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Genetic and Clinical Predictors of CD4 Recovery During Suppressive cART: WIHS

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Background

- Depending on how immunologic recovery is defined, 17-75% of recipients of virologically suppressive cART (<80 copies experience very slow or minimal recovery of circulating CD4 T cells.
- Definitions of adequate immunologic treatment responses vary with respect to threshold (CD4 count after some period of treatment), net increase(change in cell count from pre-treatment to on-treatment), duration of treatment before response is assessed, and whether immune functionis included.
- Worse CD4 cell recovery has been linked to: greater age, more chronic inflammation, co-infections, longer time from infection to treatment, fibrosis of lymph nodes, persistent perturbation of mucosal lymphoid tissue, AIDS-defining and non-defining cancer, and other complications.
- Several host genetic mechanisms have been implicated in CD4 restoration during cART including *CCR5, CCR5 promoter* and *CCR2*genotypes, *CCL3L1* gene copy number, *SDF-1-3'A* genotype, CX_3CR1 genotypes, homozygosity for *HLA-Bw4*, IL-7Rα genotype, TRAIL, TNFα genotypes Bcl-2-interacting molecule, IL-15, IL-15Rα genotypes and IFNα, IL-2, IL-15R and mitochondrial haplotypes.
- We investigated factors that influenced CD4 recovery in a group of WIHS participants with well characterized treatment response phenotypes, including whole exome sequencing.

Methods

- 95 WIHS participants contributed to this study based on:
  - Reported receiving a potent combination ART regimen.
  - Consistent HIV RNA<50 copies for at least 2 years.
  - We selected 48 women with the most rapid and consistent gains in CD4 cells and 47 women with the least gain in CD4 cells over the two years of viral suppression.
  - Interpolations were used to replace missing CD4 or HIV RNA values if no change in therapy is reported at the following visit, and CD4/HIV RNA values are unchanged at the following visit.
- WIHS core data on race, age, CD4 nadir count, history of clinical AIDS, maximal viral load, current smoking, HCV coinfection, exposure to ddI or d4T, nucleosides in regimen, self reported adherence to cART during study interval, and level of anti-Müllerian hormone (biomarker of ovarian function) were evaluated as potential predictors of CD4 response.
- Whole exome sequencing (50X) (Figure 1) was performed using reposited PBMCs.
- Logistic regression was performed to model univariate associations. Stepwise logistic regression was used to build a multivariate model of the contributions of study variables to CD4 outcome group (rapid response vs. slow response).
- Gene-wise and transcript-level burden testing for rare (MAF<5%) and bothcommon and rare variants were conducted using CMC and KBAC regression, respectively.
- Bioinformatic analyses were used to assess 1st and 2nd order interactions of HIV with genomic characteristics, HIV integration and HIV CD4 T-cell expression studies.

Results

- Figure 2 illustrates an example phenotype plot from a participant who demonstrated rapid CD4 recovery after initiation of virologically suppressive cART. The period of response is denoted by a green capitalized **H**.
- Figure 3 illustrates an example of slow CD4 recovery during suppressive cART. The period of slow response is denoted by a lower care green **h**.
- Table One provides the results of univariate analyses of associations between non-genetic factors and CD4 response group. Greater age, years of smoking prior to cART, current smoking, CD4 nadir below 350 and adherence to cART regimen below 95% were all associated with slower CD4 recovery. Interestingly, race, maximal viral load, active HCV infection (DNA+), receipt of deoxy-nucleosides, were not associated with CD4 recovery group.
- The final stepwise logistic regression model (Table Two) selected lesser age and >95% self-reported adherence to the cART regimen as predictive of rapid CD4 recovery. Greater age has been consistently identified as a factor associated with lesser CD4 recovery, perhaps due to somatic aging and reduced thymic function.
- The association of imperfect adherence and slow CD4 recovery is interesting since this study was limited to participants with viral load values below assay detection. This may indicate that low intensity viremia persisted in these women, or that virologic control was better at the time of WIHS sampling than at other times in the 6 month study interval.
- Full exome sequencing results are illustrated by Figure 4. which demonstrates specific genes and gene locations that included variability that was associated with CD4 response group.
- Table 3 summarizes these genes. Of note 4 genes are known to interact directly with HIV or its products. An additional 11 genes relate to pathways that are known to interact with HIV or its products. 5 of the genes that demonstrated variability that was associated with the CD4 response group have no known interaction with HIV. These findings support the concept that persistent viral replication differentiates the rapid CD4 responders from the slow.

