Tuberculosis and Chronic Hepatitis B Virus Infection in Africans and Variation in the Vitamin D Receptor Gene

R. Bellamy,1 C. Ruwende,1 T. Corrah, K. P. W. J. McAdam, M. Thursz, H. C. Whittle, and A. V. S. Hill

The active metabolite of vitamin D, 1,25 dihydroxyvitamin D3, is an important immunoregulatory hormone [1]. Its effects are exerted by interaction with the vitamin D receptor, which is present on human monocytes and activated T and B lymphocytes. Variation in the vitamin D receptor gene was typed in 2015 subjects from large case-control studies of three major infectious diseases: tuberculosis, malaria, and hepatitis B virus. Homozygotes for a polymorphism at codon 352 (genotype tt) were significantly underrepresented among those with tuberculosis ($\chi^2 = 6.22$, 1 df, $P = .01$) and persistent hepatitis B infection ($\chi^2 = 6.25$, 1 df, $P = .01$) but not in subjects with clinical malaria compared with the other genotypes. Therefore, this genetic variant, which predisposes to low bone mineral density in many populations, may confer resistance to certain infectious diseases.

Twin and adoption studies suggest that host genetic factors, including race, are important determinants of variable susceptibility to infectious diseases [2–4]. Twin studies of tuberculosis (TB) and chronic hepatitis B virus (HBV) infection identified a strong host genetic component to individual variability in disease susceptibility [3–5]. Previous studies concentrated on the role of major histocompatibility complex (MHC) genes in these diseases in humans [6].

Recently, genetic variation in bone mineral density has been shown to be associated with single base change polymorphisms in the vitamin D receptor (VDR) gene in many but not all populations [7]. Epidemiologic evidence suggests there is a link between vitamin D deficiency and susceptibility to TB (reviewed in [8, 9]), and prior to the availability of anti-TB chemotherapy, treatment results with vitamin D suggested it had beneficial effects, particularly in persons with cutaneous TB. Vitamin D is best known for its role in regulating calcium metabolism, but it is also an important immunoregulatory hormone [1]. The active metabolite of vitamin D, 1,25D$_3$, activates monocytes, stimulates cell-mediated immunity, and suppresses lymphocyte proliferation, immunoglobulin production, and cytokine synthesis. In vitro studies have shown that vitamin D metabolites can enhance the ability of human monocytes to restrict the growth of intracellular Mycobacterium tuberculosis. Alveolar macrophages in the lungs of TB patients produce 1,25D$_3$, which could therefore be involved in the host immune response to M. tuberculosis in vivo.

We typed two VDR polymorphisms in 2015 West African subjects from The Gambia in case-control studies of TB and two other major infectious diseases, malaria and chronic HBV infection. These studies were designed to investigate the role of genetic factors in host susceptibility to infectious diseases, and there was no intent to type a large number of candidate gene polymorphisms. NRAMP1 gene polymorphism is associated with TB in this population [10], and several other candidate genes have also been studied [11, 12]. MHC and glucose-6-phosphate dehydrogenase gene polymorphisms were previously shown to be associated with severe malaria in the Gambian malaria susceptibility study, and complement receptor 1, interleukin-1 receptor antagonist, and intercellular adhesion molecule 1 gene polymorphisms have also been studied [13, 14].

Methods

Patients and controls. Adults (>16 years old) with smear-positive pulmonary TB were identified from 3 TB clinics in the western region of The Gambia in and around the capital, Banjul. Subjects were only included in the study after experienced microscopists identified acid-fast bacilli in their sputum. The majority of persons with diagnosed TB in The Gambia present late with advanced pulmonary disease. As a result, the subjects in this study had more severe illness than generally seen in the developed world. The mean...
age of the patients was 34.7 ± 13.2 years, and 67.4% were male. Persons known to be infected with human immunodeficiency virus (HIV) were not recruited. All consenting patients (>95%) were screened for HIV antibodies; those found to be HIV-positive (<10%) were excluded from the study. The HIV prevalence in this African population is relatively low, 1.5% in adults. Six persons included in the study were later found to be HIV-positive; however, their exclusion from the analyses did not substantially affect the results.

