with amphipathic properties that are derived from the C-terminal region of the human cationic antimicrobial protein, which is broken down by serine protease and proteinase 3;<sup>30</sup> CAMPs can be divided into six categories known as human neutrophil proteins 1 to 4 (HNP1-4) and human defensins 5 and 6 (HD5 and 6). Human CAMPs have antimicrobial activity against both gram-negative and gram-positive bacteria, and their bactericidal activity requires activation by protein hydrolysis of their precursors.<sup>31</sup> CAMPs are responsible for modulating the intestinal microbiota and improving bacterial clearance at barrier sites.<sup>29</sup> Due to their positive charge, CAMPs preferentially interact with negatively charged bacterial membranes and form pores via detergent-like effects.<sup>30</sup> A recent study used superresolution single-particle tracking tools to demonstrate CAMP-mediated penetration and rigidification of the bacterial cytoplasm through the electrostatic linking of chromosomal DNA and a subset of ribosomes.<sup>32</sup> CAMPs stimulate mucus synthesis in the gastric mucosa by upregulating the mRNA expression of mucin 1 (MUC1), inhibiting HP growth, disrupting bacterial biofilms, and inducing morphological alterations in the HP membrane.<sup>33</sup> Additionally, they alleviate gastric mucosal infections, reduce HP colonization in the gastric mucosa, and decrease the production of gastric mucosal cytokines.<sup>34</sup>

The relationship between CAMPs and VitD<sub>3</sub> is not fully known and may be related to the following mechanisms: 1) VitD<sub>3</sub> combined with 4-phenylbutyrate results in the activation of functional VDR signaling to trigger the expression of cathelicidin, a secondary messenger for autophagy activation through transcriptional activation of ATGs (autophagy-related) and the enhancement of autophagic flux in human monocytes/macrophages;<sup>35</sup> 2) VitD<sub>3</sub> alone enhances CAMP expression;<sup>36</sup> and 3) in mammalian cells, 1,25-dihydroxyvitamin D<sub>3</sub> stimulates the production of heterodimers of the vitamin D receptor (VDR) and the retinoid X receptor (RXR), which mostly bind to retinoic acid-related orphan receptors  $\alpha$  and  $\gamma$  (ROR $\alpha$  and ROR $\gamma$ ).<sup>37</sup> VDR-RXR coupled with 1 $\alpha$ , 25-dihydroxy vitamin D<sub>3</sub> binds to vitamin D<sub>3</sub> response elements (VDREs) located 59 bp upstream of target genes, acts as a transcription factor, and upregulates the gene expression of various proteins, such as CAMPs (Figure 1).<sup>37</sup>



**Figure 1** VitD<sub>3</sub> inhibits HP by promoting the secretion of antimicrobial peptides (AMPs). Vitamin D<sub>3</sub> alone<sup>36</sup> or in combination with retinoic acid<sup>37</sup> or 4-PBA<sup>35</sup> results in the activation of functional VDR signaling to trigger the expression of CAMP, while VitD<sub>3</sub> upregulates  $\beta$ -defensins by binding to VDR after TLR2/1 or NOD2 signaling activates NF- $\kappa$ B signaling in human monocytes, which ultimately eradicates HP.<sup>38,39</sup> CAMP, cathelicidin antimicrobial peptide; HBDs, human  $\beta$ -defensins; VDR, vitamin D receptor; VDREs, vitamin D<sub>3</sub> response elements; 4-PBA, 4-phenylbutyrate; TLR, Toll-like receptor; NOD2, nucleotide-binding oligomerization domain containing 2; NF- $\kappa$ B, nuclear factor  $\kappa$ B; RXR, retinoid X receptor; ROR, retinoic acid-related orphan receptor; MUC1, mucin1.

