

Abstract

(modified from original)







Background: HIV-1 infection is associated with declines in CD4⁺ T-cell numbers and a generalized immunosuppressive state. The advent of HAART has significantly improved immune function and quality of life in individuals infected with HIV-1, despite being linked to a variety of metabolic side effects. To more fully understand the molecular mechanism by which HIV-1 infection and HAART alter cellular function, the expression of ~7000 genes were followed longitudinally in PBMCs collected from patients before and after initiating or interrupting HAART.

Methods: Gene expression levels were determined using Affymetrix's HuFL GeneChip. PBMCs were collected from 6 healthy control subjects, 5 protease inhibitor-naïve patients (AVBIO study) pre- and 6 months post-initiation of HAART and in 6 long-term HAART recipients pre- and 1 month post-therapy interruption and following re-initiation of HAART (NOHRT study). Differentially expressed genes were identified by using statistical techniques that uses repeated permutations of the data to generate gene lists with <7.5% predicted false-positive calls.

Results: Global effects of HIV infection and the use of HAART were determined by grouping patient samples as either ON or OFF HAART and comparing patterns of expression to those of six HIV-negative controls. HIV viremia modulated 121 genes including 23 stress response genes that collectively show a heightened state of immune activation. Roughly one-half of the genes induced by viremia were unaffected by HAART, indicating that HIV infection induces persistent changes in the immune system, some of which may favor virus production during HAART-mediated viral suppression or therapy interruption. HIV and HAART led to the modulation of 23 genes involved in biochemical pathways that integrate retinoid metabolism, lipid and cholesterol metabolism, and energy production.

Conclusions: These results expand our view of in vivo transcriptional consequences of HIV infection, link differential gene expression to metabolic toxicities associated with HIV and HAART, and suggest molecular targets that may be amenable to therapeutic intervention.

Expression Profiling of Chronic HIV Infection, HAART Initiation, and HAART Interruption and Restart

Sample	Ctrl	NAIVE		NOHRT		
		D0	WK24	D0	WK5	FW24
HAART	-	-	+	+	-	+
μ -array						
N	6	5	5	6	6	6

1. What are the global effects of HIV-1 infection and HAART-mediated viral suppression?

2. What distinguishes chronically infected protease-naïve patients from long-term HAART recipients interrupting therapy?

Clinical Phenotypes Presented by HIV-1 Infected Individuals and/or HAART Recipients

Clinical Phenotype	HIV	HAART	Reference
Dementia	+		<i>Nuovo , 1996</i>
Increased BMR	+		<i>Macallan, D.C., 1999</i>
Loss of Appetite	+		<i>Macallan, D.C., 1999; Polsky, B., 2001</i>
Mitochondrial Toxicity	+	+	<i>White, A.J., 2001; Herman, J.S., 2001</i>
Myopathy	+	+	<i>White, A.J., 2001</i>
Nephropathy	+	+	<i>Saif, M.W., 2001</i>
Thrombosis	+	+	<i>Saif, M.W., 2001</i>
Wasting	+	+	<i>Hodgson, L.M., 2001; Polsky, B., 2001</i>
Hypercholesterolemia		+	<i>van der Valk, M., 2001</i>
Hyperlipidemia		+	<i>van der Valk, M., 2001</i>
Insulin Resistance		+	<i>van der Valk, M., 2001</i>
Lactic Acidemia		+	<i>White, A.J., 2001</i>
Lipodystrophy		+	<i>van der Valk, M., 2001</i>
Pancreatitis		+	<i>White, A.J., 2001</i>

Patient Cohorts

- **Patients:**
 - **6 HIV-negative controls**
 - **6 patients voluntarily interrupting and re-initiating therapy. All patients had viral rebounds of >100,000 copies of RNA/ml plasma within 1 month of terminating therapy. Samples were taken Pre-therapy interruption, 1 month post-therapy interruption and 6 months following re-initiation of therapy.**
 - **5 protease inhibitor-naive patients. Samples were taken before and 6 months after initiating HAART (4 drug regime).**
- **HIV-negative controls were used as the reference comparisons for HIV-infected patient samples in order to develop a “story” of how each of the sample comparisons from patients with HIV-1 infection (with and without therapy) vary from that of a “non-activate, healthy immune system”.**

