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Review

Vitamin D deficiency and the pathogenesis of Crohn's disease

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ABSTRACT

Vitamin D has emerged as a key regulator of innate immune responses to pathogen threat. The hormonal form of vitamin D signals through a nuclear receptor transcription factor and regulates gene transcription. Several papers have shown that vitamin D signaling is active both upstream and downstream of pattern recognition receptors, vanguards of innate immune responses. Crohn's disease (CD) is a relapsing-recurring inflammatory bowel disease (IBD) that arises from dysregulated intestinal innate immunity. Indeed, genetic studies have identified several CD susceptibility markers linked to mechanisms of innate immune responses to infection. Interest in links between vitamin D deficiency and CD has grown substantially, particularly in the last five years. While a number of studies have consistently revealed an association between CD and vitamin D deficiency, recent experimental work has uncovered a compelling mechanistic basis for the contribution of vitamin D deficiency to the pathogenesis of the disease. Moreover, a number of intervention trials have provided generally solid evidence that robust vitamin D supplementation may be of therapeutic benefit to patients with CD. This review summarizes these laboratory and clinical findings.

Contents

1. Overview

There has been growing interest, particularly since 2011, in links between incidence of Crohn's Disease (CD) and vitamin D deficiency (Supplementary Fig. 1). Although it was originally thought that the vitamin D deficiency associated with Crohn's was merely a product of the disease because of malabsorption, as detailed below, an expanding series of laboratory findings have suggested that deficiency may also contribute to pathogenesis of CD. These results further imply that adequate vitamin D supplementation may be of therapeutic benefit in treatment of patients with established disease. Indeed, results of initial clinical studies presented herein suggest that supplementation is of benefit in disease management.

2. Crohn's disease

Crohn's disease is a relapsing-recurring chronic inflammatory bowel condition, which may affect any part of the gastrointestinal tract, and can be accompanied by abdominal pain, diarrhea, bowel obstruction and weight loss. It is also associated with increased

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risk of colon cancer. While CD is widely considered to be an autoimmune disease, auto-antibodies have not been detected, and it has been argued that its symptoms are more likely arise from a dysregulation of intestinal innate immunity [1,2]. Indeed, genome-wide association (GWAS) studies have provided an extensive list of CD susceptibility markers, many of which are linked to mechanisms of innate immune responses to infection [3]. It is likely that a combination of environmental cues and infection triggers the full manifestation of the disease. Notably, CD has been linked to low sun exposure [4], and within North America and Europe the incidence of both CD rises with increasing latitude [5–7]. Within the U.S., place of residence at age 30 is strongly associated with risk [5], and further north in Canada the rates of IBD are among the highest in the world and are rising [8]. In this sense, observations of CD risk are consistent with other long-standing links between sun exposure, human health, and resistance to disease [9–13].

3. Vitamin D

Vitamin D, which can be obtained from limited dietary sources or from supplementation, is, strictly speaking, not a vitamin as it can also be produced by exposure of skin to solar ultraviolet B (UVB) rays. In the presence of adequate solar UVB, the last intermediate in cholesterol biosynthesis, 7-dehydrocholesterol, undergoes a photochemical and thermal conversion to produce secosteroidal vitamin D3 [14]. However, in temperate regions during the fall and winter much or all of the UVB radiation is absorbed by the oblique passage of the sun's rays through the ozone layer. During this period, known as vitamin D winter, which can last much of the year in northern locations such as Scandinavian countries, surface penetration of UVB is inadequate for vitamin D synthesis [14]. Thus, levels of circulating vitamin D metabolites vary seasonally and deficiency can develop during vitamin D winter in the absence of adequate dietary sources or supplementation.

Vitamin D obtained from dietary or cutaneous routes is not biologically active and must undergo two sequential modifications; (i) largely hepatic 25-hydroxylation to produce the major circulating metabolite, 25-hydroxyvitamin D (25D), and (ii) 1α-hydroxylation in peripheral tissue catalyzed by the enzyme CYP27B1 to produce hormonally active 1,25-dihydroxyvitamin D (1,25D) [15,16]. 1,25D thus produced binds to and activates the vitamin D receptor (VDR). The VDR is a nuclear receptor and ligand-regulated transcription factor [15], and many of the physiological actions of vitamin D can be understood via the capacity of 1,25D to induce VDR binding to DNA and regulation of the expression of specific target genes. As discussed below, both expression profiling studies to identify 1,25D-regulated genes and genome-wide analysis of VDR binding sites have provided links between vitamin D signaling and CD.

