Tuberculosis is highly prevalent worldwide, accounting for nearly two million deaths annually. Vitamin D influences the immune response to tuberculosis, and vitamin D deficiency has been associated with increased tuberculosis risk in different populations. Genetic variability may influence host susceptibility to developing active tuberculosis and treatment response. Studies examining the association between genetic polymorphisms, particularly the gene coding for the vitamin D receptor (VDR), and TB susceptibility and treatment response are inconclusive. However, sufficient evidence is available to warrant larger epidemiologic studies that should aim to identify possible interactions between VDR polymorphisms and vitamin D status.

INTRODUCTION

According to the World Health Organization (WHO), there were 9.2 million incident cases and 1.7 million deaths from tuberculosis (TB) worldwide in 2006. The current treatment of tuberculosis involves the use of multiple drugs on a daily dose for 6–8 months. Interruption of the treatment leads to multidrug-resistant tuberculosis, a problem that has already been reported in over 100 countries in the world. Better treatments and vaccines for TB are currently under development to battle against the reemergence of tuberculosis and especially against new forms of multidrug-resistant tuberculosis.

*Mycobacterium tuberculosis*, the etiologic agent of TB, is a facultative intracellular bacterial parasite that can be spread by inhalation of a minimal dose (1–5 bacilli). The first immune defense response to TB infection begins with the innate immune system, involving the epithelial cells and alveolar macrophages in the airways. This initial response is strengthened by recruitment of neutrophils, which are among the first cells to arrive at the site of infection.

Macrophages phagocytize the bacilli, but the normal destruction of bacilli by macrophages can be interrupted by the defense mechanisms of the mycobacteria. One of the potential pathways through which the mycobacteria prevent their own destruction involves glycosylated phosphatidylinositol lipoarabinomannan, a compound of the mycobacterial cell membrane. Lipoarabinomannan is translocated to the phagosome wall, interrupting the normal maturation of the phagosome and its further fusion with the lysosome. Another potential mycobacterial defense mechanism involves inhibition of Ca\(^{2+}\) signaling events, which are also required for phagosome maturation. Thus protected from host defenses, the viable mycobacteria reproduce inside the macrophages and can also migrate to other tissues. However, a localized inflammatory response promotes the recruitment of T lymphocytes, which leads to the formation of a granuloma to wall off the spread of the infection. The TB infection is usually contained inside the granuloma, and the infection may remain dormant, or latent, for many years. However, immunodeficiency secondary to an event such as coinfection with human immunodeficiency virus (HIV) or malnutrition, can lead to activation of the disease.

Although tuberculosis is a highly infective disease, only 1 in 10 infected persons may become sick with active TB. The susceptibility to active disease can be influenced by environmental and genetic factors or by
gene-environment interactions. Genetic variability may influence not only host susceptibility to active TB but also host response to treatment. Polymorphisms of many different gene candidates have been studied, and the gene for the vitamin D receptor (VDR) is of great interest.

ROLE OF VITAMIN D IN TUBERCULOSIS

Even before the discovery of the etiologic cause of tuberculosis by Robert Koch in 1903, vitamin D from cod liver oil and from exposure to sun or radiation was used to treat tuberculosis. Several recent studies in different populations have associated a deficiency in vitamin D with increased risk of tuberculosis. In a recent meta-analysis by Nnoaham and Clarke, a pooled effect size of vitamin D was estimated to be 0.68 (95% CI 0.43–0.93), indicating that vitamin D levels were 0.68 SD lower in persons with tuberculosis than in controls. However, these findings cannot be considered conclusive since the association may be confounded by important variables, such as smoking and sunlight exposure, which were not accounted for in the analysis.

It is well-established that immune cells can produce the hormonally active metabolite of vitamin D. Macrophages and other immune cells can express 1α-hydroxylase, the enzyme that converts circulating 25-hydroxyvitamin D₃ into 1,25-dihydroxyvitamin D₃, the active form of vitamin D. Moreover, *M. tuberculosis* infection activates Toll-like receptors (TLR1/2) that mediate the activation of different cells in the innate immune system and their expression of cytokines and antimicrobial peptides. Recent observations indicate that activation of this cell surface receptor also upregulates expression of both the 1α-hydroxylase enzyme and the VDR in monocytes and macrophages, leading to both increased levels of active vitamin D and increased potential binding of 1,25-dihydroxyvitamin D with the VDR.

Although the biological mechanisms through which vitamin D modulates the immune system to fight *Mycobacterium* infection are still under study, two possible mechanisms have emerged as the most likely. For instance, 1,25-dihydroxyvitamin D₃ appears to reduce the viability of *M. tuberculosis* by enhancing the fusion of the phagosome and lysosome in infected macrophages. The capacity of *Mycobacterium* infection to prevent macrophage maturation and formation of the phagolysosome is completely reversed in the presence of 1,25-dihydroxyvitamin D₃. The pathways used to promote vitamin D-induced phagolysosome formation are independent of the classical interferon-gamma (IFN-γ)-dependent macrophage activation and involve products of phosphatidylinositol-3-kinases (PI3K), which help to regulate the transport of endosomes to lysosomes.