## VitD<sub>3</sub> Inhibits HP by Promoting the Secretion of $\beta$ -Defensins

Human  $\beta$ -defensins (HBDs), which are polypeptides with approximately 35 amino acid residues and are primarily expressed in epithelial tissues, act as the first line of defense between people and their surroundings. In the human genome, more than 50 HBD genes have been discovered. Of these, the functions of HBD1, HBD2, HBD3, and HBD4 are the best characterized,<sup>40</sup> with HBD-3 having a particularly strong impact on HP.<sup>41</sup> However, the specific mechanism by which defensins kill their target cells is still unknown. HBD3 has been shown to have chemo-inductive effects on dendritic cells, memory T cells, and mast cells,<sup>40</sup> and the constructive defensin HBD1 and the inducible defensins HBD2 and HBD4 have potent antimicrobial effects against *E. coli* but have no or minimal effects on HP.<sup>42</sup> HBD2 accumulates on the cell surface of HP strains and leads to slight growth inhibition, even though it does not appear to be efficient at reducing the viability of bacteria.<sup>42,43</sup> As recently shown by Cullen et al, the modification of lipid A by removing phosphate groups, which results in a comparably less negative surface charge, may increase the resistance of HP to HBD2.<sup>44</sup> Pero et al demonstrated that the C-terminal domain (RRKK) of HBD3 and the inner domain of HBD1 (PIFTKIQGT) are essential for antimicrobial activity.<sup>45</sup> The removal of six amino acids at the N-terminus of HBD3 did not decrease the activity. Consequently, Pero et al developed an analog that maintained HBD1-mediated killing and was resistant to high NaCl concentrations, similar to HBD3. The activity of this analog (3NI) was not evaluated against HP, and this analog may be a promising tool against HP that can be used to combat infection and reverse DNA methylation.<sup>45</sup> By electrostatically interacting with the negative charge of the microbial plasma membrane, the positive residues in HBDs enter the lipid bilayer through the amphipathic region of the membrane, increasing membrane permeability and killing the microbe.<sup>46,47</sup> However, altering membrane permeability is not the only mechanism by which HBDs kill pathogens. It is possible that HBDs can inhibit the synthesis of RNA, DNA, and proteins and induce the synthesis of cytokines, such as IFN- $\gamma$ , in epithelial cells, which contributes to the elimination of bacteria and viruses. It is also possible that several mechanisms cooperate to cause pathogen death.<sup>48</sup>

The relationship between defensins and VitD<sub>3</sub> is more complicated, and it has been reported that after Toll-like receptor 2/1 (TLR2/1) or nucleotide-binding oligomerization domain containing 2 (NOD2) signaling activates nuclear factor  $\kappa$ B (NF- $\kappa$ B) signaling in human monocytes, VitD<sub>3</sub> increases the abundance of  $\beta$ -defensins by binding to vitamin D response elements in the promoter and enhancer regions of VDR-regulated defensin genes.<sup>38,39</sup> However, the details of the pathway are unclear, and further research is needed (Figure 1).

## Antibacterial Activity of Vitamin D<sub>3</sub> Decomposition Products Against HP

Vitamin D group has been shown to decompose nonbiologically via exposure to high humidity and high temperature.<sup>49</sup> Kouichi et al carried out the nonbiological degradation of vitamin D<sub>3</sub> species and investigated the antibacterial activity of the degraded vitamin D<sub>3</sub> species against HP.<sup>50</sup> Vitamin D<sub>3</sub> was dispersed for 1 week into distilled water warmed at 70°C to obtain abundant amounts of vitamin D<sub>3</sub> decomposition products (VDPs). Then, the VDPs were divided into four fractions by column chromatography, of which VDP fraction-1 had the most pronounced inhibition of HP. VDP fraction-1 was further divided into nine aliquots by column chromatography, and the two aliquots with the most significant HP inhibition were purified and categorized as VDP1, VDP2 and VDP3. VDP3 seems to be structurally unstable and volatilizes easily, as it disappears during purification.<sup>50</sup>