Vitamin D was discovered as the cure for nutritional rickets and is best known as a critical regulator of calcium homeostasis and skeletal health [16]. Indeed, CYP27B1 expression in the kidneys is induced by parathyroid hormone in response to a drop in circulating Ca++ concentrations, and renal production of 1,25D has long been considered to be the major source of circulating, i.e. endocrine, 1,25D. However, there has been somewhat of a paradigm shift in recent years in our understanding of the roles of vitamin D in human physiology, as well as its metabolism and its endocrine function. This started with the observation that CYP27B1 is widely expressed [17], in particular in tissues unrelated to calcium homeostasis, suggesting the 1,25D may be synthesized and catabolized locally. Similarly, the VDR is widely expressed and, importantly for the purposes of this review, both CYP27B1 and the receptor are produced throughout the immune system [18,19]. As described below, experiments in the last 10 years or so have shown that vitamin D metabolism and signaling play key roles in immune system function. Because of its relevance to the etiology of CD we will focus on the role of vitamin D in regulation of innate immunity.

4. Vitamin D metabolism and innate immunity

Perhaps the most critical finding providing evidence that 1,25D signaling contributes to innate immune responses came from the group of Robert Modlin, who showed in 2006 that stimulation of macrophages through the pattern recognition receptor toll-like

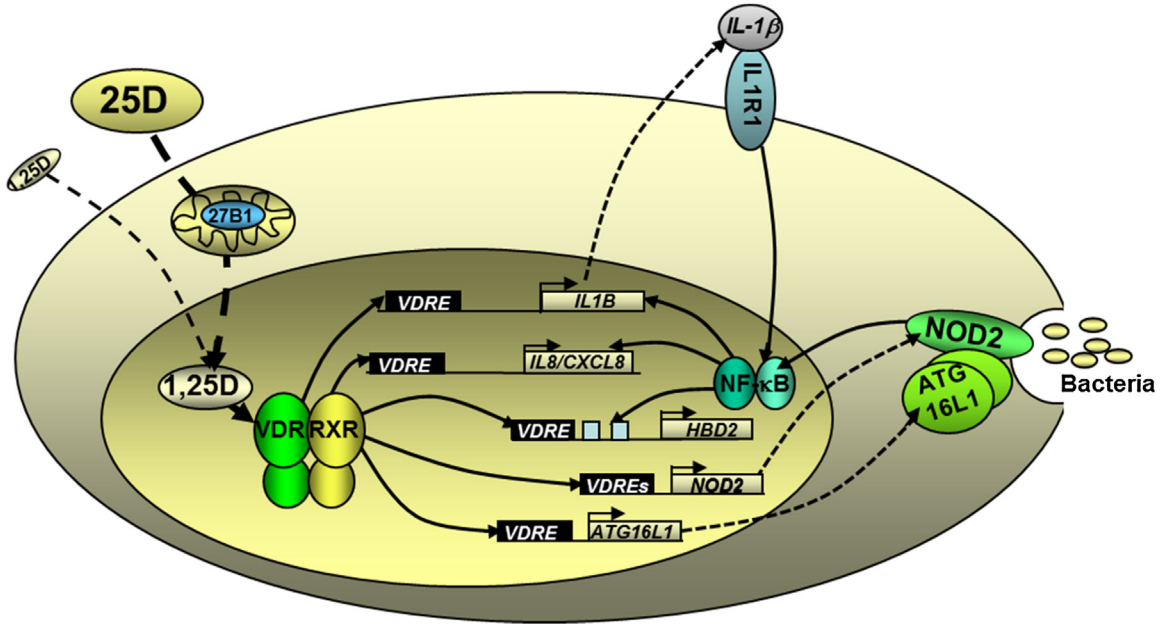

Fig. 1. Schematic representation of the network of genes relevant to Crohn's disease regulated by signaling by the hormone-bound VDR and its downstream target *NOD2/CARD15*, which encodes the pattern recognition receptor NOD2. See text and associated references for details.

receptor 2 (TLR2) leads to expression of CYP27B1 and endogenous production of 1,25D from circulating levels of 25D [20,21]. Pattern recognition receptors can be transmembrane or intracellular proteins that detect the presence of infection either by recognizing pathogen-specific molecules (patterns) or more common molecular species such as nucleic acids in inappropriate locations (e.g. endosomal RNA) [22]. The observations of Modlin and collaborators followed up on findings from my own group [23] and that of Adrian Gombart [24] that the 1,25D-bound VDR is a direct inducer of two human genes encoding antimicrobial peptides LL-37 (*CAMP*; cathelicidin antimicrobial peptide) and human beta defensin 2 (*DEFB4/HBD2*), and incubation with 1,25D leads to release of antimicrobial activity from treated cells. Collectively, these findings were very important because they showed that local 1,25D synthesis and signaling could occur as part of an innate immune response to pathogen, leading to antimicrobial peptide release, and, moreover, that 1,25D production in the immune system was controlled by non-calcium homeostatic inputs. Indeed, it is now known that *CYP27B1* gene expression in myeloid cells can be regulated by a complex cytokine network [25].

