Association between Vitamin D Receptor Gene Polymorphisms and Response to Treatment of Pulmonary Tuberculosis

Daniel E. Roth,¹,⁺ Giselle Soto,¹ Fanny Arenas,² Christian T. Bautista,¹,⁺ Jaime Ortiz,³ Richard Rodriguez,³ Lilia Cabrera,¹ and Robert H. Gilman¹,²

¹Asociación Benéfica Proyectos en Informática, Salud, Medicina y Agricultura, and ²Tuberculosis Research Laboratory, Department of Microbiology, Cayetano Heredia University, and ³Hospital de Apoyo Maria Auxiliadora, Lima, Peru

Background. Polymorphisms in the gene that encodes the vitamin D receptor (VDR) may influence the host response to Mycobacterium tuberculosis infection.

Methods. In a Peruvian community with a high incidence of tuberculosis (TB), VDR TaqI and FokI polymorphisms were compared among 103 patients with pulmonary TB and 206 matched healthy control subjects. Associations of VDR polymorphisms with treatment outcome were analyzed among 78 patients undergoing treatment of pulmonary TB.

Results. Sputum mycobacterial culture and auramine stain conversions were significantly faster among participants with the FokI FF genotype, compared with participants with the non-FF genotypes. Sputum culture conversion was faster among participants with the TaqI Tt genotype, compared with those with the TT genotype. Increased probability of culture conversion during TB treatment was independently associated with the TaqI Tt genotype (age- and sex-adjusted relative risk, 4.28; 95% confidence interval, 1.88–9.75; P = .001). VDR polymorphisms were not significantly associated with susceptibility to TB in the case-control study.

Conclusions. VDR gene polymorphisms are associated with the time to sputum culture and auramine stain conversion during TB treatment. To our knowledge, the present study is the first report of a specific host gene influence on the outcome of TB treatment. These findings demonstrate the potential clinical relevance of immunomodulatory functions of vitamin D metabolites acting via the VDR in the host response against pulmonary TB.

Host genes interact with environmental factors to determine the capacity of an individual to respond to infection with Mycobacterium tuberculosis [1]. Genetic loci implicated in human susceptibility to tuberculosis (TB) include NRAMP [1–3], HLA-DQB1 [4], and the genes encoding interferon-γ [5] and the vitamin D receptor (VDR) [6]. The VDR is of particular interest because epidemiologic and laboratory studies suggest an association between vitamin D metabolism and immunity to TB. Vitamin D deficiency has been associated with susceptibility to and severity of TB [7–9], and 1,25-dihydroxyvitamin D, the active vitamin D metabolite, is a potent immunomodulator that acts via the VDR to stimulate macrophages to restrict mycobacterial growth in vitro [10–12].

Associations between polymorphisms in the VDR gene and the immune response against TB provide evidence of vitamin D–related gene-environment interactions in the host response to TB [13]. In the VDR gene, a polymorphism resulting from FokI endonuclease activity [14] was associated with the extent of pulmonary TB, as determined by radiography, in Gujarati Indians living in the United Kingdom [7]. Among Chinese patients, susceptibility to TB has been associated with the FokI ff genotype [15]. The VDR tt genotype, identified by use of the TaqI restriction endonuclease [16], was associated with a decreased risk of active TB.
in The Gambia [6]; however, among Gujarati Indians, non-tt genotypes were only significantly associated with susceptibility to TB when the 25-hydroxyvitamin D serum concentration was <10 nmol/L [7]. The association between resistance to TB and the t allele has not been reproduced in smaller studies in other countries [17, 18], emphasizing the complexity of characterization of host genetic susceptibility to TB across diverse ethnic groups living under varied social conditions [19].

To our knowledge, no studies, to date, have reported the influence of host genes on outcomes of TB treatment. We therefore prospectively studied a cohort of Peruvian adult patients with pulmonary TB, to determine the effect of VDR polymorphisms on the response to treatment of TB in a resource-poor community with a very high incidence of TB. We also conducted a matched case-control study to determine whether the reported association between VDR polymorphisms and susceptibility to TB was reproducible in Peru.

