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Vitamin D and TMEM16J Host Variants Associated with TB and Death in HIV-Infected and –Exposed Infants

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Background

Research Questions:

- What is the prevalence of Vitamin D insufficiency and deficiency (VDID) in infants in South Africa?
- Is VDID an independent risk factor for Tuberculosis (TB) infection, TB disease and death in HIV infected and HIV-exposed, un-infected infants?
- Are host vitamin D receptor (VDR) and PKP3-SIGIRR-TMEM16J genetic variants independent risk factors for latent TB infection (LTBI), TB disease and death among HIV infected and HIV-exposed, un-infected infants?

VDID is prevalent among HIV–infected adult populations but prevalence in infants in Africa not well studied

Vitamin D and VDRs have important roles in the human immune system

- Low vitamin D increases susceptibility to TB and VDRs are upregulated when toll-like receptors (TLRs) are activated against TB
- VDRs play a role in adaptive and innate immunity

Recently PKP3-SIGIRR-TMEM16J Variants, which have a negative regulatory effect on TLR pathways and which initiate host immune response to pathogens such as *Mycobacterium tuberculosis,* have been associated with the risk of TB in adults (Horne JID 2012)

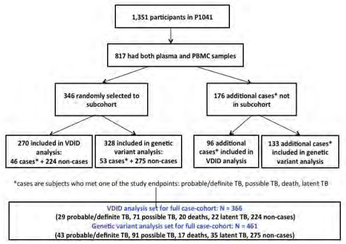
Relationship between VDID, VDR and TMEM16J variants and pediatric TB and death are not known

Methods

Study Population & Design

- Case-cohort within IMPAACT P1041
  - Randomly selected, pre-specified sub-cohort (n=346) with adequate plasma and peripheral blood monocyte (PBMC) samples + all additional cases who developed outcomes in P1041
- P1041 was a multi-center, prospective, phase II/III, randomized, double-blind placebo-controlled study to determine the efficacy of primary Isoniazid (INH) prophylaxis to prevent Tuberculosis (TB) and Latent TB Infection (LTBI) among HIV-1 perinatally-exposed infants (both infected [HIV+] and uninfected [HIV-]) in South Africa (Madhi NEJM 2011)
- All infants BCG vaccinated with no known previous exposure to TB at entry
- 96 weeks follow-up for outcomes
- **Lab Assessments**
- Serum vitamin D levels were determined using the DiaSorin immunoassay
- 5 vitamin D related SNPs: rs1544410, rs2228570, rs2282679, rs12785878 and rs10741657, and 3 SNPs in the PKP3-SIGIRR-TMEM16J region associated with TB susceptibility rs10902158, rs7105848, and rs7111432-G/A (TMEM16J) were assessed by real-time PCR

Figure 1. Study flow chart



Vitamin D Definitions (using 25(OH) Vit D)

- Sufficient: ≥32 ng/ml (80 nmol/l)
- VDID: <32 ng/ml
  - Insufficient: 20-31 ng/ml
  - Deficiency: <20 ng/ml

Methods

Outcomes:

1. ***Definite TB:*** **Mycobacterium tuberculosis isolated from any site**
2. ***Probable TB:*** **Positive AFB stain plus:**
   - i. 2 algorithm clinical criteria; OR
   - ii. at least one abnormal CXR feature suggestive of TB
3. ***Possible TB:*** **CXR findings suggestive of PTB and**
   - i. score >=6 on screening algorithm; OR
   - ii. Reactive TST
4. ***Latent TB Infection*****: +TST(>10mm) at week 96 in the absence of clinical TB**
5. ***Death*** **by 96 weeks**

Algorithm to Screen for and Diagnose Clinical Tuberculosis	Score				
	0	1	2	3	4
Weeks of illness (including cough)	<2	2-4		>4	
Nutrition (% weight for age)/malnutrition	>80%	60-80%	<60% or drop >2centiles		
Family history of TB	None	Reported by family		Sputum proven	
Tuberculin Skin Test				>5mm	
Fever not responding to Rx for >2 weeks			X		
Pre-specified confirmed or suspected EPTB					X