5. Links between 1,25D-regulated gene transcription and Crohn's disease

Subsequent work has shown that the activated VDR stimulates the transcription of several genes implicated in innate immune signaling. Interleukin 1β (IL-1β) is one of the primary cytokines produced by the innate immune system in response to pathogen threat. Transcription of the *IL1B* gene and subsequent secretion of its protein product is cooperatively induced by a combination of 1,25D and infection of macrophages [26]. This study showed that the *IL1B* gene is a direct target of the VDR. The primary translation product of IL-1β undergoes maturation by proteolytic cleavage catalyzed by caspase 1 associated with a complex called the inflammasome. Several variations of the inflammasome exist, which are generally regulated through association with different types of pattern recognition receptors [27], one of which is encoded by the *NLRP3* (NLR, pyrin-domain containing 3) gene. NLRP3 is one of the primary receptors activated in response to microbial invasion and other danger signals [27,28]. Notably, in the context of Crohn's disease, genetic studies using a candidate gene approach provided evidence that common variants in the region of the *NLRP3* gene contribute to CD susceptibility [29].

More compelling evidence came from the discovery that the gene encoding the pattern recognition receptor NOD2 (nucleotide oligomerization domain protein 2; a.k.a. CARD15, IBD1) is a direct target of the VDR [30]. This is noteworthy for multiple reasons (see Fig. 1). Most importantly, genetic studies of CD susceptibility revealed that mutations in the *NOD2* gene disrupting the pattern recognition domain contribute strongly to development of the disease [31,32]. Binding to NOD2 by its ligand, muramyl dipeptide (MDP), a lysosomal breakdown product of bacterial peptidoglycan, leads to activation of the transcription factor NF-κB. NF-κB in turn activates the transcription of the gene encoding the antimicrobial peptide *DEB4/HBD2*, which is also a CD susceptibility locus [33]. The region on chromosome 8 encoding DEFB4 has undergone a series of gene-duplication events, and exhibits a wide variation in copy number among healthy individuals (median number of 4). Analysis of CD patients revealed that in subjects with colonic, but not ileal disease, 3 copies or fewer was significantly associated with increased risk of developing CD [33]. *DEFB4/HBD2* is the same antimicrobial peptide gene shown previously to be a direct target of 1,25D signaling [23], and incubation of cells with 1,25D to induce NOD2 followed by addition of MDP in the presence of 1,25D led to synergistic induction of *DEFB4/HBD2* gene transcription. This effect was completely absent in cells from CD patients homozygous for inactivating mutations in the pattern recognition domain of NOD2 [30]. Thus, 1,25D signaling activated both ends of the NOD2-DEFB4 innate immune pathway associated with CD susceptibility.

The induction of *NOD2* gene expression by 1,25D is also significant because its protein function has been linked to autophagy [34,35]. Autophagy is a process characterized by the generation of autophagosomes, which target several intracellular pathogens, as well as other damaged organelles and proteins for degradation in lysosomes. Notably, other CD susceptibility loci *ATG16L1* and *IRGM* encode proteins required for autophagy [36–38]. Importantly, low intestinal VDR expression is associated with abnormal Paneth cells and reduced production of ATG16L1, and a recent study found that the *ATG16L1* gene is also a target of the VDR target [39]. The induction of *NOD2* and *ATG16L1* gene expression by 1,25D is made even more compelling by the observation that the two gene products are functionally linked, as NOD2 direct can recruit ATG16L1 to the plasma membrane at the site of bacterial entry [40] (see also Fig. 1). These findings are important because they link vitamin D signaling directly to a process whose deficiency can increase the risk of development of CD. They are also consistent previous work showing that 1,25D can stimulate autophagy [41,42], in part through induced expression of the antimicrobial peptide CAMP [43]. Intriguingly, a recent, comprehensive GWAS study of two cohorts comprising 1812 individuals linked variants of the *VDR* gene locus to alterations in the human gut microbiome [44]. As part of this study, the *VDR* was observed to be upregulated in colonic biopsies of IBD patients, including those with CD. The study also provided evidence for common genetic variants, including the *VDR*, across phenotypes such as body-mass index (BMI), CD, and variations in the intestinal microbiome [44].