Unrelated blood donors from the Royal Victoria Hospital, Banjul, were recruited as controls. The transfusion center serves the same geographic region from which the study subjects were recruited. All blood donors were male, and their mean age was 30.3 ± 7.5 years. The controls were retrospectively matched to TB patients as far as numbers allowed. The Gambian ethnic groups consist of Mandinka (38.5% among TB patients, 37.4% among controls), Wolof (19.1%, 18.4%), Jola (17.9%, 16.9%), Fula (12.0%, 12.2%), Manjago (2.5%, 3.3%), Serrahule (2.7%, 3.8%), and West African other (6.9%, 6.6%). These ethnic groups are genetically closely related. Persons who did not belong to a West African racial group (<0.5%) were not recruited. All of the blood donors were HIV-negative.

Children <10 years old with cerebral malaria, severe malarial anemia (hemoglobin <5 g/dL), and mild symptomatic malaria were recruited from hospitals and clinics in The Gambia, as described previously [13]. Controls were children of the same age range and ethnic groups who presented to the same hospitals and clinics with illnesses unrelated to malaria [13]. Controls had a wide variety of infectious and noninfectious diseases, including acute respiratory infections, gastroenteritis, and malnutrition. The mean ages (in years) of the children by study group were as follows: cerebral malaria, 3.9 ± 2.0; severe malarial anemia, 2.2 ± 1.6; mild malaria, 4.0 ± 2.8; and controls, 3.3 ± 2.4. Of children with cerebral malaria, 55.2% were boys, as were 47.6% of those with severe malarial anemia, 52.4% with mild malaria, and 53.3% of controls. The ethnic group distribution was Mandinka (39.0% of those with cerebral malaria, 40.5% of severe malarial anemia patients, 47.6% of mild malaria patients, and 42.9% of controls), Wolof (15.5%, 11.3%, 10.8%, 12.9%), Jola (16.8%, 18.5%, 15.2%, 16.4%), Fula (11.8%, 12.8%, 9.5%, 12.2%), Manjago (4.5%, 4.6%, 5.1%, 5.7%), Serrahule (6.4%, 5.1%, 5.1%, 4.1%), and other (5.9%, 6.7%, 6.4%, 6.1%).

All malaria cases and controls from whom serum was available were screened for HBV surface antigen (HBsAg) and anti–HBV-core antibody (anti-HBc) by ELISA, as described previously [6]. We defined persistent HBV carriage serologically as HBsAg-positive and anti-HBc-positive and cleared infection as HBsAg-negative and anti–HBc-positive. The mean ages of HBsAg-positive and anti–HBc-positive HBsAg-negative children were 4.3 ± 2.6 years and 4.6 ± 2.8 years, respectively. By ethnic group, those HBsAg-positive and -negative, respectively, were as follows: Mandinka (42.3% and 46.9%), Wolof (14.8% and 16.9%), Jola (22.5% and 12.5%), Fula (4.9% and 7.5%), Manjago (4.4% and 4.4%), Serrahule (5.5% and 4.4%), and other (4.9% and 7.5%). Serum from adult blood donors was also screened for anti-HBc and HBsAg.

By ethnic group, Mandinka were (25.8% HBsAg-positive and 28.2% HBsAg-negative), Wolof (32.3% and 27.0%), Jola (12.9% and 14.4%), Fula (16.1% and 6.9%), Manjago (6.5% and 5.7%), Serrahule (6.5% and 6.3%), and other (0% and 8.6%).

Sample processing. In total, 10 mL of venous blood was collected into potassium/EDTA tubes, and DNA was extracted using Nucleon II (Scotlab, Glasgow, UK). A 340-bp fragment of the VDR gene was amplified by the polymerase chain reaction (PCR) using primers 5'-CAGAGCATGGACGGAGCAAG-3' and 5'-GGTTCCGGACGGATGTACGT-3'. The codon 352 polymorphism is due to a silent T to C base change, and the intron 8 polymorphism is due to a G to T base change. We detected these polymorphisms by transfer of PCR product to nylon membrane and hybridization with digoxigenin-labeled, sequence-specific oligonucleotides 5'-GGCGCTAGTGGGACGCATC-3' and 5'-GCGCTAGTGGGACGCATC-3', which detect the alleles T and t, and 5'-GAGGGGGCCACCTG-3' and 5'-GAGGTGCCAGCTG-3', which detect a and A, respectively (Morrison N, personal communication). Signal was detected using an antidigoxigenin antibody chemiluminescence system (Boehringer Mannheim, Lewes, UK).