**PATIENTS AND METHODS**

**Patient recruitment.** The present study involved patients with confirmed pulmonary TB who were seen at the Hospital de Apoyo Maria Auxiliadora (HAMA), a referral center for a community with a low socioeconomic status (typical annual household income, US$1200–US$2000; R.H.G., unpublished observations) on the outskirts of Lima, Peru, where there is a very high incidence of TB [20]. All patients were enrolled in the Peruvian National Tuberculosis Control Program (TCP), on the basis of the presence of symptoms suggestive of TB and a sputum specimen determined to be positive for acid-fast bacilli by use of the Ziehl-Neelsen stain. In conjunction with a longitudinal study to detect multidrug-resistant TB (MDR-TB) [21], recruitment was conducted during 2 separate periods: January through May 2000 and August 2000 through April 2001. Of the 170 patients who were consecutively enrolled in the TCP during these periods, 118 were eligible for participation in the study on the basis of the following criteria: age 15–45 years, HIV-negative status, no known pregnancy, and a willingness to participate in the study (table 1).

Patients were recruited into the study on the day of treatment initiation. Demographic data, previous history of TB and/or previous contact with individuals with TB, and the presence or absence of a scar characteristic of bacille Calmette-Guérin vaccination were recorded. The study physician obtained an initial history and performed a physical examination, and standard posterior-anterior chest radiography, collection of 2 additional sputum samples, and venous blood sampling were completed within 2 days of treatment initiation. Written, informed consent was obtained for all procedures and genetic analyses. The study protocol conformed to the guidelines of the Declaration of Helsinki, and it was approved by the institutional research ethics committee of the Asociación Benéfica Proyectos en Informática, Salud, Medicina y Agricultura and HAMA.
were randomly selected from a community census and were located within the catchment area of HAMA. For each patient recruited in Las Pampas de Miraflores, a shantytown that is currently employed depending on the regimen). Follow-up of the last patient enrolled ended in October 2001.

Specimen collection and analysis. On the 2 consecutive days before each clinic visit, patients collected 2 first morning sputum samples in sealed plastic containers. At the clinic, the specimens were placed on ice packs, and they then were transported to the TB research laboratory at the Cayetano Heredia University (Lima, Peru) within 24 h of collection. All samples were examined for the presence of mycobacteria by use of a standard auramine staining technique [24], with the exception of specimens obtained at treatment initiation and completion, which were examined by the TCP by use of a Ziehl-Neelsen stain. M. tuberculosis culture was performed on Lowenstein-Jensen slopes and in Middlebrook 7H9 broth base, by use of

Table 2. Characteristics, at baseline, and vitamin D receptor (VDR) genotype distributions among participants included in case-control and cohort studies.