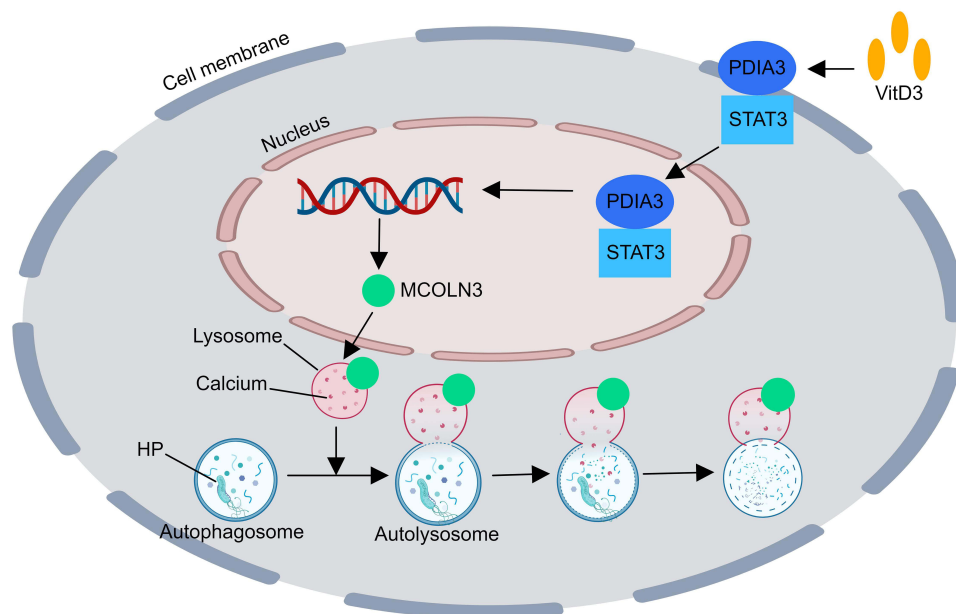
VDP1 and VDP2 have antibacterial effects on HP with free cholesterol (FC), but VDP2 has a weaker effect; however, the antibacterial effect of VDP2 against the FC-retaining HP was completely abolished, whereas VDP1 was unaffected.<sup>50</sup> Phosphatidylethanolamine (PE) is a myristic acid molecule that is the most common glycerophospholipid in the cell membrane of HP; has a high binding affinity for nonesterified steroidal compounds such as free cholesterol, pregnenolone, and progesterone at the carbon-3 position; and plays an important role in the interaction between bacterial cells and steroidal compounds.<sup>51,52</sup> Dimyristoyl PE (DMPE) is one of the most prevalent PE species among HP cell membrane lipids. Among the steroidal compounds, progesterone and its analogs induce the cell lysis of HP by binding to the myristoyl PE of bacterial cells.<sup>51,53</sup> VDP1 binds to the DMPE in the HP membrane, induces leakage from the cell membrane, and ultimately lyses bacterial cells; however, VDP2 has a low binding affinity with DMPE.<sup>50</sup>

The anti-HP effect of VDP1 is extremely selective, and commonplace bacteria can survive even at high concentrations of VDP1.<sup>54</sup> Additionally, VDP1, which contains Grundmann's ketone, is a highly nonpolar ketone.<sup>50</sup> Grundmann's ketone has antimicrobial effects, especially against fungi, gram-positive bacteria, and HP.<sup>55</sup> Furthermore, any cytotoxic side effects from VDP1 are likely to be extremely weak in human cells.<sup>50</sup>