Statistical analysis was performed in a stepwise manner, initially comparing overall genotype frequencies using a 3 × 2 χ² test and then, if a significant overall difference between cases and controls was detected (P < .05), individual genotypes were compared using 2 × 2 χ² analysis. To allow for any potential confounding effect of ethnic group, χ² analysis was also performed using the Mantel-Haenszel test, stratifying by ethnic group. In the case of the children in the HBV study, stratification was also done by malaria status.

### Results

We typed 408 sputum smear-positive adult pulmonary TB cases and 414 adult blood donor controls from the same region and ethnic groups for single base change polymorphisms at codon 352 and within intron 8. The codon 352 genotypes are designated TT, Tt, and tt and the intron 8 polymorphism genotypes, AA, Aa, and aa. The genotype associated with low bone mineral density, tt, was significantly underrepresented in the TB cases compared with controls (27/408 and 49/414, respectively, Mantel-Haenszel χ² = 6.22, 1 df, P = .01; table 1). The odds ratio (OR) for patients of genotype tt having TB compared with genotypes TT/Tt combined was 0.53 (95% confidence interval [CI], 0.31–0.88), suggesting that tt homozygotes may be resistant to clinical TB. Allele t was in linkage disequilibrium with allele A, a noncoding polymorphism in intron 8 (P < 10⁻⁵), but this allele was not significantly associated with TB, malaria, nor HBV phenotypes. There are several polymor-

<table>
<thead>
<tr>
<th>Table 1. TB and VDR genotype in subjects in The Gambia.</th>
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<tr>
<td>Genotype</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>Tt</td>
</tr>
<tr>
<td>tt</td>
</tr>
<tr>
<td>Total</td>
</tr>
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NOTE. Overall distribution of VDR genotypes significantly different between cases and controls (3 × 2 χ² = 6.98, 2 df, P = .03; corrected for ethnic group Mantel-Haenszel χ² = 4.93, P = .03). tt genotype was reduced in TB cases vs. controls (Yates’s correction 2 × 2 χ² = 6.06, 1 df, P = .01; M-H χ² = 6.22, P = .01; odds ratio = 0.53 [95% confidence interval, 0.31–0.88]).
phisms in the 3' region of the VDR gene, and it is not known which of these produces altered expression of the gene. The A/a polymorphism may be in weaker linkage disequilibrium than T/t with the unknown functional polymorphism.

To investigate whether VDR gene polymorphism was associated with TB only or also with other infectious diseases, 368 children with cerebral malaria, 168 with severe malarial anemia (including 49 who also had cerebral malaria), 292 with mild symptomatic malaria, and 414 controls were typed for VDR polymorphisms. No association was found between VDR polymorphism and susceptibility to uncomplicated (mild) malaria or with either form of severe malaria (data not shown).

Of the children in the malaria study with available serum, 964 were screened for anti-HBC antibody to identify those who had prior HBV infection. We tested 329 HBV-infected anti-HBc-positive children and 201 anti-HBc-positive adults from the blood donor controls for HBsAg to detect persistent HBV carriers. Of 175 HBsAg-positive children, 12 (6.9%) and 90 (51%) were tt and TT homozygotes, respectively, compared with 18 (12%) and 69 (45%) in 154 HBsAg-negative children (table 2). Among 31 HBsAg-positive adults, 3 (9.7%) and 14 (45%) were tt and TT homozygotes, respectively, compared with 28 (16%) and 68 (40%) among 170 HBsAg-negative adults. Overall, tt homozygotes were significantly underrepresented among HBsAg-positive subjects ($\chi^2 = 6.25; P = .01; OR, 0.43; 95% CI, 0.22–0.85$).