<table>
<thead>
<tr>
<th>Characteristic or genotype distribution</th>
<th>Case-control study</th>
<th></th>
<th></th>
<th>Cohort study, patients with TBb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients with TB</td>
<td>PPD-positive</td>
<td>PPD-negative</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(n = 103)</td>
<td>control subjects</td>
<td>control subjects</td>
<td>patients with TBb (n = 78)</td>
</tr>
<tr>
<td>Age, mean ± SD, years</td>
<td>25.4 ± 6.9</td>
<td>25.4 ± 7.6</td>
<td>25.5 ± 7.1</td>
<td>25.4 ± 6.7</td>
</tr>
<tr>
<td>Males</td>
<td>60 (62/103)</td>
<td>60 (62/103)</td>
<td>60 (62/103)</td>
<td>62 (48/78)</td>
</tr>
<tr>
<td>“Single” civil statusb</td>
<td>64 (66/103)</td>
<td>46 (44/95)</td>
<td>54 (54/101)</td>
<td>71 (55/71)</td>
</tr>
<tr>
<td>Currently employedd</td>
<td>79 (80/101)</td>
<td>93 (89/96)</td>
<td>95 (96/101)</td>
<td>79 (61/77)</td>
</tr>
<tr>
<td>Completed high school educationd</td>
<td>64 (65/101)</td>
<td>57 (55/96)</td>
<td>50 (49/99)</td>
<td>68 (52/77)</td>
</tr>
<tr>
<td>Reported history of TB</td>
<td>18 (18/103)</td>
<td>10 (9/90)</td>
<td>11 (10/95)</td>
<td>19 (15/78)</td>
</tr>
<tr>
<td>Known contact with an individual with TBc</td>
<td>41 (42/103)</td>
<td>4 (3/75)</td>
<td>5 (4/88)</td>
<td>37 (29/78)</td>
</tr>
<tr>
<td>Visible scar characteristic of BCG vaccination present</td>
<td>89 (92/103)</td>
<td>91 (87/96)</td>
<td>84 (86/102)</td>
<td>86 (67/78)</td>
</tr>
<tr>
<td>Size of transverse PPD reaction, mm of induration ± SDd</td>
<td>11.6 ± 7.5</td>
<td>17.9 ± 7.1</td>
<td>3.1 ± 3.1</td>
<td>11.5 ± 7.6</td>
</tr>
<tr>
<td>TaqI genotypeg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>90 (93/103)</td>
<td>86 (89/103)</td>
<td>83 (85/103)</td>
<td>91 (71/78)</td>
</tr>
<tr>
<td>Tt</td>
<td>10 (10/103)</td>
<td>14 (14/103)</td>
<td>17 (17/103)</td>
<td>9 (7/78)</td>
</tr>
<tr>
<td>tt</td>
<td>0</td>
<td>0</td>
<td>1 (1/103)</td>
<td>0</td>
</tr>
<tr>
<td>FokI genotypeg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>9 (9/103)</td>
<td>7 (7/103)</td>
<td>7 (7/103)</td>
<td>12 (9/78)</td>
</tr>
<tr>
<td>Ft</td>
<td>32 (33/103)</td>
<td>42 (43/103)</td>
<td>36 (37/103)</td>
<td>28 (22/78)</td>
</tr>
<tr>
<td>ff</td>
<td>59 (61/103)</td>
<td>52 (53/103)</td>
<td>57 (59/103)</td>
<td>60 (47/78)</td>
</tr>
</tbody>
</table>

NOTE. Data are % of study participants (no. of study participants with characteristic or genotype/no. of study participants assessed), unless indicated otherwise. BCG, bacille Calmette-Guérin; PPD, purified protein derivative; TB, tuberculosis.

For the demographic characteristics or VDR genotype distributions shown, the only difference between the 78 patients included in the study and the 34 patients excluded from the study because of inadequate data was that there were proportionally fewer study participants with a civil status of “single” in the group of excluded patients (15 [46%] of 33 patients; P = .017). Among all patients initially recruited for the study (N = 170; see table 1), the 92 patients who were excluded from the cohort analysis were similar to the 78 patients who were included in the cohort analysis in all respects, with the exception that the patients excluded from analysis were older (mean age ± SD, 32.0 years ± 14.2 years; P < .0001), were less likely to have a civil status of “single” (44 [48%] of 91 patients; P = .003), and were slightly less likely to have completed high school education (44 [48%] of 91 patients; P = .042).

a For the demographic characteristics or VDR genotype distributions shown, the only difference between the 78 patients included in the study and the 78 patients who were included in the cohort analysis was that there were proportionally fewer study participants with a civil status of “single” in the group of excluded patients (15 [46%] of 33 patients; P = .017). Among all patients initially recruited for the study (N = 170; see table 1), the 92 patients who were excluded from the cohort analysis were similar to the 78 patients who were included in the cohort analysis in all respects, with the exception that the patients excluded from analysis were older (mean age ± SD, 32.0 years ± 14.2 years; P < .0001), were less likely to have a civil status of “single” (44 [48%] of 91 patients; P = .003), and were slightly less likely to have completed high school education (44 [48%] of 91 patients; P = .042).

b There is a significant difference among the 3 case patient and control groups ( ).
c There is a significant difference among the 3 case patient and control groups ( ).
d There is a significant difference between the patients with TB and the PPD-negative control subjects (64% vs. 50%; ).
e Contact occurred within the past 2 years. There is a significant difference among the 3 case patient and control groups ( ).
f There is a significant difference among the 3 case patient and control groups ( ).
g Alleles at both VDR loci conformed to the Hardy-Weinberg equilibrium.