The links between vitamin D signaling and the etiology of CD have been further enforced by the use of genome-wide approaches to identify VDR binding sites by immunoprecipitation of receptor-DNA complexes from chemically cross-linked cells (chromatin immunoprecipitation; ChIP) followed by high-throughput DNA sequencing (ChIPseq). The first study of this kind was carried out in human lymphoblastoid cells [45]. It is of interest because of its efforts to link VDR binding sites with genetic loci associated with human disease. The study found that VDR binding sites were significantly associated with loci associated with several inflammatory immune-related disorders, including CD. Notably, a strong, intronic VDR binding site was identified in the *PTPN2* gene [45], a locus strongly associated with CD [46]. PTPN2 (a.k.a. IBD21) encodes a non-receptor protein tyrosine phosphatase, whose expression is induced by exposure of cells to RNA of viral origin [47], thus implicating regulating of the gene in innate immune responses to pathogen threat. Further work of this kind in myeloid cells provided evidence that 1,25D regulates a cluster of cytokine genes that include *CXCL8/IL8* [48,49]. This is relevant to CD because other findings have revealed that induction of the *IL8/CXCL8* gene and that encoding IL-1β, another target of the VDR [26], also lie downstream of NOD2 signaling [50]. Taken together, the studies cited above reveal that the hormone-bound VDR through its stimulation of *NOD2* transcription regulates directly and indirectly a network of genes relevant to innate immune signaling in Crohn's disease (summarized in Fig. 1).

6. Clinical studies examining vitamin D deficiency, supplementation and Crohn's disease

Associations between CD and vitamin D deficiency have been well-established for a number of years. It was often assumed that the deficiency was the product of the disease due to (a combination of) intestinal malabsorption of dietary vitamin D [51], or lack of sun exposure in cases of active disease, particularly in patients living at

higher latitudes. However, the above laboratory findings provide strong evidence that vitamin D deficiency may contribute to the pathogenesis of the CD, and that, conversely, sufficient vitamin D supplementation may boost innate immunity and suppress inflammation and CD symptoms. Since 2010, a number of clinical studies have provided evidence that robust vitamin D supplementation may be of therapeutic benefit for patients with CD.

The results of a large prospective cohort study of 72,719 women enrolled in the Nurses' Health Study were published in 2011 [52]. These women were followed from 1986 through 2008, a period during which 122 cases of CD were documented. Circulating 25D levels were assessed based on a combination of a lifestyle survey and by direct measurement of plasma 25D. 25D levels were broken into quartiles with the lowest and highest medians being 22.3 and 32.2 ng/mL, respectively (~55.8 and 80.5 nM). The study found that for women with a predicted 25D level >30 ng/mL (75 nM) the multivariate-adjusted hazard ratio was 0.38 (95% CI, 0.15–0.97) when compared with women with predicted levels of less than 20 ng/mL (50 nM). The authors concluded that higher predicted levels of circulating 25D significantly reduced the risk of CD in this female population. Similarly, a retrospective cohort study of 403 patients with CD concluded that vitamin D deficiency was common in the patient population and was independently associated with lower health-related quality of life (HRQOL) and greater disease activity [53]. A retrospective study in a pediatric population reached similar conclusions concerning CD and disease activity; 47% of the children were found to vitamin D deficient or insufficient and vitamin D deficiency was associated with greater corticosteroid exposure in this population [54]. High levels of vitamin D insufficiency/deficiency were also found in a population of children newly diagnosed with IBD and it was concluded that serum vitamin D level is significantly lower than in healthy children [55]. However, it should be noted that in this type of a population vitamin D deficiency may have been caused by malabsorption and lack of physical activity because of active disease prior to presentation in the clinic. An analysis of vitamin D status and markers of inflammation and disease activity and in CD patients found a significant inverse correlation between 25D concentrations with intestinal inflammation as determined by fecal calprotectin measurement [56]. This association held for patients in clinical remission, but not for those with active CD. In addition, 25D levels did not correlate with disease activity score or systemic inflammation as measured by circulating C-reactive protein in this group. Finally, another study conducted on a multi-institutional cohort of 2809 patients with IBD concluded that vitamin D deficiency (<20 ng/mL or <50 nM) was associated with an increased risk of malignancy, and that each increment of 1 ng/mL in 25D was associated with an 8% reduction in risk of developing colorectal cancer [57].

While the above data generally support a role for vitamin D sufficiency in reducing CD incidence and disease activity (and the associated increase of colorectal cancer), the most compelling results in this regard come from intervention trials. One treatment center observed a significant association between the vitamin D supplementation ($P = 0.02$), circulating 25D ($P < 0.05$), and quiescent disease activity in a pediatric IBD population [58]. Similarly, in a US military veterans population with IBD vitamin D3 supplementation was associated with lower use of use of laboratory, pharmacy, radiology and other fee-based services [59]. In a multivariable analysis of a cohort of >1500 CD patients, 25D levels of <20 ng/mL (50 nM) were associated with a statistically significantly increased risk of both surgery and IBD-related hospitalization compared with patients with 25D levels of >30 ng/mL. More interestingly, patients with CD with low 25D levels normalized by supplementation also had reduced rates of surgery compared with those who were untreated [60].

