Vitamin D-Receptor Gene Polymorphisms: Association With Virologic Failure and Mortality

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BACKGROUND

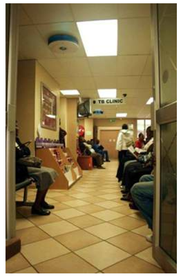
- Host genetic factors play an important role in HIV infection and disease progression.
- Due to its extensive role in the immune system, vitamin D may have a potential role in altering HIV disease progression.
- Vitamin D actions are mediated by the vitamin D receptor (VDR).
- Polymorphic variations exist in the VDR gene, each of which may have different significance in disease susceptibility and progression.
- Different loci of the VDR gene have different functions so that associations of HIV disease progression with specific polymorphisms may indicate unique mechanisms.

OBJECTIVES

- To examine if VDR gene polymorphisms in the 5' coding region, corresponding to Fok1 and the 3' Untranslated region, corresponding to Bsm1, Apa1, and Taq1 are associated with virologic failure and mortality among HIV-infected African patients

METHODS

- 142 HIV+ patients initiating ART between November 2008 - March 2009 at the Themba Lethu Clinic, Johannesburg, South Africa
- Followed up to March 2013



Eligibility criteria :

- age ≥ 18 years
- CD4 ≤ 350 cells/mm3
- first-line ART
- pregnant women were excluded
- ART naïve patients only

Study outcomes (Disease progression):

1. Failure to achieve viral suppression (<50 copies/ml) within 12 months after initiation of ART.
2. Mortality during the follow-up period

Person-time accrued from ART initiation until the earliest of: (1) the outcome – first follow-up viral load level > 50 copies/ml or death; (2) loss to follow-up (defined as 4 months late for last scheduled clinic visit); (3) transfer; or (4) close of dataset 31 March 2013.

Genotyping:

- Genomic DNA was extracted from whole blood using standard methods and stored at -20oC until required.
- DNA from each person was analysed for the polymorphisms in the VDR gene using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP).
- Restriction digestion of the PCR product was performed according to the manufacturer's instructions and results visualised on 2% agarose gels incorporating GelRed™ (Anatech, Johannesburg, South Africa).

Statistical analysis :

- Kaplan-Meier curves and the log-rank test used
- Cox proportional regression models constructed for univariate and multivariate analysis
- Multivariate models adjusted for: age, gender, BMI, WHO stage, CD4 count, hemoglobin level, employment status and TB status.
- Tests to estimate Hardy-Weinberg equilibrium conducted by genotype
- Statistical analysis in STATA 12
- Genomic DNA was extracted from whole blood using standard methods and stored at -20oC until required.

RESULTS

Table 1 : Characteristics of study cohort at baseline and end of follow-up

At ART initiation	n (%) or median (IQR)
Total	142
Female	99 (69.7%)
Tuberculosis	40 (28.2%)
WHO III/IV	53 (39.5%)
EFV-3TC-d4T	114 (82.6%)
CD4 count (cells/ml)	78 (36 – 140)
BMI (kg/m^2)	21.2 (18.3 – 24.6)
Haemoglobin (g/dl)	11.1 (9.6 – 12.4)
Age (years)	38 (31.4 – 45.2)
End of Follow-up	
Follow-up time (months)	40.4 (IQR 13.3 – 41.7)
Death	18 (12.7%)
Lost to follow-up	25 (17.6%)

Figure 1: Kaplan-Meier curves for VDR genotype and virologic failure

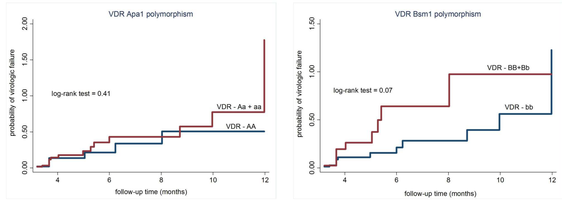


Table 1: Associations between Vitamin D Receptor Gene Polymorphisms and virologic failure within 12 months post-ART initiation

Genotype	cHR (95% CI) p-value	aHR* (95% CI) p-value
AA AA/Aa	1.00 1.40 (0.62 – 3.14) 0.41	1.00 1.40 (0.58 – 3.36) 0.45
bb BB/Bb	1.00 2.04 (0.94 – 4.44) 0.07	1.00 3.05 (1.18 – 7.90) 0.02
FF Ff/ff	1.00 1.56 (0.65 – 3.72) 0.32	1.00 2.25 (0.83 – 6.07) 0.11
TT Tt/tt	1.00 1.04 (0.45 – 2.41) 0.93	1.00 0.99 (0.40 – 2.45) 0.98

Figure 2: Kaplan-Meier curves for VDR genotype and mortality

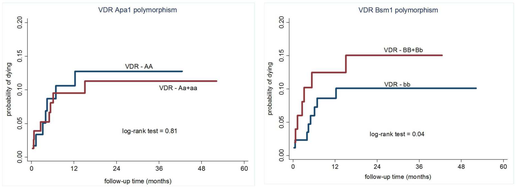


Table 1: Associations between Vitamin D Receptor Gene Polymorphisms and mortality during the follow-up period post-ART initiation

Genotype	cHR (95% CI) p-value	aHR* (95% CI) p-value
AA AA/Aa	1.00 0.88 (0.32 – 2.43) 0.81	1.00 0.71 (0.22 – 2.28) 0.57
bb BB/Bb	1.00 2.32 (1.01 – 5.32) 0.04	1.00 3.51 (1.10 – 11.20) 0.03
FF Ff/ff	1.00 1.27 (0.41 – 4.00) 0.68	1.00 1.23 (0.35 – 4.28) 0.75
TT Tt/tt	1.00 0.72 (0.26 – 2.00) 0.53	1.00 0.49 (0.16 – 1.52) 0.22

KEY FINDINGS

- Individuals carrying non-bb variants of the *Bsm1* polymorphism in the VDR vs. the bb variant:
  - 3.0 fold greater risk of virologic failure
  - 3.5 fold greater risk of mortality
- We could not demonstrate associations between disease progression and the *Apa1*, *Fok1* and *Taq1* variants

CONCLUSIONS

- Patients exhibiting the VDR B allele may be less responsive to the immunoregulatory actions of vitamin D and hence show less favorable disease progression.
- Also, it is possible that the VDR B allele may be a marker allele which is linked to a functional allele located nearby in the same gene influencing HIV disease progression.
- Understanding this role of VDR genotypes among HIV-infected patients may offer new insights for developing therapeutic strategies to alter disease progression.

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