In addition, 1,25-dihydroxvitamin D may enhance the production of LL-37, an antimicrobial peptide of the cathelicidin family. Antimicrobial peptides, such as defensins and cathelicidins, are involved as a first line of defense in the prevention of infections, including tuberculosis. Although cathelicidins are widely distributed in mammals, LL-37 is the only member of the cathelicidin family that has been identified in humans, where it is found in alveolar macrophages, lymphocytes, neutrophils, and epithelial cells. In addition to having direct bactericidal activity, LL-37 also modulates the immune response by attracting monocytes, T cells, and neutrophils to the site of infection. The presence of 1,25-dihydroxyvitamin D₃ in neutrophils and macrophages upregulates in a dose-dependent manner the hCAP-18 gene that codes for LL-37, which suggests that 1,25-dihydroxyvitamin D induction of LL-37 may play a role in host defense against TB infection.

VITAMIN D RECEPTOR GENE POLYMORPHISMS AND TUBERCULOSIS

The vitamin D receptor (VDR) gene is found in the chromosomal 12q13 region. VDR gene polymorphisms are commonly found in many population groups, although the prevalence of certain VDR genotypes varies among different populations. Most of the gene polymorphisms studied are based only on restriction fragment length polymorphism (RFLP) analysis, which does not identify the functional effects of these changes. Genetic alterations of the VDR gene may lead to defects in gene activation or to changes in the protein structure of the VDR, both of which could affect the cellular functions of 1,25-dihydroxyvitamin D. Various VDR polymorphisms could also be linked to each other or to unidentified genes that are important determinants of disease risk.

In the 3' end of the VDR gene, several polymorphisms (*BsmI, ApaI,* and *TaqI*) with strong linkage disequilibrium (LD) have been studied. Although these nucleotide changes in the VDR gene are predicted to be “silent” and to have no effect on the structure of the expressed VDR protein, they may be involved in regulating VDR gene expression, or they could potentially be linked with other truly functional nucleotide sequences in the VDR gene. It has been suggested that the mRNA coded from the *TaqI* T allele of the VDR gene is more stable than the mRNA from the T allele of the VDR gene.

A non-silent VDR gene polymorphism is the *FokI* polymorphism, found in exon 2, which is at a translation initiation start site and is predicted to change the structure of the coded protein. A thymine-to-cytosine (T→C) change found in the F allele leads to an alternative trans-
VITAMIN D RECEPTOR GENE POLYMORPHISMS AND RESPONSE TO TREATMENT OF PULMONARY TUBERCULOSIS

VDR polymorphisms may affect not only host susceptibility to tuberculosis, but also the response to treatment. Two studies have focused on the association of polymorphisms in the FokI and TaqI VDR genes\textsuperscript{12} and TB susceptibility; one of them also evaluated the association with the Apal VDR gene polymorphism.\textsuperscript{9}

Roth et al.\textsuperscript{9} studied the association between FokI and TaqI VDR gene polymorphisms and susceptibility to TB and sputum conversion time following treatment among inhabitants of an area in the outskirts of Lima, Peru, where the incidence of TB is very high.\textsuperscript{12} A case-control study design was used to evaluate the association between VDR gene polymorphisms and susceptibility to TB. Study cases were 103 persons, aged 15–45 years, with confirmed TB (excluding HIV-positive patients and pregnant women) who were receiving directly observed therapy, as recommended by the WHO.\textsuperscript{28} Two age- and sex-matched controls were chosen for each case, one with a positive tuberculin test (PPD) and the other with a negative test. The prevalence of the TaqI t allele in the VDR gene was lower among the PPD-positive controls than among the PPD-negative controls and even lower among the TB cases, suggesting that the t allele was somehow protective against TB susceptibility. However, there was no significant difference in TB susceptibility between the FokI VDR genotypes ff versus FF (OR = 0.84, 95% CI 0.34–2.86) or Ff versus FF (OR = 0.64, 95% CI 0.25–1.62), or the TaqI VDR genotype Tt versus TT (OR = 0.61, 95% CI 0.28–1.29).

Although the FokI VDR genotype had no apparent effect on susceptibility to TB, quite different effects were seen regarding the response to TB treatment. To evaluate this effect, the researchers\textsuperscript{9} conducted a cohort study among 78 patients with confirmed pulmonary TB (positive sputum test and TB symptoms). Kaplan-Meier survival analysis for the total treatment-time follow-up period showed that patients with the VDR FokI FF genotype had significantly faster conversion times in the sputum cultures and in the auramine staining tests compared to those with the VDR FokI ff genotype, thus supporting the notion that the F allele, which results in the expression of a shorter VDR protein, somehow enhances the treatment response compared to the FokI f allele, which codes for a slightly longer VDR protein. With respect to the TaqI polymorphisms, no study participants had the tt genotype. Among TB patients, the conversion of culture tests – but not the auramine staining tests – was significantly faster for those with the TT VDR genotype as compared to the Tt genotype. In conclusion, there was a beneficial effect on the host response to TB among participants with the TT or FF VDR genotypes, especially for culture conversion, as compared to the Tt and the ff VDR genotypes.\textsuperscript{12}