The gold-standard in judging potential therapeutic efficacy is the double-blind placebo-controlled trial. Two such studies have been published to date. In a trial published in 2010 [61], 94 patients with CD in remission were randomized to receive either 1200 IU/day for 12 months. This protocol led to a significant increase in circulating 25D levels and a reduction in the relapse rate from 14/48 in the placebo group to 6/46 in the treatment group, just outside the limit of statistical significance ($p < 0.06$). In another small-scale double-blind randomised placebo-controlled trial, 27 CD patients in remission were assigned to 2000 IU/day of vitamin D or placebo for a period of 3 months [62]. The vitamin D dose was sufficient to significantly increase 25D levels in this population. Several parameters were measured, and treatment was associated with increased circulating levels of the antimicrobial peptide LL-37 (CAMP), and maintenance of intestinal permeability (whereas it increased in the placebo group). In addition, for CD patients achieving at least 75 nmol/L 25D levels, treatment was significantly associated with higher quality of life scores with a non-significant reduction in Crohn's Disease Activity Index (CDAI) scores [62].

7. Conclusions

In conclusion, a range of laboratory studies has provided strongly supportive evidence that deficient vitamin D signaling may contribute to the pathogenesis of CD. The results of clinical interventions are very promising, but limited, largely because of the size and number of double-blind placebo-controlled trials performed to date. Vitamin D supplementation is inexpensive, safe, and does not require regular intervention of a physician, making its potential therapeutic use even more compelling. Moreover, vitamin D insufficiency/deficiency is common in CD patients, and initial results suggest that placebo-controlled trials do not have to be conducted over extended periods of time, which facilitates implementation and the analysis. Thus, the combined preclinical and clinical data provide strong support for conducting large-scale double-blind placebo-controlled intervention trials to test the therapeutic efficacy of vitamin D supplementation in CD. These trials will have to use sufficiently robust levels of supplementation and take into account possible malabsorption in a CD patient population; e.g. 2000–4000 IU/day, or possibly higher in patients with malabsorption [63]. It would also be important to test different doses of vitamin D supplements to determine the range that optimizes clinical efficacy and to take into account the different age groups (e.g. pediatric vs adult) suffering from the disease.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2016.12.015.

References

[1] F. Coulombe, M.A. Behr, Crohn's disease as an immune deficiency? Lancet 374 (2009) 769–770.
[2] D.J. Marks, Defective innate immunity in inflammatory bowel disease: a Crohn's disease exclusivity? Curr. Opin. Gastroenterol. 27 (2011) 328–334.
[3] D.P.B. McGovern, S. Kugathasan, J.H. Cho, Genetics of inflammatory bowel diseases, Gastroenterology 149 (1163–1176) (2015) e1162.
[4] P. Jantchou, F. Clavel-Chapelon, A. Racine, M. Kvaskoff, F. Carbonnel, M.-C. Boutron-Ruault, High residential sun exposure is associated with a low risk of incident Crohn's disease in the prospective E3N cohort, Inflamm. Bowel Dis. 20 (2014) 75–81.
[5] H. Khalili, E.S. Huang, A.N. Ananthakrishnan, L. Higuchi, J.M. Richter, C.S. Fuchs, A.T. Chan, Geographical variation and incidence of inflammatory bowel disease among US women, Gut 61 (2012) 1686–1692.

