Poster 823

Genomics Links Autophagy with Neurocognitive Impairment in HIV-Infected Children

Stephen A. Spector[1,4], Sean S. Brummel[5], Pratima Rawat[1], Kumud K. Singh[1], Jihoon Kim[2], Kelly A. Frazer[1], Sharon Nichols[3], George R. Seage III[5], Paige Williams[5], Russell B. Van Dyke[6], Olivier Harismendy[2], Rodney N. Trout[1]

[1]Departments of Pediatrics[1], Medicine[2] and Neuroscience[3] University of California San Diego, La Jolla, California, USA, [4]Rady Children's Hospital, San Diego, California, USA; [5]Harvard T.H. Chan School of Public Health, Boston, MA USA, Department of Pediatrics, [6]Tulane School of Medicine, New Orleans, LA, USA

BACKGROUND

- HIV associated neurocognitive impairment (NCI) is a common complication of perinatal infection.
- The pathogenesis of NCI appears to be multifactorial.
- Few studies have examined the genetic risk factors associated with the development of NCI in perinatally HIV-infected (PHIV) infants and children.
- NCI persists in the ART era despite sustained viral suppression on combination antiretroviral therapy.
- Chronic inflammation has been increasingly implicated as an important mechanism in the pathogenesis of HIV-related NCI.
- We and others have identified dysfunctional autophagy as a mechanism that drives the chronic inflammation observed in HIV-related NCI.

OBJECTIVE

To identify host genetic variants associated with NCI in PHIV children (2 months – 18 years).

METHODS

- One Discovery Cohort (DC) and two Validation Cohorts (VC) of PHIV children were assessed.
- Discovery Cohort: Participants from PACTG 152 and PACTG 300 studies that predated effective cART; neurocognitive assessments were performed prior to the initiation of any ARVs (NEJM 1997;336:1704; J Pediatr 1998;133:500; Pediatr 1999:104:32).
- Validation Cohorts:
  - VC1: Pediatric HIV/AIDS Cohort Study (PHACS) Master Protocol (AMP): a contemporary cohort of PHIV children enrolled between the ages of 7 and 16 yrs and followed longitudinally.
  - VC2: PACTG 338 and PACTG 377: Enrolled HIV infected children between 4 mo (P377) or 24 mo (P338) and 17 yrs with CDC category 1 or 2 disease who were stable on ART and had never received a protease inhibitor (AIDS Res Hum Retro 2000; 16:1113; PIDJ 2002;21 :119; Pediatr 2005; 115: 380).
- Neuropsychological Measures: Cognitive status was determined using standardized global cognitive score for age (CSA). A CSA of <70 was considered cognitive impairment.
- Genome wide exome sequencing was performed on the Discovery Cohort consisting of 217 HIV-infected children with CSA <70 and 247 controls matched for age, CD4+ count and viral load with CSA ≥70.
- Validation cohort 1: SNPS meeting criteria in DC were assessed.
- Validation cohort 2: SNPs confirmed in VC1 were assessed in VC2.

STATISTICAL METHODS

- 674 significant variants in 831 genes were prioritized with cutoff criteria; Fisher's exact test P-value for dominant inheritance model) <0.005 and Hardy-Weinberg Equilibrium P-value greater than 0.05.
- SNPs reaching p ≤0.001 and odds ratios ≥1.5 were further identified yielding 29 SNPs in 24 genes.
- The 29 SNPs were evaluated in VC1 which identified 3 SNPs of interest
- The 3 SNPs were re-evaluated in VC2
- Counts, means and percentages were used to summarize analyses
- Logistic regression was used to estimate adjusted odds ratios
- The combined, across study odds ratio estimate was computed using inverse variance weights

RESULTS

Baseline characteristics of Discovery and Validation cohorts

	Discovery Cohort	Validation Cohort 1	Validation Cohort 2
N	464	451	357
Female	236 (50.9%)	241 (53.4%)	192 (53.8%)
Race/Ethnicity			
Black	286 (61.6%)	324 (75.7%)	190 (53.2%)
Hispanic	123 (26.5%)	109 (24.2%)	120 (33.6%)
White	51 (11%)	99 (22%)	44 (12.3%)
Unknown/Other	4 (0.9%)	23 (5.1%)	3 (0.8%)
CD4+ count/mm^3 (median)	859	733	668
Log HIV RNA (median)	**5.4**	**2.5**	**4.2**
Age (years, median)	**1.5**	**12**	**6**
Cognitive score (CSA, median)	**71.5**	**86**	**83**
CSA <70	**217 (46.8%)**	**54 (13.7%)**	**54 (15.1%)**
CSA ≥70	**247 (53.2%)**	**340 (86.3%)**	**303 (84.9%)**

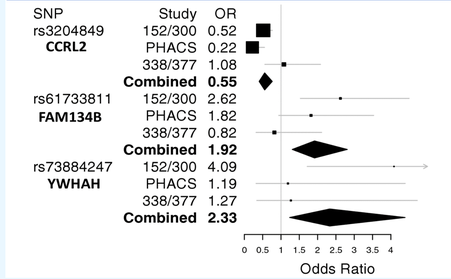
29 SNPs identified in 24 genes in Discovery Cohort