Patient Characteristics

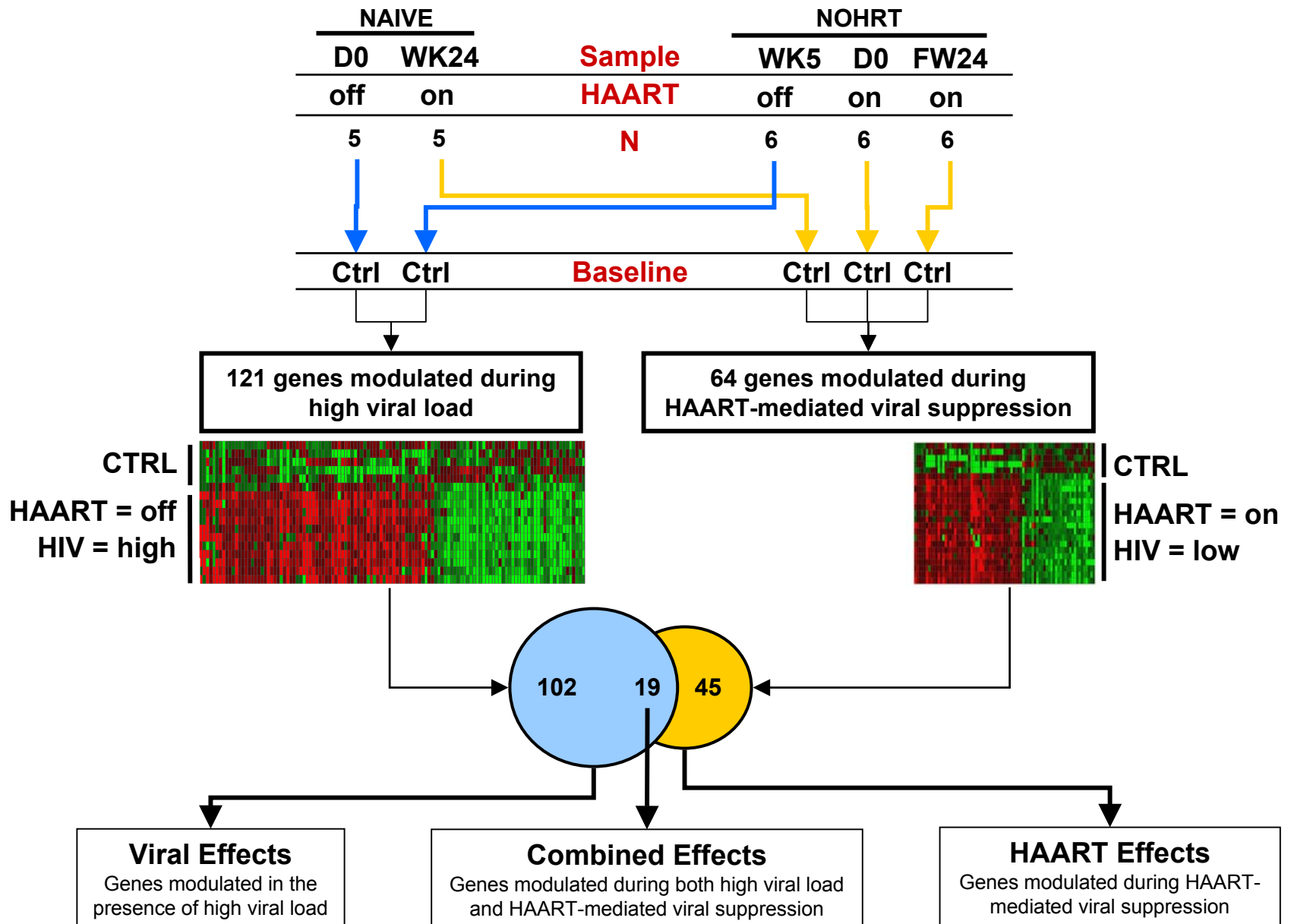
Cohort	Patient	Viral Load ¹		CD4 ² Tcell number ⁴	
		OFF HAART	ON HAART ³	OFF HAART	ON HAART ³
PI-naïve	1	351,000	< 500	479	620
	2	329,276	< 50	11	113
	3	186,200	< 500	7	102
	4	12,990	< 500	616	840
	5	19,280	60	784	1156
	Mean	88,371 ⁴	206	379	566
HAART interrupted	6	3,202,910	< 50 (<50)	582	869 (817)
	7	263,904	< 50 (<50)	1177	1597 (1244)
	8	158,099	< 50 (<50)	356	664 (666)
	9	114,062	< 50 (<50)	656	714 (708)
	10	113,269	< 50 (<50)	465	821 (781)
	11	127,125	< 50 (<50)	333	465 (477)
	Mean	245,603 ⁴	< 50 (<50)	595	855 (782)
Mean Total		154,330	50 ⁵	487	744 ⁵