TB treatment and surveillance. All patients received antituberculosis chemotherapy, according to the Peruvian protocol for directly observed therapy, short course (DOTS) [22], which follows World Health Organization guidelines [23]. Patients with new diagnoses of TB followed a 6-month regimen, and patients with previous diagnoses of TB followed an 8-month regimen. Follow-up visits were scheduled at 1 month, 2 months, and 4 months after treatment initiation, as well as at treatment completion (at 6 or 8 months after treatment initiation, depending on the regimen). Follow-up of the last patient enrolled ended in October 2001.

Healthy control participants. Within 1 week of the recruitment of each patient with TB into the cohort study, 2 age-matched (±5 years) and sex-matched control subjects were recruited in Las Pampas de Miraflores, a shantytown that is located within the catchment area of HAMA. For each patient with TB who was enrolled, several potential control participants were randomly selected from a community census and were administered purified protein derivative (PPD) skin tests until 1 control participant for whom results of the PPD skin test were negative (induration, <10 mm) and 1 control participant for whom results of the PPD skin test were positive (induration, ≥10 mm) were identified. None of the control participants had symptoms of TB or was known to have active TB.
the Micro Observation Direct Susceptibility test (MODS), as described elsewhere [25]. When mycobacterial colonies were present, susceptibility to antimicrobial agents was assessed using the microplate tetrazolium assay [26]. “MDR-TB” was defined as resistance to, at least, isoniazid (0.4 mg/mL) and rifampin (1.0 mg/mL).

**VDR analysis.** Human DNA was extracted from 300-μL whole blood specimens, by use of the Puregene DNA Isolation Kit (Gentra Systems). DNA amplified by polymerase chain reaction (PCR) contained sequences with the previously described VDR restriction fragment–length polymorphisms (RFLPs) identified by *TaqI, FokI,* and *BsmI* restriction endonucleases [14, 16]. Cycling conditions for all reactions involved 30 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. PCR products were digested in an excess of restriction enzyme for 3 h (at 65°C with *TaqI* and *BsmI*; at 37°C with *FokI*). Products were visualized on 2.5% agarose gel containing Tris-acetate-EDTA buffer and 1.5%–2% ethidium bromide. The presence of a restriction site was assessed by agarose gel containing Tris-acetate-EDTA buffer and 1.5%–2% ethidium bromide. The presence of a restriction site was as-

<table>
<thead>
<tr>
<th>Polymorphism, genotype</th>
<th>Culture (n = 78)</th>
<th>Auramine-stained smears (n = 78)</th>
<th>Culture (n = 78)</th>
<th>Auramine-stained smears (n = 78)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>FokI</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FF</td>
<td>15.5 (14.0–17.0)</td>
<td>15.0 (13.9–16.1)</td>
<td>78 (7/9)</td>
<td>100 (9/9)</td>
</tr>
<tr>
<td>Ff</td>
<td>44.0 (18.1–69.9)</td>
<td>18.0 (12.0–24.0)</td>
<td>41 (9/22)</td>
<td>64 (14/22)</td>
</tr>
<tr>
<td>ff</td>
<td>46.5 (45.3–47.7)</td>
<td>16.5 (14.8–18.2)</td>
<td>30 (14/47)</td>
<td>62 (29/47)</td>
</tr>
<tr>
<td><em>TaqI</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>46.0 (44.7–47.3)</td>
<td>17.5 (16.3–18.7)</td>
<td>34 (24/71)</td>
<td>66 (47/71)</td>
</tr>
<tr>
<td>Tt</td>
<td>15.5 (14.9–16.1)</td>
<td>15.5 (14.2–16.8)</td>
<td>86 (6/7)</td>
<td>71 (5/7)</td>
</tr>
<tr>
<td>Total</td>
<td>45.5 (44.2–46.8)</td>
<td>17.5 (16.4–18.7)</td>
<td>39 (30/78)</td>
<td>67 (52/78)</td>
</tr>
</tbody>
</table>

- Derived from Kaplan-Meier survival curves.
- Thirty days after treatment initiation.
- Significant difference between FF and either Ff or ff (P = .023).
- Significant difference between FF and either Ff or ff (P = .025).
- Significant difference between Tt and TT (P = .012).