[6] W.C. Lim, S.B. Hanauer, Y.C. Li, Mechanisms of disease: vitamin D and inflammatory bowel disease, Nat. Clin. Pract. Gastroenterol. Hepatol. 2 (2005) 308–315.
[7] E.V. Loftus, Clinical epidemiology of inflammatory bowel disease: incidence, prevalence, and environmental influences, Gastroenterology 126 (2004) 1504–1517.
[8] C.N. Bernstein, A. Wajda, L.W. Svenson, A. MacKenzie, M. Koehoorn, M. Jackson, R. Fedorak, D. Israel, J.F. Blanchard, The epidemiology of inflammatory bowel disease in Canada: a population-based study, Am. J. Gastroenterol. 101 (2006) 1559–1568.
[9] A.R. Masten, Sunlight in tuberculosis, Chest 1 (1935) 8–23.
[10] M.F. Holick, High prevalence of Vitamin D inadequacy and implications for health, Mayo Clin. Proc. 81 (2006) 353–373.
[11] I.A.F. van der Mei, A.L. Ponsonby, T. Dwyer, L. Blizzard, R. Simmons, B.V. Taylor, H. Butzkueven, T. Kilpatrick, Past exposure to sun, skin phenotype, and risk of multiple sclerosis: case-control study, Br. Med. J. 327 (2003) 316–320.
[12] A. Juzeniene, L.W. Ma, M. Kwitniewski, G.A. Polev, Z. Lagunova, A. Dahlback, J. Moan, The seasonality of pandemic and non-pandemic influenzas: the roles of solar radiation and vitamin D, Int. J. Infect. Dis. 14 (2010) E1099–E1105.
[13] E. Giovannucci, Y. Liu, E.B. Rimm, B.W. Hollis, C.S. Fuchs, M.J. Stampfer, W.C. Willett, Prospective study of predictors of vitamin D status and cancer incidence and mortality in men, J. Natl. Cancer Inst. 98 (2006) 451–459.
[14] L. Tavera-Mendoza, J.H. White, Cell defenses and the sunshine vitamin, Sci. Am. 297 (2007) 62–72.
[15] R. Lin, J.H. White, The pleiotropic actions of vitamin D, BioEssays 26 (2004) 21–28.
[16] M.F. Holick, Vitamin D deficiency, N. Engl. J. Med. 357 (2007) 266–281.
[17] D. Zehnder, R. Bland, M.C. Williams, R.W. McNinch, A.J. Howie, P.M. Stewart, M. Hewison, Extrarenal expression of 25-Hydroxyvitamin D3-1α-Hydroxylase, J. Clin. Endocrinol. Metab. 86 (2001) 888–894.
[18] M. Hewison, Vitamin D and the immune system: new perspectives on an old theme, Endocrinol. Metab. Clin. North Am. 39 (2010) 365–379.
[19] J.H. White, Vitamin D metabolism and signaling in the immune system, Rev. Endocr. Metab. Disord. 13 (2012) 21–29.
[20] P.T. Liu, S. Stenger, H. Li, L. Wenzel, B.H. Tan, S.R. Krutzik, M.T. Ochoa, J. Schauber, K. Wu, C. Meinken, et al., Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response, Science 311 (2006) 1770–1773.
[21] P.T. Liu, S.R. Krutzik, R.L. Modlin, Therapeutic implications of the TLR and VDR partnership, Trends Mol. Med. 13 (2007) 117–124.
[22] T. Kawasaki, T. Kawai, Toll-like receptor signaling pathways, Front. Immunol. 5 (2014).
[23] T. Wang, F. Nestel, V. Bourdeau, Y. Nagai, Q. Wang, J. Liao, L. Tavera-Mendoza, R. Lin, J. Hanrahan, S. Mader, et al., Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression, J. Immunol. 173 (2004) 2909–2912.
[24] A. Gombart, N. Borregaard, H. Koeffler, Human cathelicidin antimicrobial peptide (CAMP) gene is a direct target of the vitamin D receptor and is strongly up-regulated in myeloid cells by 1,25-dihydroxyvitamin D3, FASEB J. 19 (2005) 1067–1077.
[25] R.F. Chun, P.T. Liu, R.L. Modlin, J.S. Adams, M. Hewison, Impact of vitamin D on immune function: lessons learned from genome-wide analysis, Front. Physiol. 5 (2014).
[26] M. Verway, M. Bouttier, T.-T. Wang, M. Carrier, M. Calderon, B.-S. An, E. Devemy, F. McIntosh, M. Divangahi, M.A. Behr, et al., Vitamin D induces interleukin-1 beta expression: paracrine macrophage epithelial signaling controls M-tuberculosis infection, PLoS Pathog. 9 (2013).
[27] K. Schroder, J. Tschopp, The inflammasomes, Cell 140 (2010) 821–832.
[28] L. Franchi, R. Munoz-Planillo, G. Nunez, Sensing and reacting to microbes through the inflammasomes, Nat. Immunol. 13 (2012) 325–332.
[29] A.C. Villani, M. Lemire, G. Fortin, E. Louis, M.S. Silverberg, C. Collette, N. Baba, C. Libioulle, J. Belaiche, A. Bitton, et al., Common variants in the NLRP3 region contribute to Crohn's disease susceptibility, Nat. Genet. 41 (2009) 71–76.
[30] T.-T. Wang, B. Dabbas, D. Laperriere, A.J. Bitton, H. Soualhine, L.E. Tavera-Mendoza, S. Dionne, M.J. Servant, A. Bitton, E.G. Seidman, et al., Direct and indirect induction by 1,25-Dihydroxyvitamin D3 of the NOD2/CARD15-defensin β2 innate immune pathway defective in crohn disease, J. Biol. Chem. 285 (2010) 2227–2231.
[31] J.-P. Hugot, M. Chamaillard, H. Zouali, S. Lesage, J.-P. Cezard, J. Belaiche, S. Almer, C. Tysk, C.A. O'Morain, M. Gassull, et al., Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease, Nature 411 (2001) 599–603.
[32] Y. Ogura, N. Inohara, A. Benito, F.F. Chen, S. Yamaoka, G. Núñez, Nod2, a nod1/Apaf-1 family member that is restricted to monocytes and activates NF-κB, J. Biol. Chem. 276 (2001) 4812–4818.
[33] K. Fellermann, D.E. Stange, E. Schaeffeler, H. Schmalzl, J. Wehkamp, C.L. Bevins, W. Reinisch, A. Teml, M. Schwab, P. Lichter, et al., A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to crohn disease of the colon, Am. J. Hum. Genet. 79 (2006) 439–448.
[34] R. Cooney, J. Baker, O. Brain, B. Danis, T. Pichulik, P. Allan, D.J.P. Ferguson, B.J. Campbell, D. Jewell, A. Simmons, NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation, Nat. Med. 16 (2010) 90–97.
[35] M. Verway, M.A. Behr, J.H. White, Vitamin D, NOD2, autophagy and Crohn's disease, Expert Rev. Clin. Immunol. 6 (2010) 505–508.