1. RNA copies/ml plasma
2. Cells/ml blood
3. Parentheses indicate values obtained prior to interrupting HAART
4. Geometric mean

Methods

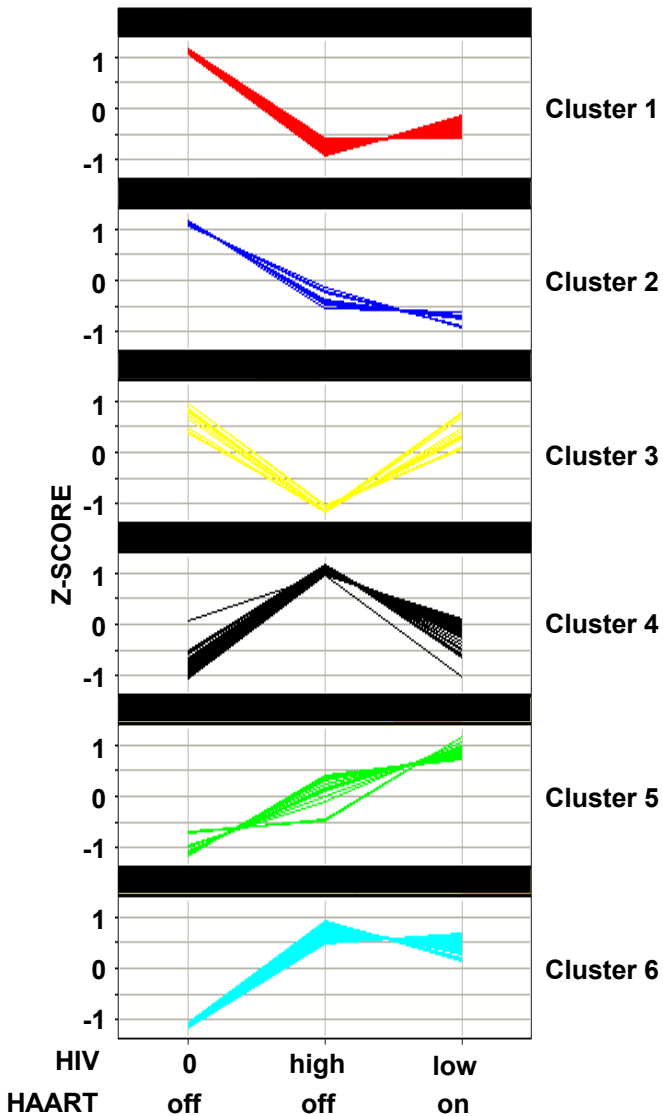
- **Expression levels of approximately 6800 genes were determined using the Affymetrix HuFL GeneChip and expression values determined using the DChip software package.**
- **ON (n=17) and OFF HAART (n=11) groups were compared to HIV-negative controls (n=6) to identify genes that significantly deviated from normal.**
- **Extensive pre-filtering was used to eliminate genes with changes in expression levels near the limits of detection and genes that did not differ from controls ($p > 0.10$, Student T-test).**
- **Pre-filtered genes were analyzed with the Statistical Analysis of Microarray (SAM) software package that uses an iterative data permutation method to calculate T-statistic scores.**
- **Gene lists were selected using SAM that had median false discovery rates (FDR) of $< 7.5\%$.**