**Data analysis.** In the cohort analysis, the growth of mycobacteria in culture of at least 1 of the 2 sputum specimens collected at each follow-up visit was considered to be a positive culture result. The identification of mycobacteria, by auramine staining, in at least 1 of the 2 specimens was considered to be a positive auramine smear result. At enrollment and at completion of treatment, a positive result of Ziehl-Neelsen staining for acid-fast bacteria was accepted as a substitute for a positive auramine smear result. The auramine stain and culture conversion times were defined as the number of days from the initiation of therapy until the midpoint between the date that the last positive result was obtained and either the date that the first of 2 consecutive negative results were obtained [27] or the date that a single negative result was obtained, if the negative result occurred at treatment completion or before treatment withdrawal. The total surveillance period for each patient was calculated as the number of days from the date of treatment initiation until the date that the last sputum sample was submitted. Culture conversion time was the primary outcome measure [28].

Of the 112 eligible patients with TB, 103 were included in the final case-control study, and 78 could be included in the cohort study (table 1). For 19 participants who missed scheduled sputum examinations between initial recruitment and the time at which microbiologic resolution was first documented, calculated auramine stain or culture conversion times were highly imprecise. These 19 participants thus were excluded from the primary cohort analysis, yet they were included in sensitivity analyses to verify the robustness of the results.

Either χ² or Fisher’s exact tests were used to compare differences in categorical variables. Differences in continuous variables were compared using the Student’s *t* test and 1-way analysis of variance (ANOVA). For the case-control study, a univariate conditional logistic regression model was applied for analysis of matched-pair data, and the odds ratio (OR) was calculated to
measure the association between VDR alleles and susceptibility to active TB. For the cohort study, the Kaplan-Meier survival curve method was used to estimate the probability of specimens becoming culture or auramine stain negative during follow-up. For specific subgroups based on VDR genotype, survival curves were compared using the log-rank test. Cox proportional hazards regression analysis was applied to determine the relative risk (RR) associated with the following potential predictors of culture conversion time: \( \text{TaqI} \) genotype (TT vs. Tt or tt); \( \text{FokI} \) genotype (FF vs. Ff or ff); age; sex; occupation (employed vs. unemployed); civil status (single vs. other); education level (completed secondary school education vs. did not complete secondary school education); previous history of TB; contact with individuals with TB within the past 2 years; treatment scheme (6-month regimen vs. 8-month regimen); severity of disease, as determined by radiography (presence vs. absence of cavitary lesions); body mass index at baseline; number of TB-associated symptoms at recruitment; number of TB-associated findings noted on physical examination at recruitment; and antibiotic susceptibility of the first mycobacterial strain cultured (susceptible to all drugs vs. resistant to at least 1 drug). All factors for which \( P < .20 \) in univariate analysis were included in a multivariate forward stepwise Cox regression analysis, to determine the independent predictors of culture conversion. All reported confidence intervals (CIs) are 95% CIs, and all reported \( P \) values are 2 sided; \( P < .05 \) was considered to be statistically significant. Statistical analyses were conducted using SPSS (version 10.0; SPSS) and EGRET (version 2.0; Cytel Software).

RESULTS

Matched case-control study. Each of the 103 patients with TB who were included in the study was linked with 2 age-matched (±5 years) and sex-matched control participants, for a total of 309 participants in the case-control analysis. The patient and control groups were, by design, identical with regard to age and sex distributions, but they differed in other respects, such as civil status, employment status, education level, rate of previous contact with individuals with TB within the past 2 years, and the mean size of the transverse PPD reaction (table 2). The raw distributions of \( \text{TaqI} \) and \( \text{FokI} \) genotypes were similar among the 3 groups of study participants (table 2). Univariate analysis for matched data, performed by conditional logistic regression, revealed that neither of the VDR polymorphisms was significantly associated with susceptibility to active TB (\( \text{FokI} \) FF genotype vs. Ff or ff genotypes: OR, 1.32; 95% CI, 0.55–3.18; \( P = .538; \text{TaqI} \) Tt or tt genotypes vs. TT genotype: OR, 0.61; 95% CI, 0.29–1.30; \( P = .199 \)). Significant associations were also absent among men and women when analyzed separately (data not shown).