Figure 1: Graphic DepictionWhole Exome Sequencing

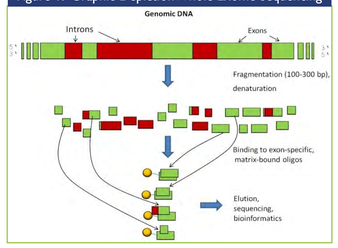


Figure 2: Example of Rapid CD4 Recovery Phenotype

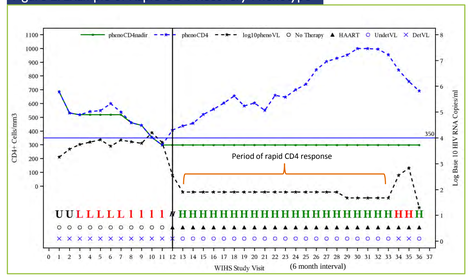


Figure 3: Example of Slow CD4 Recovery Phenotype

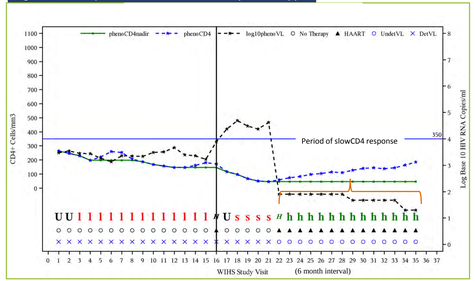


Table One: Univariate Analyses of Non-genetic Factors Associated with CD4 Response

Variable	Total (N=95)	Rapid Responders (n=48)	Slow Responders (n=47)	P-value
Age	42.0 ± 8.4	39.2 ± 7.5	44.8 ± 8.4	0.0010
Years smoked pre-cART	13.7 ± 13.5	10.3 ± 11.8	17.2 ± 14.4	0.015
AMH Undetectable	0.3 ± 0.4	0.2 ± 0.4	0.3 ± 0.5	0.067
CD4 nadir	219.7 ± 155.8	255.3 ± 187.0	183.4 ± 106.0	0.12
Max VL pre-cART (log10)	4.6 ± 0.8	4.6 ± 0.8	4.5 ± 0.9	0.79
Race				1.00
White(Non-Hispanic)	24 (25.3%)	12 (25.0%)	12 (25.2%)	
White(Hispanic)	14 (14.7%)	7 (14.6%)	7 (14.9%)	
African-American (NH)	31 (32.6%)	16 (33.3%)	15 (31.9%)	
Other (Hispanic)	26 (27.4%)	13 (27.1%)	13 (27.7%)	
Current Smoker (pre-cART) (%yes)	40 (42.1%)	15 (31.3%)	25 (53.2%)	0.030
AIDS before index visit (%yes)	42 (44.2%)	17 (35.4%)	25 (53.2%)	0.081
CD4 nadir < 350 (%yes)	78 (82.1%)	35 (72.9%)	43 (92.0%)	0.018
HCV active cART (%yes)	29 (30.5%)	11 (22.9%)	18 (38.3%)	0.10
Nucleoside treatment (%yes)	95 (100.0%)	48 (100%)	47 (100%)	1.00
ddI or d4T (%yes)	71 (74.7%)	32 (66.7%)	39 (83.0%)	0.067
>95% adherent (%yes)	56 (58.9%)	33 (68.8%)	23 (48.9%)	0.0090

Table Two: Final stepwise logistic regression model of non genetic factors associated with rapid CD4 recovery