Global Effects of HIV-1 Infection and HAART-Mediated Viral Suppression

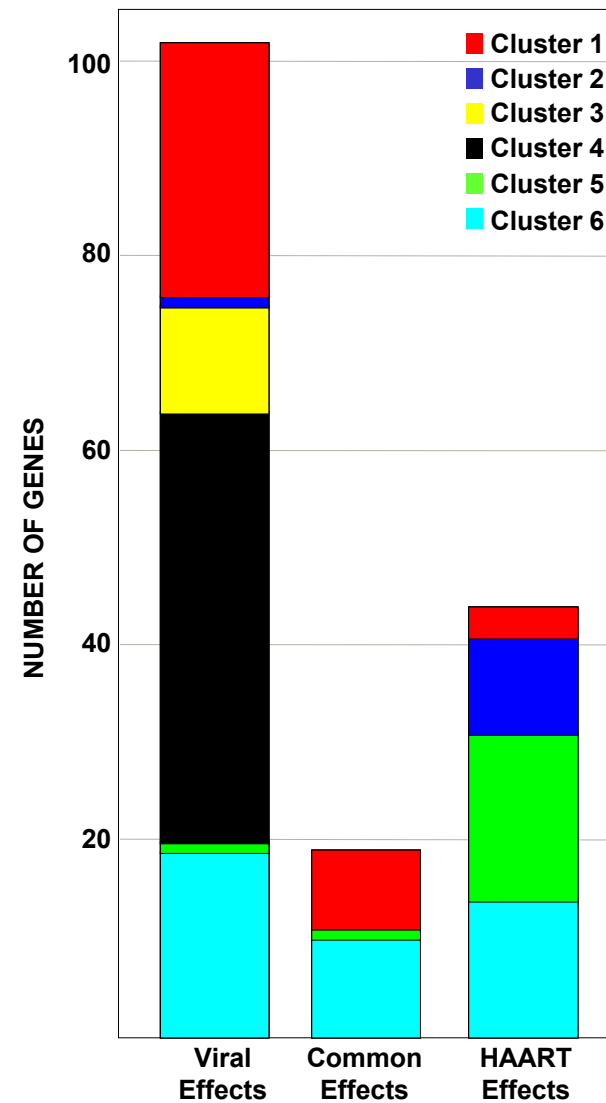


Profiles of Differentially Expressed Genes During High Viral Load and HAART-Mediated Viral Suppression