Chromosome Number	Gene	rs Number	Reference	Comparison	Odds Ratio	P-value
1	CDC27	rs3085	A	G	3.19	5.2E-04
1	IGSF3	rs201676764	C	T	3.61	3.1E-05
1	IGSF3	rs61786588	A	G	3.84	1.9E-06
1	IGSF3	rs61786589	C	G	11.59	3.3E-05
2	GRHL1	rs2303920	G	A	1.63	7.6E-04
2	LINC01237	rs4973668	C	G	2.19	4.8E-04
2	STEAP3	rs113158407	G	A	8.98	2.7E-04
3	CCRL2	rs3204849	T	A	0.52	8.1E-04
4	TMPRSS11B	rs12331141	C	T	0.48	1.8E-04
4	TMPRSS11B	rs2319797	A	T	0.48	1.7E-04
5	FAM134B	rs61733811	C	G	2.62	3.1E-04
6	LOC105377920	rs1051484	T	C	0.48	3.7E-04
7	ESYT2	rs2305473	T	C	1.92	5.7E-04
7	ESYT2	rs2305477	A	C	1.98	3.3E-04
7	KMT2C	rs111493987	C	A	3.77	2.0E-06
7	VWDE	rs73294382	T	C	4.36	7.6E-04
8	PABPC1	rs76261471	A	C	0.35	4.1E-05
10	DCLRE1A	rs17235066	T	C	2.50	3.8E-04
10	FAM21A	rs199520696	C	T	2.68	3.0E-04
11	MUC5B	rs202127660	A	G	3.93	2.5E-05
14	IGHV7-81	rs201928713	T	C	0.44	1.4E-04
16	PPL	rs61734749	C	T	9.10	2.5E-04
16	UBN1	rs35575708	C	T	9.10	2.5E-04
17	CDC27	rs201613328	C	A	2.79	4.0E-05
17	CDC27	rs78525224	A	T	3.97	8.2E-06
17	ICT1	rs34496172	C	T	2.39	1.2E-04
19	ZNF358	rs11555037	A	G	7.40	2.2E-04
21	LTN1	rs61735768	T	C	2.14	9.2E-04
22	YWHAH	rs73884247	A	G	4.09	5.3E-04

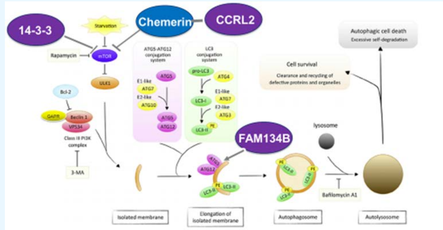
3 SNPs of interest from Validation Cohort 1

Chromosome Number	Gene	rs Number	Reference group	Comparison Group	Discovery Cohort Odds Ratio (95% CI)	Validation Cohort 1 Odds Ratio (95% CI)
3	CCRL2	3204849	T/T	A/A or A/T	0.52 (0.35, 0.76)	0.34 (0.18, 0.63)
5	FAM134B (RETREG1)	61733811	C/C	G/G or C/G	2.68 (1.56, 4.62)	1.26 (0.70, 2.25)
22	YWHAH (14-3-3)	73884247	A/A	A/A or A/T	4.09 (1.72, 9.73)	1.31 (0.52, 3.32)

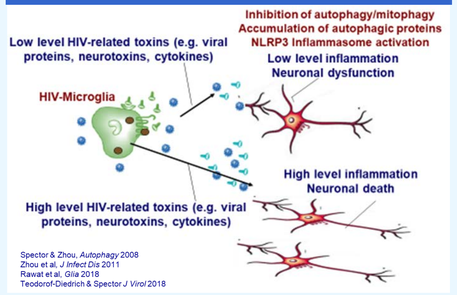
Forest plot of results from Discovery and Validation Cohorts



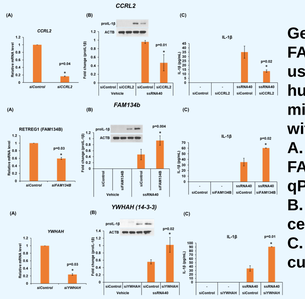
Schematic diagram of autophagy and CCRL2, FAM134B and YWHAH (14-3-3)



Schematic model of autophagy and inflammation in HIV-related NCI



Knockdown of YWHAH (14-3-3), CCRL2 & FAM134B in microglial cells alters Inflammatory response as measured by Interleukin-β (IL-1β)



Gene silencing of CCRL2, FAM134B and YWHAH (14-3-3) using siRNA transfection in human monocyte derived microglial cells (MG) treated with HIV ssRNA40 for 24 hr.
A. CCRL2, YWHAH and FAM134B expression in MG by qPCR.
B. Expression of proIL-1B in cell lysates by immunoblotting.
C. Quantification of mIL-1B in culture supernatants by ELISA.

CONCLUSIONS

- Genome wide exome sequencing in a Discovery Cohort of perinatally HIV-infected infants and children identified 29 SNPs potentially associated with cognitive impairment defined as CSA<70. Of these, 3 SNPs, CCRL2 (rs3204849), FAM134B (rs6173381) and YWHAH (rs73884247), remained associated with CSA<70 in 2 validation cohorts
- CCRL2, FAM134B and YWHAH are all potentially involved in inflammation through the modulation of autophagy
- Consistent with the effects on NCI, silencing experiments demonstrate that loss of function FAM134B and YWHAH results in increased inflammation while loss of function of CCRL2 results in decreased inflammation.
- These findings suggest that genetic variants that alter the inflammatory response of microglial cells to HIV ssRNA alter the risk for HIV associated cognitive impairment that may be mediated by alterations in autophagy.

ACKNOWLEDGEMENTS

This work was supported in part by R01NS077874, R01NS084912 and R01NS104015, the PHACS Network supported by The Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD) under cooperative agreements HD052104 (PHACS Coordinating Center, Tulane University School of Medicine) and HD052102 (PHACS Data and Operations Center, Harvard T.H. Chan School of Public Health), and the IMPAACT Network funded by the National Institute of Allergy and Infectious Diseases (NIAID) [U01 AI068632], the Eunice Kennedy Shriver National Institute of Child Health and Human Development (NICHD), and the National Institute of Mental Health (NIMH) [AI068632].