### Discussion

In this study, persons with VDR genotype tt were underrepresented among TB cases and overrepresented among HBsAg-negative subjects. This suggests that persons with this genotype may be resistant to TB and persistence of HBV infection. The possibilities that vitamin D supplementation could prevent TB or chronic HBV infection merit further investigation.

The study design did not allow us to distinguish between susceptibility to *M. tuberculosis* infection and susceptibility to disease reactivation and progression. The adult blood donor controls would have been exposed to *M. tuberculosis*, as they live in a region in which TB is endemic. However, they should be regarded as typical of the healthy general population and not as a TB-resistant group. Tuberculin tests in The Gambia cannot be used to identify adults previously infected by *M. tuberculosis*, because most of the population has been vaccinated with bacille-Calmette Guérin and environmental mycobacteria are common. The majority of the population can be infected by *M. tuberculosis* if the frequency of exposure is very high. As only 10% of the population infected with *M. tuberculosis* will ever develop clinical disease, persons with TB have greater susceptibility to this organism than the general population. Therefore, it was more likely that this study would identify genetic factors that determine susceptibility to the development of clinical disease than susceptibility to infection.

### Table 2. VDR and hepatitis B surface antigen (HBsAg) status among anti-hepatitis B core-positive subjects.

<table>
<thead>
<tr>
<th></th>
<th>% children HBsAg Negative</th>
<th>% children HBsAg Positive</th>
<th>% adults HBsAg Negative</th>
<th>% adults HBsAg Positive</th>
<th>% combined HBsAg Negative</th>
<th>% combined HBsAg Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT</td>
<td>69 (45)</td>
<td>90 (51)</td>
<td>68 (40)</td>
<td>14 (45)</td>
<td>137 (42)</td>
<td>104 (50)</td>
</tr>
<tr>
<td>Tt</td>
<td>67 (44)</td>
<td>73 (42)</td>
<td>74 (44)</td>
<td>14 (45)</td>
<td>141 (44)</td>
<td>87 (42)</td>
</tr>
<tr>
<td>tt</td>
<td>18 (12)</td>
<td>28 (16)</td>
<td>3 (9.7)</td>
<td>46 (14)</td>
<td>46 (14)</td>
<td>15 (7.3)</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>175</td>
<td>170</td>
<td>31</td>
<td>324</td>
<td>206</td>
</tr>
</tbody>
</table>

NOTE. VDR genotypes significantly different between HBsAg-positive carriers and those who cleared virus ($3 \times 2 \chi^2 = 7.14, 2 df, P = .03$; corrected for ethnic group Mantel-Haenszel [M-H] $\chi^2$ test = 6.10, $P = .01$). Those with tt genotype were significantly protected from chronic HBV infection (Yates’s correction $2 \times 2 \chi^2 = 5.25, 1 df, P = .02$; M-H $\chi^2 = 6.25, P = .01$; odds ratio [OR] $= 0.43$ [95% confidence interval, 0.22–0.85]). For children, stratifying by malaria status did not significantly affect tt association (ORs with and without malaria stratification, 0.54 and 0.56, respectively).

Because 90% of those infected will never develop clinical disease, even persons who have been infected with *M. tuberculosis* but remain disease free throughout life are likely to differ very little from the healthy general population in terms of genetic susceptibility. A group of TB-susceptible persons provides much greater power than would a TB-resistant group.

An association between VDR genotype and bone mineral density has been found in many but not all populations studied [7]. This population heterogeneity might be explained by gene-environment interaction and could relate to factors such as vitamin D or calcium intake [7]. Further studies will be required, to determine whether this heterogeneity also occurs in susceptibility to TB and persistent HBV infection.

Recent studies have also shown associations between VDR genotypes and HIV infection (Ali et al., unpublished data) and leprosy type (the tt genotype is associated with tuberculoid and the TT genotype with lepromatous leprosy) [15], suggesting that the VDR polymorphism may be of immunoregulatory importance for many disease processes. Further studies will be required to investigate how VDR polymorphism may influence susceptibility to infectious diseases.

### Acknowledgments

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### References