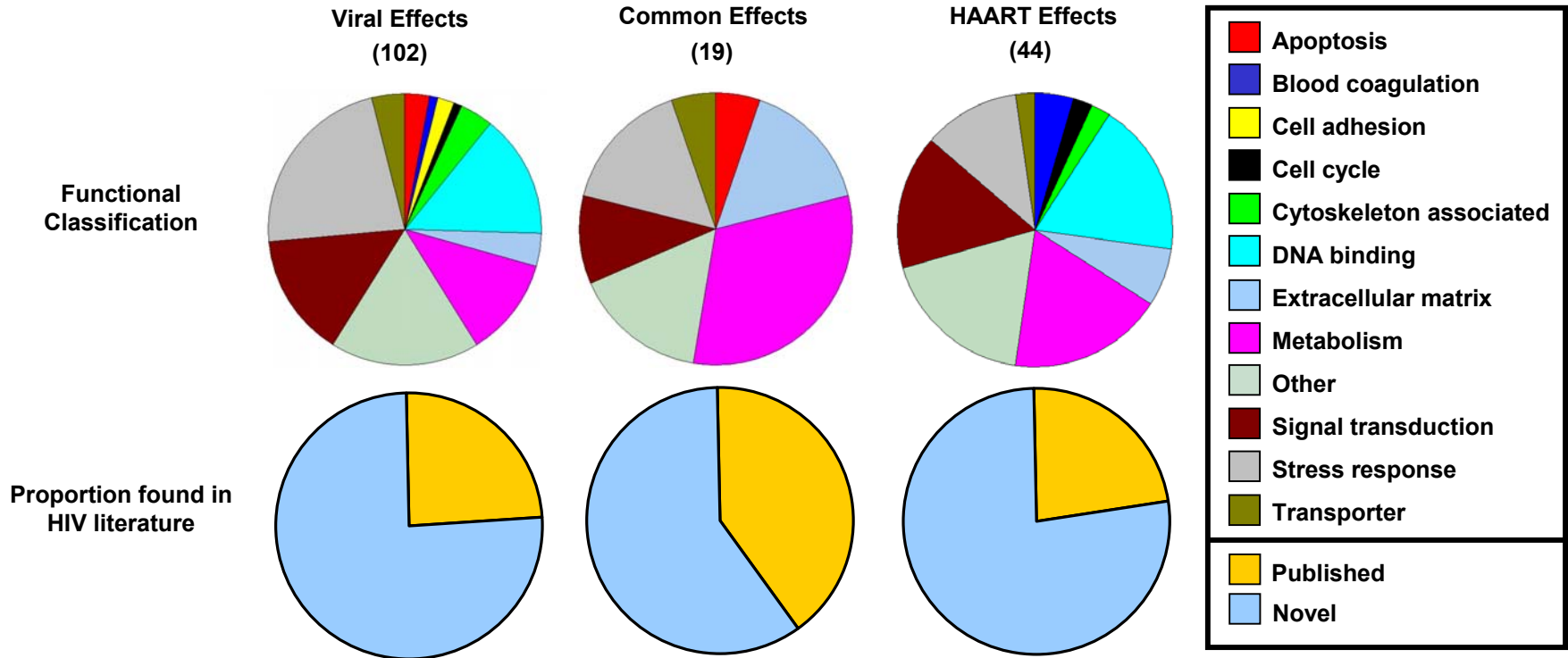
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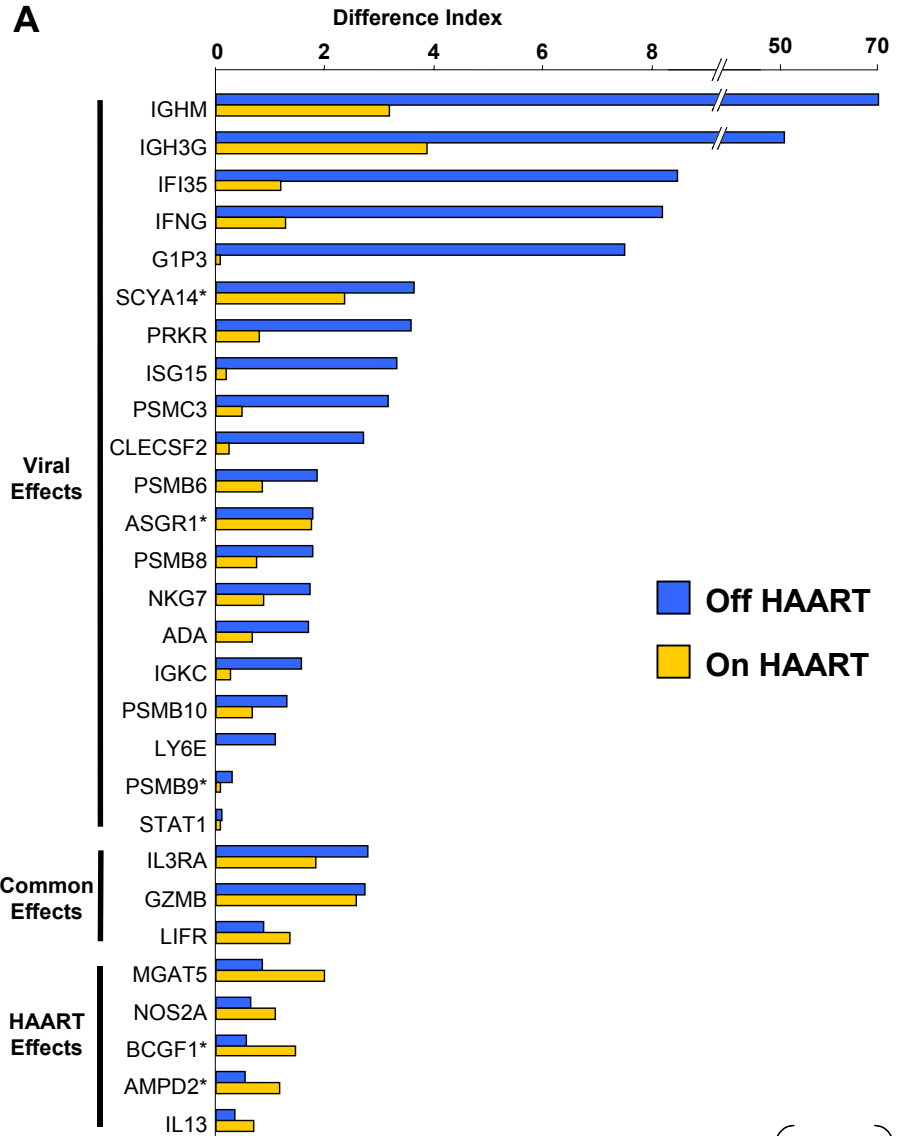
B



Functional Classification of Differentially Expressed Genes During High Viral Load and HAART-Mediated Viral Suppression