## VitD3 Enhances Lysosomal Degradation of HP via $\text{Ca}^{2+}$ -Dependent Lysosomal Acidification

Autophagy is characterized by the formation of double-membrane vesicles known as autophagosomes. This process is an evolutionarily conserved self-degradation process that is utilized by host cells to maintain cellular homeostasis and protect them from pathogen invasion.<sup>56</sup> Lysosomes are membrane-bound organelles that contain more than 50 different degradative hydro-lases. Autophagosomes mature into autolysosomes after merging with lysosomes, and pathogens trapped inside are destroyed by lysosomal proteases after being identified by the innate immune system,<sup>57</sup> but this process requires an acidic environment in the lysosomal lumen.<sup>58</sup> The autophagy pathway was formerly thought to be a host defense mechanism against infections, but recent research has shown that bacteria are capable of disrupting this process and actively replicating themselves within cells.<sup>59</sup> Initially, HP was thought to be a noninvasive pathogen attached to the surface of gastric epithelial cells; however, in the last decade or so, during HP infections, the pathogen was found to invade the cells,<sup>60</sup> induce autophagy in several gastric cell lines,<sup>61</sup> and cause an abnormal accumulation of  $\text{Ca}^{2+}$  in the lysosomes of its infected cells; in these cases, lysosomal acidification is impaired, which prevents HP from being degraded in lysosomes, allowing it to continue to multiply and persist in infections.<sup>62</sup>

Hu et al demonstrated that vitamin D restores the impaired lysosomal activity of epithelial cells by stimulating the protein disulfide isomerase family A member 3 (PDIA3) receptor, leading to the redistribution of the PDIA3-STAT3 (signal transducer and activator of transcription 3) complex into the nucleus and thereby enhancing the production of the mucolipin 3 (MCOLN3) protein.<sup>62</sup> STAT3 is a transcription factor that relays signals from activated cytokine and growth factor receptors in the plasma membrane to the nucleus, where it regulates gene transcription,<sup>63</sup> which leads to the upregulation of MCOLN3 protein expression in a promoter-specific manner in HFE145 cells.<sup>62</sup> Mucolipins (MCOLNs), a subfamily of transient receptor potential channels, are predicted to encode  $\text{Ca}^{2+}$  channels that predominantly function in endolysosomal vesicles.<sup>64</sup> The MCOLN3 channel is predominantly expressed on late endosomal and lysosomal membranes (>75%).<sup>64</sup>  $\text{Ca}^{2+}$  release from endolysosomes via the MCOLN3 channel is necessary for lysosomal acidification and maturation.<sup>64,65</sup> Upregulation of the MCOLN3 channel by VitD3 is required for  $\text{Ca}^{2+}$  release from lysosomes and consequently normalizes lysosomal acidification. A highly acidic environment ( $\text{pH} < 5.0$ ) in the lumen is extremely important for the lysosome to execute its digestive action and to export recycled cargos. The hydrolytic enzymes within lysosomes are also activated under acidic pH conditions.<sup>66</sup> The restoration of the acidic environment of lysosomes damaged by HP infection increased HP degradation by lysosomes (Figure 2).



**Figure 2** The mechanism by which VitD3 enhances the lysosomal degradation of HP via  $\text{Ca}^{2+}$ -dependent lysosomal acidification.<sup>62</sup> The membrane receptor PDIA3 is activated by VitD3 therapy, which also causes the PDIA3-STAT3 complex to relocate into the nucleus and upregulates MCOLN3 protein expression. This process restores  $\text{Ca}^{2+}$  release from lysosomes and causes lysosomal acidification. Lysosomes eliminate HP to restore the acidity of the environment. PDIA3, protein disulfide isomerase family A member 3; STAT3, signal transducer and activator of transcription 3; MCOLN3, mucolipin 3.



## Conclusions

In summary, the role of VitD3 in the eradication of HP is certain and includes its classical pathway of binding to VDR to regulate the body's immune defense and increase the secretion of AMPs, while the nonclassical pathway is a novel signaling pathway that initiates activation of the PDIA3-STAT3-MCOLN3-Ca<sup>2+</sup> axis to reactivate the acidification and degradation of lysosomes and the collapse of the membrane structure of HP cells via degradation products. VitD3 represents a novel approach and a new option for the eradication of HP, but there are still some pathways or mechanisms that remain unclear and need further in-depth research and development.

## Disclosure

The authors report no conflicts of interest in this work.

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