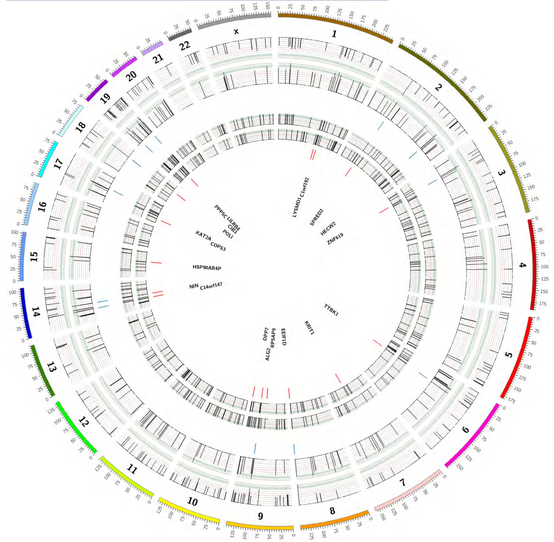
variable	odds ratio	95% CI (odds ratio	p-value
Age at start of phenotype	0.933	0.878 – 0.992	0.026
>95% adherent (self reported)	2.688	1.038 – 7.0	0.042

Nadir CD4, maximal VL, smoking years, race, HCV infection, clinical AIDS not selected by stepwise logistic regression. Age treated as linear

Table 3: Genes that were associated with CD4 response

Gene	Gene Name	Position	HIV interaction
LYSMD1	LysM, putative peptidoglycan-binding, domain containing 1	1:151131224	unknown
C1orf192	FLTP, chromosome 1 open reading frame 192	1:161333521	unknown
SPRED2	sprouty-related, EVH1 domain containing 2	2:65536985	secondary
HECW2	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2	2:197062977	secondary
ZNF619	zinc finger protein 619	3:40517604	unknown
TTBK1	tau tubulin kinase 1	6:43210222	secondary
KRIT1	KRIT1, ankyrin repeat containing	7:91827283	secondary
EEF1D	eukaryotic translation elongation factor 1 delta (guanine nucleotide exchange protein)	8:144660867	primary
RPSAP9	ribosomal protein SA pseudogene 9	9:79012515	unknown
ALG2	alpha-1,3/1,6-mannosyltransferase	9:101977707	secondary
DPP7	dipeptidyl-peptidase 7	9:140003992	secondary
C14orf147	serine palmitoyltransferase, small subunit A	14:34902144	secondary
NIN	ninein (GSK3B interacting protein)	14:51185481	secondary
ADAM10/ HSP90AB4P	ADAM metallopeptidase domain 10/heat shock protein 90kDa alpha (cytosolic), class B member 4, pseudogene	15:58886403	primary
COPS3	COP9 signalosome subunit 3	17:17148938	primary
KAT2A	K(lysine) acetyltransferase 2A	17:40264129	primary
POLI	polymerase (DNA directed) iota	18:51794849	secondary
CIB3	calcium and integrin binding family member 3	19:16271179	unknown
PPP5C	protein phosphatase 5, catalytic subunit	19:46849251	secondary
LILRB4	leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 4	19:55173271	secondary

Figure 4. Ideogram plot of 22 autosomes and the X chromosome



Discussion

- This study affirmed the strong, inverse relationship between age and CD4 cell recovery during virologically suppressive cART;
- Self reported nonadherence predicted slow CD4 recovery, despite the limitation of this study to women who had viral loads <80 copies during treatment. This finding may indicate that viral load, measured at the time of study visit, fails to reflect viremia that occurred between visits. Alternatively, this finding may indicate that very low intensity viral replication, below standard VL assay quantitation, may adversely impact CD4 recovery.
- Genetic analyses further support the concept that persistent HIV replication strongly influences CD4 recovery.
- Whole exome sequencing and bioinformatic analyses may identify novel candidate genes and physiological pathways that may influences CD4 expansion. These data can support improved understanding of the mechanisms of CD4 cell regulation, and may lead to new therapeutic strategies to improve CD4 recovery in HIV infection.
- We are now conducting quantitative studies to determine if very low level viremia, or episode viremia is occurring in this study group to further clarify the mechanisms of CD4 response failure.
- Clinicians should continue efforts to maximize regimen adherence, including educating patients that intermittent viral suppression is not enough, even when clinical viral load results appear optimal.

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