[36] J. Hampe, A. Franke, P. Rosenstiel, A. Till, M. Teuber, K. Huse, M. Albrecht, G. Mayr, F.M. De La Vega, J. Briggs, et al., A genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in ATG16L1, Nat. Genet. 39 (2007) 207–211.
[37] J.D. Rioux, R.J. Xavier, K.D. Taylor, M.S. Silverberg, P. Goyette, A. Huett, T. Green, P. Kuballa, M.M. Barmada, L.W. Datta, et al., Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis, Nat. Genet. 39 (2007) 596–604.
[38] M. Parkes, J.C. Barrett, N.J. Prescott, M. Tremelling, C.A. Anderson, S.A. Fisher, R. G. Roberts, E.R. Nimmo, F.R. Cummings, D. Soars, et al., Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility, Nat. Genet. 39 (2007) 830–832.
[39] J. Sun, VDR/vitamin D receptor regulates autophagic activity through ATG16L1, Autophagy 12 (2016) 1057–1058.
[40] L.H. Travassos, L.A.M. Carneiro, M. Ramjeet, S. Hussey, Y.-G. Kim, J.G. Magalhaes, L. Yuan, F. Soares, E. Chea, L. Le Bourhis, et al., Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry, Nat. Immunol. 11 (2010) 55–62.
[41] M. Hoyer-Hansen, M. Jaattela, AMP-activated protein kinase – a universal regulator of autophagy? Autophagy 3 (2007) 381–383.
[42] M. Hoyer-Hansen, M. Jaattela, Connecting endoplasmic reticulum stress to autophagy by unfolded protein response and calcium, Cell Death Differ. 14 (2007) 1576–1582.
[43] J.M. Yuk, D.M. Shin, H.M. Lee, C.S. Yang, H.S. Jin, K.K. Kim, Z.W. Lee, S.H. Lee, J.M. Kim, E.K. Jo, Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin, Cell Host Microbe 6 (2009) 231–243.
[44] J. Wang, L.B. Thingholm, J. Skieceviciene, P. Rausch, M. Kummen, J.R. Hov, F. Degenhardt, F.-A. Heinsen, M.C. Ruhlemann, S. Szymczak, et al., Genome-wide association analysis identifies variation in vitamin D receptor and other host factors influencing the gut microbiota, Nat. Genet. (2016) (advance online publication).
[45] S.V. Ramagopalan, A. Heger, A.J. Berlanga, N.J. Maugeri, M.R. Lincoln, A. Burrell, L. Handunnetthi, A.E. Handel, G. Disanto, S.-M. Orton, et al., A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution, Genome Res. 20 (2010) 1352–1360.
[46] J.C. Barrett, S. Hansoul, D.L. Nicolae, J.H. Cho, R.H. Duerr, J.D. Rioux, S.R. Brant, M.S. Silverberg, K.D. Taylor, M.M. Barmada, et al., Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease, Nat. Genet. 40 (2008) 955–962.
[47] M.L. Colli, F. Moore, E.N. Gurzov, F. Ortis, D.L. Eizirik, MDA5 and PTPN2, two candidate genes for type 1 diabetes, modify pancreatic β-cell responses to the viral by-product double-stranded RNA, Hum. Mol. Genet. 19 (2010) 135–146.
[48] S. Heikkinen, S. Väisänen, P. Pehkonen, S. Seuter, V. Benes, C. Carlberg, Nuclear hormone 1α,25-dihydroxyvitamin D3 elicits a genome-wide shift in the locations of VDR chromatin occupancy, Nucleic Acids Res. 39 (2011) 9181–9193.
[49] J. Ryynänen, C. Carlberg, Primary 1,25-Dihydroxyvitamin D_3 response of the interleukin 8 gene cluster in human monocyte- and macrophage-Like cells, PLoS One 8 (2013) e78170.
[50] J. Li, T. Moran, E. Swanson, C. Julian, J. Harris, D.K. Bonen, M. Hedl, D.L. Nicolae, C. Abraham, J.H. Cho, Regulation of IL-8 and IL-1β expression in Crohn's disease associated NOD2/CARD15 mutations, Hum. Mol. Genet. 13 (2004) 1715–1725.
[51] A.D. Harries, R. Brown, R.V. Heatley, L.A. Williams, S. Woodhead, J. Rhodes, Vitamin D status in Crohn's disease: association with nutrition and disease activity, Gut 26 (1985) 1197–1203.
[52] A.N. Ananthakrishnan, H. Khalili, L.M. Higuchi, Y. Bao, J.R. Korzenik, E.L. Giovannucci, J.M. Richter, C.S. Fuchs, A.T. Chan, Higher predicted vitamin D status is associated with reduced risk of Crohn's disease, Gastroenterology 142 (2012) 482–489.
[53] A. Ulitsky, A.N. Ananthakrishnan, A. Naik, S. Skaros, Y. Zadvornova, D.G. Binion, M. Issa, Vitamin D deficiency in patients with inflammatory bowel disease: association with disease activity and quality of life, JPEN J. Parenter. Enter. Nutr. 35 (2011) 308–316.
[54] A.D. Levin, V. Wadhera, S.T. Leach, H.J. Woodhead, D.A. Lemberg, A. Czarina Mendoza-Cruz, A.S. Day, Vitamin D deficiency in children with inflammatory bowel disease, Dig. Dis. Sci. 56 (2011) 830–836.
[55] W. El-Matary, S. Sikora, D. Spady, Bone mineral density, vitamin D, and disease activity in children newly diagnosed with inflammatory bowel disease, Dig. Dis. Sci. 56 (2011) 825–829.
[56] T. Raftery, M. Merrick, M. Healy, N. Mahmud, C. O'Morain, S. Smith, D. McNamara, M. O'sullivan, Vitamin D status is associated with intestinal inflammation as measured by fecal calprotectin in Crohn's disease in clinical remission, Dig. Dis. Sci. 60 (2015) 2427–2435.
[57] A.N. Ananthakrishnan, S.C. Cheng, T. Cai, A. Cagan, V.S. Gainer, P. Szolovits, S.Y. Shaw, S. Churchill, E.W. Karlson, S.N. Murphy, et al., Association between reduced plasma 25-hydroxy vitamin D and increased risk of cancer in patients with inflammatory bowel diseases, Clin. Gastroenterol. Hepatol. 12 (2014) 821–827.
[58] C.M. Samson, P. Morgan, E. Williams, L. Beck, R. Addie-Carson, S. McIntire, A. Booth, E. Mendez, C. Luzader, G. Tomer, et al., Improved outcomes with quality improvement interventions in pediatric inflammatory bowel disease, J. Pediatr. Gastroenterol. Nutr. 55 (2012) 679–688.
[59] D. Youssef, B. Bailey, A. Atia, A. El-Abbassi, T. Manning, A.N. Peiris, Differences in outcomes between cholecalciferol and ergocalciferol supplementation in