Cohort study. The characteristics, at baseline, of the cohort of patients with TB were similar, in all respects, to the characteristics of the group of patients with TB included in the case-control study (table 2). Of the 78 patients included, 64 (82%) followed the standard 6-month treatment regimen, and 14 (18%) followed the 8-month regimen. Five (6%) did not have a negative auramine smear result and 9 (12%) did not have a nega-
Figure 3. Kaplan-Meier survival curve demonstrating the decreasing proportion of positive results of mycobacterial sputum cultures among patients with tuberculosis assigned to subgroups on the basis of their TaqI genotype (the solid line denotes the Tt genotype; the long-dashed line, the TT genotype; and ■, censored case patients). Time 0 denotes treatment initiation. The culture conversion times for patients with TT and Tt genotypes were significantly different (log-rank value, 14.8; \( P = .0001 \)).

Figure 4. Kaplan-Meier survival curve demonstrating the decreasing proportion of positive results of auramine staining of sputum samples among patients with tuberculosis assigned to subgroups on the basis of their TaqI genotype (the solid line denotes the Tt genotype; the long-dashed line, TT genotype; and ■, censored case patients). Time 0 denotes treatment initiation. The auramine stain conversion times for patients with the TT and Tt genotypes were similar (log-rank value, 0.30; \( P = .583 \)).

Of the potential risk factors analyzed by univariate Cox regression (see the Patients and Methods section), 4 were strongly associated with a significantly higher probability of culture conversion during treatment (RRs are adjusted for age and sex): TaqI TT genotype (RR, 4.28; 95% CI, 1.88–9.75; \( P = .001 \)), FokI FF genotype (RR, 2.35; 95% CI, 1.15–4.83; \( P = .020 \)), completion of secondary school education (RR, 2.47; 95% CI, 1.42–4.28; \( P = .001 \)), and susceptibility to all antibiotics (RR, 3.49; 95% CI, 1.63–7.48; \( P = .001 \)). The effect of increased body mass index was statistically significant but minor (RR, 1.04; 95% CI, 1.00–1.08; \( P = .30 \)). The multivariate Cox regression analysis showed that, of the aforementioned factors, all but the FokI FF genotype remained significant independent predictors of culture conversion (data not shown).

**DISCUSSION**

The present study has demonstrated that VDR polymorphisms are significantly associated with the time to microbiologic resolution of pulmonary TB after initiation of a DOTS protocol among Peruvian adults. To our knowledge, this is the first study to describe an association between a host gene and response to TB treatment.

During treatment surveillance, a patient with the TaqI Tt genotype was >4 times more likely to have a negative mycobacterial culture result than was a patient with the homozygous TT genotype, an effect that was independent of other predictor variables examined. A relatively weaker effect of the FokI polymorphism was observed, such that a patient with the FF genotype had more than twice the probability of having a negative culture result before withdrawal, loss to follow-up, or completion of treatment. Clinical status at baseline (number of TB symptoms and physical findings; body mass index; or extent of disease, as determined by chest radiography) was not associated with either polymorphism (data not shown). Five (6%) of the 78 patients had MDR-TB at treatment initiation.

Kaplan-Meier survival curves demonstrated that the FokI FF genotype was associated with significantly faster conversion of mycobacterial cultures and auramine smears, compared with the Ff or ff genotypes (table 3 and figures 1 and 2). Unadjusted univariate analysis demonstrated that, by 1 month (30 days) after treatment initiation, participants with the FF genotype (compared with participants with either the Ff or ff genotype) had a significantly higher probability of culture conversion (RR, 5.6; 95% CI, 1.2–25.2) (table 3). The TaqI TT genotype was associated with a significantly shorter conversion time, compared with the TT genotype, but the trend did not reach significance for the auramine stain conversion time (table 3 and figures 3 and 4). Patients with the Tt genotype were significantly more likely to have a negative culture result within 1 month of treatment initiation, compared with patients with the TT genotype (RR, 9.6; 95% CI, 1.2–75.9) (table 3). For both TaqI and FokI, the results remained similar and statistically significant in sensitivity analyses that incorporated the 19 case patients excluded because of missing data; likewise, the results were essentially unchanged after the exclusion of the 5 patients with MDR-TB, according to the initial culture results (analyses not shown).