Results

Table. Baseline Characteristics of full case-cohort

Characteristic	Infants chosen for subcohort (N=346*)	Case in Subcohort? No (N=292)	Case in Subcohort? Yes (N=54)	Non-subcohort cases (N=176)
Site				
Johannesburg	271 (78%)	225 (77%)	46 (86%)	124 (70%)
Cape Town	49 (14%)	45 (15%)	4 (7%)	42 (24%)
Durban	26 (8%)	22 (8%)	4 (7%)	10 (6%)
HIV status				
HIV-infected	148 (43%)	118 (40%)	30 (56%)	94 (53%)
HIV-uninfected	198 (57%)	174 (60%)	24 (44%)	82 (47%)
Weight z-score (WHO)				
Mean (s.d.)	-0.77 (1.43)	-0.30 (1.14)	-0.90 (1.47)	
Median (Q1, Q3)	-0.68 (-1.41, 0.14)	-0.25 (-0.99, 0.61)	-0.78 (-1.63, 0.08)	
Birth Weight<2500 gms				
Yes	58 (17%)	48 (16%)	10 (19%)	32 (7%)
No	288 (83%)	244 (84%)	44 (81%)	144 (82%)
Mother Ever had TB Diagnosis				
Yes	24 (7%)	21 (7%)	3 (6%)	12 (7%)
No	322 (93%)	271 (93%)	51 (94%)	164 (93%)
Study participant ever breastfed				
Yes	39 (11%)	35 (12%)	4 (7%)	16 (9%)
No	307 (89%)	257 (88%)	50 (93%)	176 (91%)

Results

Figure 2: Prevalence of VDID in random sub-cohort (%)

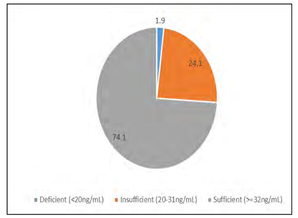


Figure 3: Summary of Cox Proportional Hazards Regression Models for VDID

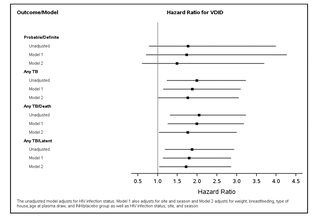


Figure 4: Frequency (%) of PKP3 genotypes in full case-cohort

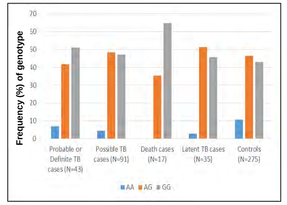


Figure 5: Frequency (%) of ANO9 genotypes in full case-cohort

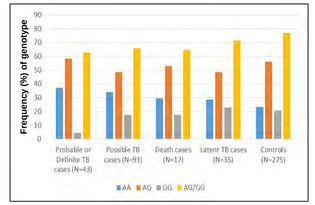


Figure 6: Summary of Cox Proportional Hazards Regression Models for rs10902158 (PKP3)

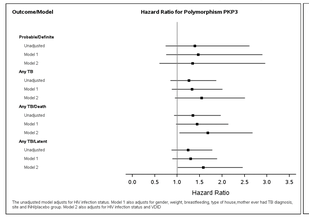
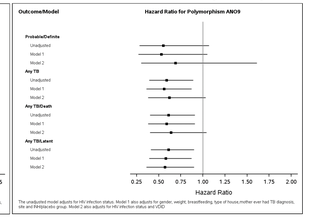


Figure 7: Summary of Cox Proportional Hazards Regression Models for rs7111432 (ANO9)



Results

- Median age at Vitamin D draw was 8 months; 11% ever breastfed; 51% HIV-infected
- VDID prevalence was 26%

VDID and outcomes

- 138 TB cases (43 definite/probable and 95 possible) and 26 deaths included in analysis
- Children with VDID had a 77% greater risk of probable/definite TB (HR 1.77, p=0.17) adjusting for HIV alone
- When possible TB was included, VDID significantly associated with TB (HR 1.99, p=0.005)
- Adjusting for HIV-status, season, site, sex, WAZ score, breastfed, type of house, prior maternal TB, age at Vitamin D draw, and INH/placebo treatment arm, VDID associated with any TB (aHR 1.75, 95% CI 1.01-3.05, p=0.046) as well as any TB or death (aHR 1.76, 95% CI 1.03-3.00, p=0.038)

Host genetic variants and outcomes

- No polymorphism associated with VDID
- Adjusting for HIV, sex, WAZ score, breastfed, type of house, prior maternal TB, and INH/placebo treatment arm, TMEM16J G-allele was protective of probable/definite TB (aHR 0.53, p=0.07), any TB (aHR 0.56, p=0.017), any TB/death (aHR 0.59, p=0.016) and any TB/latent TB (aHR 0.58, p=0.0009)
- Adjusting for the same variables, having GG polymorphism of the PKP3 variant was marginally associated with increased risk of any TB/death (aHR 1.44, p=0.07)

Conclusions and Implications

- In young children with and without HIV, VDID was associated with a nearly two-fold increased risk of any TB or death
- SNPs in the PKP3-SIGIRR-TMEM16J gene region are associated with tuberculosis and death in young HIV-infected and –exposed children
- Further studies needed to evaluate whether
  - vitamin D supplementation could reduce TB and death in HIV-exposed pediatric populations
  - these SNPs are associated with similar outcomes in diverse human pediatric populations

Acknowledgements

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