veterans with inflammatory bowel disease, Geriatr. Gerontol. Int. 12 (2012) 475–480.
[60] A.N. Ananthakrishnan, A. Cagan, V.S. Gainer, T. Cai, S.-C. Cheng, G. Savova, P. Chen, P. Szolovits, Z. Xia, P.L. De Jager, et al., Normalization of plasma 25-hydroxy Vitamin D is associated with reduced risk of surgery in Crohn's disease, Inflamm. Bowel Dis. 19 (2013) 1921–1927.
[61] S.P. Jørgensen, J. Agnholt, H. Glerup, S. Lyhne, G.E. Villadsen, C.L. Hvas, L.E. Bartels, J. Kelsen, L.A. Christensen, J.F. Dahlerup, Clinical trial: vitamin D3 treatment in Crohn's disease – a randomized double-blind placebo-controlled study, Aliment. Pharmacol. Ther. 32 (2010) 377–383.
[62] T. Raftery, A.R. Martineau, C.L. Greiller, S. Ghosh, D. McNamara, K. Bennett, J. Meddings, M. O'sullivan, Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: results from a randomised double-blind placebo-controlled study, United Eur. Gastroenterol. J. 3 (2015) 294–302.
[63] C.W. Lo, P.W. Paris, T.L. Clemens, J. Nolan, M.F. Holick, Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes, Am. J. Clin. Nutr. 42 (1985) 644–649.