Of the potential risk factors analyzed by univariate Cox regression (see the Patients and Methods section), 4 were strongly associated with a significantly higher probability of culture conversion during treatment (RRs are adjusted for age and sex): TaqI TT genotype (RR, 4.28; 95% CI, 1.88–9.75; \( P = .001 \)), FokI FF genotype (RR, 2.35; 95% CI, 1.15–4.83; \( P = .020 \)), completion of secondary school education (RR, 2.47; 95% CI, 1.42–4.28; \( P = .001 \)), and susceptibility to all antibiotics (RR, 3.49; 95% CI, 1.63–7.48; \( P = .001 \)). The effect of increased body mass index was statistically significant but minor (RR, 1.04; 95% CI, 1.00–1.08; \( P = .30 \)). The multivariate Cox regression analysis showed that, of the aforementioned factors, all but the FokI FF genotype remained significant independent predictors of culture conversion (data not shown).

**DISCUSSION**

The present study has demonstrated that VDR polymorphisms are significantly associated with the time to microbiologic resolution of pulmonary TB after initiation of a DOTS protocol among Peruvian adults. To our knowledge, this is the first study to describe an association between a host gene and response to TB treatment.

During treatment surveillance, a patient with the TaqI Tt genotype was >4 times more likely to have a negative mycobacterial culture result than was a patient with the homozygous TT genotype, an effect that was independent of other predictor variables examined. A relatively weaker effect of the FokI polymorphism was observed, such that a patient with the FF genotype had more than twice the probability of having a negative culture result before withdrawal, loss to follow-up, or completion of treatment. Clinical status at baseline (number of TB symptoms and physical findings; body mass index; or extent of disease, as determined by chest radiography) was not associated with either polymorphism (data not shown). Five (6%) of the 78 patients had MDR-TB at treatment initiation.

Kaplan-Meier survival curves demonstrated that the FokI FF genotype was associated with significantly faster conversion of mycobacterial cultures and auramine smears, compared with the Ff or ff genotypes (table 3 and figures 1 and 2). Unadjusted univariate analysis demonstrated that, by 1 month (30 days) after treatment initiation, participants with the FF genotype (compared with participants with either the Ff or ff genotype) had a significantly higher probability of culture conversion (RR, 5.6; 95% CI, 1.2–25.2) (table 3). The TaqI TT genotype was associated with a significantly shorter conversion time, compared with the TT genotype, but the trend did not reach significance for the auramine stain conversion time (table 3 and figures 3 and 4). Patients with the Tt genotype were significantly more likely to have a negative culture result within 1 month of treatment initiation, compared with patients with the TT genotype (RR, 9.6; 95% CI, 1.2–75.9) (table 3). For both TaqI and FokI, the results remained similar and statistically significant in sensitivity analyses that incorporated the 19 case patients excluded because of missing data; likewise, the results were essentially unchanged after the exclusion of the 5 patients with MDR-TB, according to the initial culture results (analyses not shown).

Of the potential risk factors analyzed by univariate Cox regression (see the Patients and Methods section), 4 were strongly associated with a significantly higher probability of culture conversion during treatment (RRs are adjusted for age and sex): TaqI TT genotype (RR, 4.28; 95% CI, 1.88–9.75; \( P = .001 \)), FokI FF genotype (RR, 2.35; 95% CI, 1.15–4.83; \( P = .020 \)), completion of secondary school education (RR, 2.47; 95% CI, 1.42–4.28; \( P = .001 \)), and susceptibility to all antibiotics (RR, 3.49; 95% CI, 1.63–7.48; \( P = .001 \)). The effect of increased body mass index was statistically significant but minor (RR, 1.04; 95% CI, 1.00–1.08; \( P = .30 \)). The multivariate Cox regression analysis showed that, of the aforementioned factors, all but the FokI FF genotype remained significant independent predictors of culture conversion (data not shown).
culture result, compared with patients with at least 1 f allele (i.e., Ff or ff genotypes). These findings suggest that variations in the VDR structure or activity modify the host response to TB. Given that the beneficial effect of either the Tt or FF genotype during treatment was more pronounced when culture conversion was considered, compared with auramine stain conversion, VDR polymorphisms likely affect mycobacterial viability, not just the quantity of expectorated microbes.