Recently, Baab et al.\textsuperscript{12} conducted a similar study among a population of women and men, aged 18–65 years, from Western Cape, South Africa, where the incidence of TB in 2003 was high (919/100,000). Study cases were 249 persons with newly diagnosed TB (excluding pregnant women and persons with HIV or other chronic diseases); controls were 352 persons with no previous history or symptoms of TB selected from clinics, households, and workplaces in the same geographical area. A cohort study was conducted among the cases of TB, starting from the day of diagnosis up to 12 months. Patients were required to provide sputum samples for Ziehl-Nielsen staining and M. tuberculosis culture on the day of diagnosis, the following 2 days, then weekly up to month 2, and then monthly until month 6. After that, two subsequent samples were collected at months 9 and 12. Conversion time (from positive to negative M. tuberculosis in sputum) was calculated in days from the day of diagnosis to an average between the dates of the last date of a positive result and the first of two successive negative results if less than 92 days had elapsed between the positive and negative results. Otherwise, the last positive day was used as the conversion day. Participants were categorized as “fast respondents” if their conversion time was before day 55 after diagnosis, and “slow respondents” otherwise.
For the case-control study, no significant associations were observed between the individual VDR genotypes and the presence or absence of pulmonary TB. Moreover, after adjusting for age and gender, no statistically significant differences were observed between the diplotype frequencies in cases and controls. However, for the response-to-treatment cohort study, Babb et al.\(^9\) found a faster smear conversion time for the VDR genotypes ApaI AA and TaqI TT and Tt as compared to the ApaI aa and TaqI tt VDR genotypes, respectively. No statistically significant difference in the culture conversion time was observed between genotypes. For the categorization between “fast respondents” and “slow respondents”, there was a significant trend to a faster smear conversion in those with a VDR FokI f allele and for a faster culture conversion in patients with the ApaI A allele. In summary, Babb et al. found no significant association between the ApaI, TaqI and FokI polymorphisms and susceptibility to TB. However, ApaI AA and TaqI Tt and TT genotypes may contribute to a faster response to treatment.\(^9\)

The association between VDR polymorphisms and response to TB treatment remains inconclusive, and larger studies are needed. In the study among Peruvians, the FokI F and TaqI T alleles were associated with a faster response to TB treatment.\(^8\) In the South African study, there was also a faster response to TB treatment among people with the TaqI T and the ApaI A alleles, whereas no definitive association was observed for the FokI genotypes.\(^12\)

Selection of the optimal method to evaluate response to treatment (auramine staining or culture of sputum) is of substantial importance given the differences found among the studies. Roth et al.\(^12\) found significant associations for the TaqI genotypes among the TB cultures – not the stained samples – suggesting that VDR genotypes are potentially associated with mycobacterial viability, rather than with the quantity of expectorated microorganisms.\(^9\) A low number of viable bacteria can give positive results in the culture, but can be negative in the auramine staining test.\(^12\)

**CONCLUSION**

Although the immune system responds quickly to the presence of *M. tuberculosis* bacilli, this pathogen has developed several ways to avoid being killed by macrophages and, instead, to reproduce inside them. Vitamin D plays an important role in the immune response to *M. tuberculosis* by promoting both formation of the phagolysosome and also production of the antimicrobial peptide LL-37, which has direct bactericidal activity and an immune-regulating function.

The available evidence does not allow for a solid conclusion concerning the relationship between various VDR polymorphisms and TB susceptibility or response to treatment. However, these findings are intriguing and support additional inquiry on this research question in order to target individuals at risk of developing TB or to devise new treatment modalities. Additional studies with sufficient statistical power to investigate the effects of various polymorphisms in the VDR gene or other relevant genes on TB are needed. These studies should recognize that the prevalence of certain genotypes varies among different ethnic groups, which influences how suitable different ethnicities may be for exploring particular gene-disease interactions. For example, in the study among South Africans, 41 participants (6.8% of the total population) had the tt VDR genotype\(^3,4,22,24\) compared to none in the study among Peruvians.\(^9\) Also, the VDR FokI f allele is known to occur less frequently in Africans than in Caucasians and Asians,\(^12\) but the studies reviewed above found that Peruvians and native Paraguayans have the lowest prevalence of the FokI ff genotype so far.\(^25\) Nevertheless, it should be possible to generalize the functional effects associated with certain polymorphisms to other populations because the physiological role and underlying molecular mechanisms of vitamin D action likely remain the same.\(^14\) The inconclusiveness of available studies highlights the critical need to better assess the vitamin D status of these study populations. Future studies should investigate possible interactions between vitamin D status, genetic polymorphisms in the VDR gene and other genes involved with vitamin D metabolism and function, and TB susceptibility and treatment response.

**REFERENCES**


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