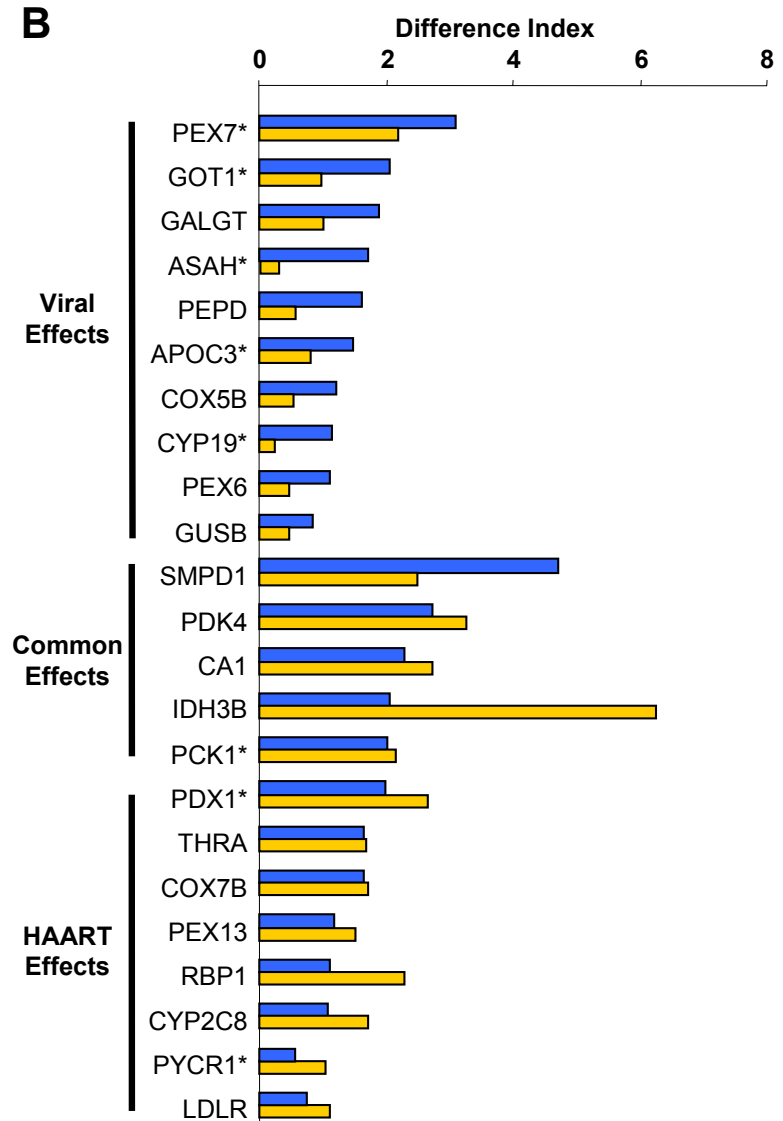


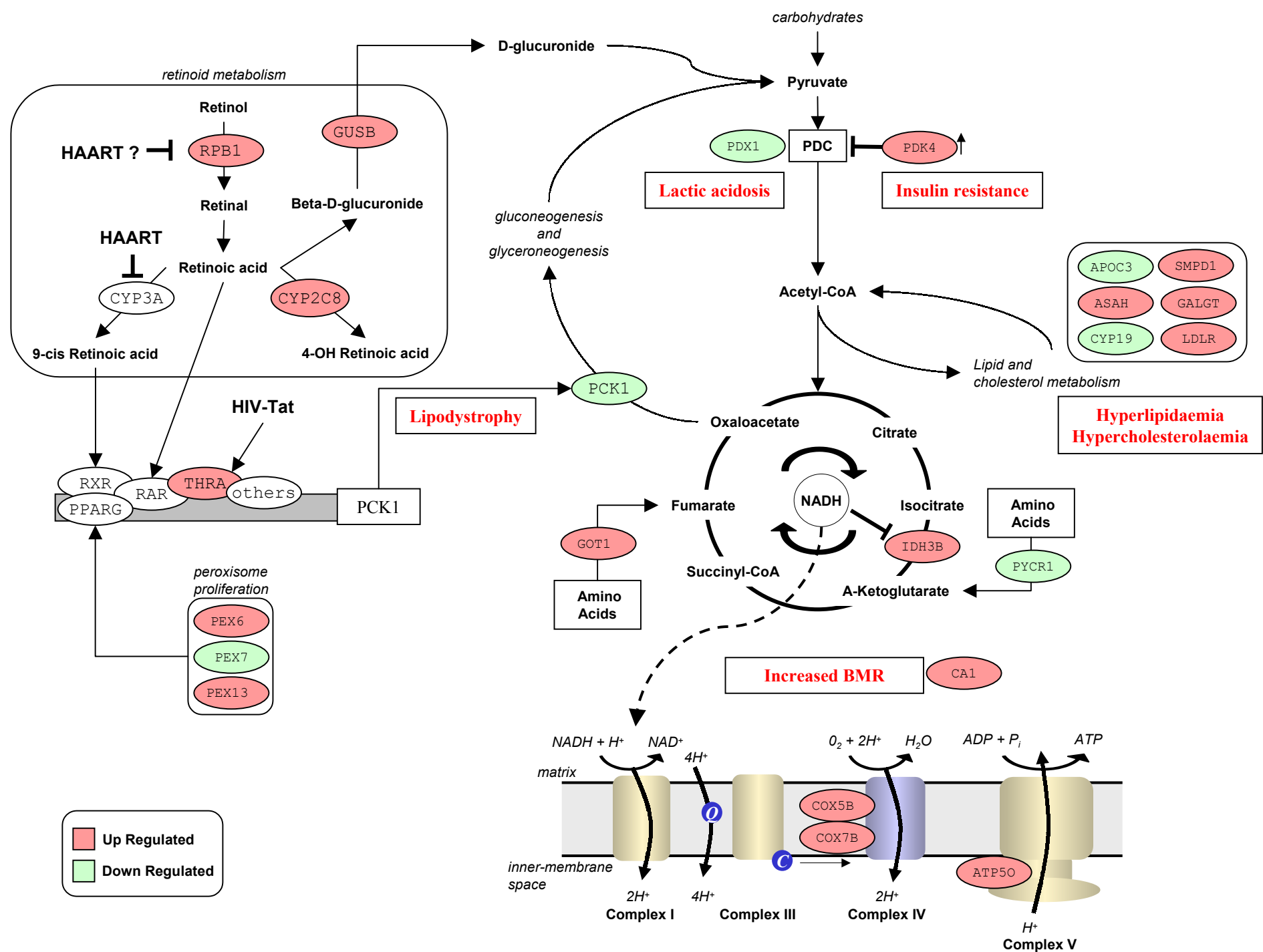
Stress Response Genes

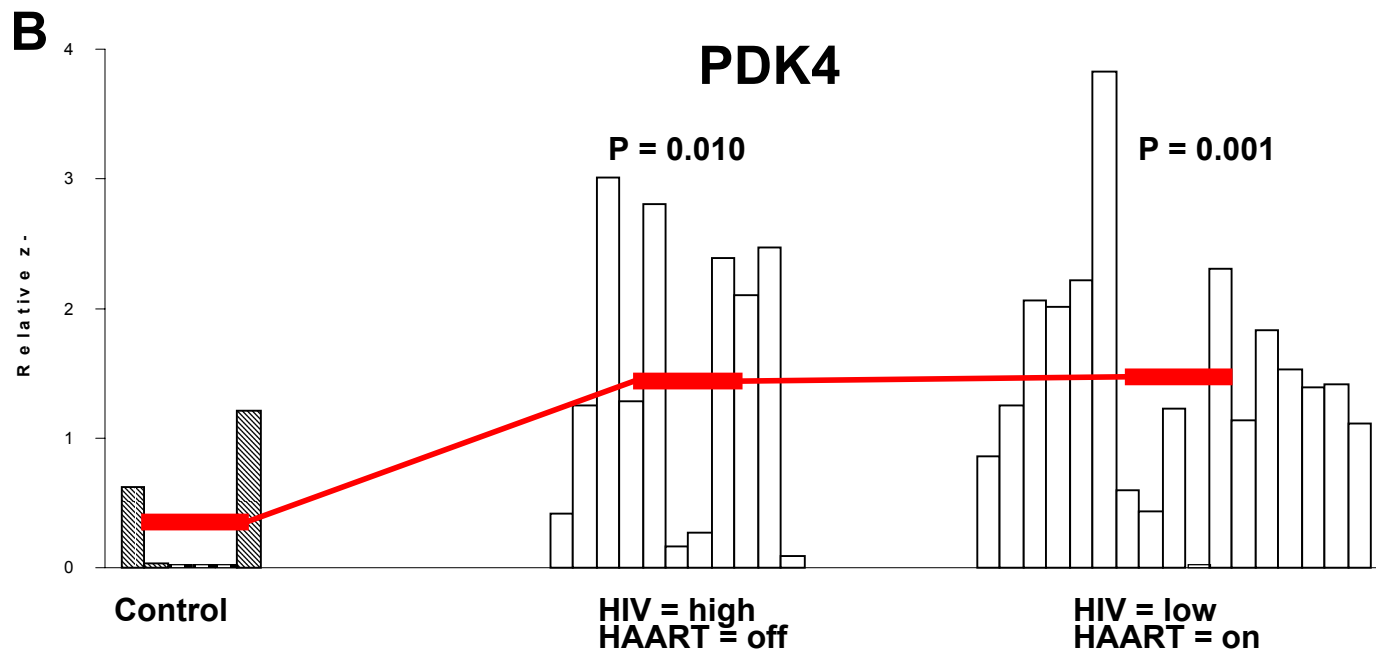
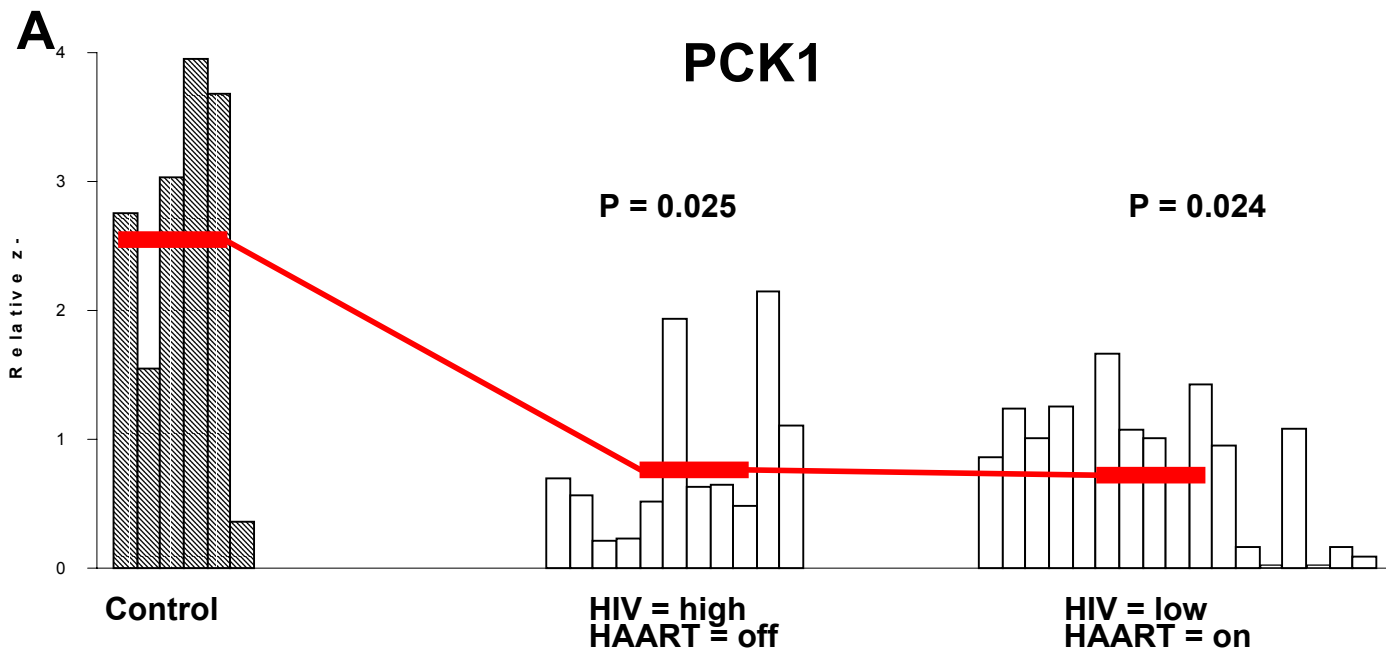


$$\text{Difference Index} = \left(\frac{\mu_t}{\mu_o} \right) \left(\frac{\mu_t - \mu_o}{\delta_t + \delta_o} \right)$$

Metabolism Genes







Conclusions

- **HIV infection induced the expression of 121 genes in common between PI-naive patients and long-term HAART recipients who volunteered to terminate therapy.**
 - **Only about half of these genes returned to normal levels during HAART, suggesting that HIV infection results in persistent changes in the immune system that are not restored following viral suppression below the limits of detection.**
 - **Some of these changes may continue to assist in low level replication of HIV during therapy and allow for HIV to rebound quickly following therapy interruption.**
- **HIV and HAART regulated the expression of 23 genes involved retinoid metabolism, lipid and cholesterol metabolism, and energy production.**
 - **Alterations in these biochemical pathways may provide clues to the causes of lipodystrophy, insulin resistance, hyperlipidaemia, hypercholesterolaemia and lactic acidosis.**