Some investigators have proposed that ethnic factors are more important than socioeconomic determinants of susceptibility to TB [29]. Genetic variability at the VDR locus could partially explain the influence of ethnicity on the immune response to TB [30]. Because socioeconomic and genetic factors may be distributed together in a nonrandom fashion, it is also possible that the Tt and FF genotypes served as markers of faster disease resolution because of a link to more favorable socioeconomic conditions. Education level (defined by completion of secondary school education) was associated with treatment outcome, perhaps because it affects treatment compliance. Body mass index had a very weak association with treatment outcome, but it might be a marker of pretreatment macronutritional status. However, we did not detect any associations between VDR polymorphisms and either education level, employment status, or body mass index, and the Tt genotype was strongly and independently predictive of a higher likelihood of sputum conversion in multivariate analysis, suggesting that there is a direct association between the VDR and the immune response to TB.

Variations in the response to TB treatment associated with VDR polymorphisms may be related to the expression of tissue matrix metalloproteinases (MMPs), host enzymes that have been implicated in the regulation of chronic inflammation. M. tuberculosis–stimulated human monocytes specifically up-regulate expression of MMP-9 [31], and circulating levels of MMP-9 appear to correlate with severity of TB disease [32]. The TaqI T allele has been found to be associated with diminished production of an anti-proteinase (TIMP-1) that is a natural inhibitor of MMP-9 [33]. The T allele might therefore predispose to a more aggressive form of TB and, hence, a slower response to treatment, through potential VDR-mediated regulation of metalloproteinase synthesis. An additional consideration is that both rifampin and isoniazid alter metabolism of vitamin D [34], so it is possible that the influence of VDR polymorphisms on the host response to TB may be affected by the treatment itself.

In contrast to the results of the cohort study, in the case-control study, we did not find a direct association between either the VDR FokI or TaqI polymorphisms and susceptibility to development of active TB; this result is consistent with the results of some previously published studies [7, 17], but not with the results of other studies [6, 15, 18]. However, we observed a trend in which the prevalence of the t allele was highest among PPD-negative healthy control participants, intermediate among PPD-positive healthy control participants, and lowest among patients with TB. As Bellamy et al. [6] first demonstrated in West Africans, the t allele may reduce susceptibility to either initial mycobacterial infection or progression of a latent infection to active disease. The highly skewed TaqI allele distribution in Peruvians, in whom the homozygous tt genotype was virtually absent, contrasts with the distribution noted in West Africans (prevalence of genotypes: TT, 45%; Tt, 43%; and tt, 12%) [6], suggesting that a relatively larger sample size may be required to demonstrate a statistically significant effect of the TaqI polymorphism on susceptibility to TB in Peru.

In summary, the present study is the first, to our knowledge, to demonstrate a host gene association with response to TB treatment in humans. The findings demonstrate the potential clinical relevance of immunomodulatory functions of vitamin D metabolites acting via the VDR in the host response against pulmonary TB. The effect of the VDR genotype on auramine stain and culture conversion times has important potential implications for both individual and public health, given that the rate of microbial clearance may alter the risk of transmission of bacteria within a community. Additional studies are needed to confirm these associations in different ethnic groups, to delineate the specific molecular mechanisms by which vitamin D metabolism influences the host response to M. tuberculosis, and to identify potential TB drug targets in the vitamin D metabolic pathways. Vitamin A and zinc supplementation during TB treatment have been shown to result in earlier sputum smear conversion [35], and there is now compelling evidence to suggest that vitamin D supplementation during TB treatment may be similarly beneficial.

Acknowledgments

We thank Carlton Evans and Mayuko Saito for their advice and comments, as well as Paula Maguila, Paula Maurtua, J. B. Phu and D. Sara for administrative support. We appreciate the generous assistance of Ross Eccleshall, of Stanford University (Stanford, CA), who helped to ensure the reliability of the vitamin D receptor polymerase chain reaction assay. The present study would not have been possible without the cooperation of the participants from Las Pampas de San Juan de Miraflores (Lima, Peru), who have helped to contribute to the generation of knowledge about health problems that profoundly affect their community.

References