DKK1 is Associated with HIV-Associated Neurocognitive Impairment

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ABSTRACT

Background: DKK1 is a soluble antagonist of the Wnt/β-catenin pathway. It binds to LRP5/6 (a co-receptor of Wnts) and sequesters it away from Wnts. We demonstrated that Wnt/b-catenin is a critical regulator of the glutamate/glutamine cycle in astrocytes, and diminished Wnt/ β-catenin signaling perturbs their neuroprotective properties. As a consequence, we hypothesized that increased DKK1 would increase the risk for neurocognitive impairment (NCI) in an HIV-infected (HIV+) cohort.

Methods: To assess the relationship between plasma DKK-1 and NCI, blood plasma samples from 41 HIV+ and 43 HIV- adults were obtained from the UCSD TMARC cohort. DKK1 and MCP-1 plasma levels were measured by immunoassay. MCP-1 was included as a comparison marker; it is a neuroinflammatory chemokine driving monocyte/macrophage infiltration into the brain. All subjects were assessed using a standardized comprehensive NC battery that adhered to Frascati guidelines. NC performance was summarized using the global deficit score (GDS) method.

Results: Levels of MCP-1 (p = 0.02) but not DKK1 (p = 0.65) were higher in HIV+ subjects than in HIV- subjects. Among HIV+ subjects, higher levels of DKK-1 (d=0.63, p = 0.05) but not MCP-1 (p = 0.59) were associated with NCI. The association between DKK1 and NCI strengthened when recursive partitioning was used to identify an informative threshold value in DKK1: among the 41 HIV+ subjects, those who had plasma DKK1 levels of at least 1,129 pg/ml had a 6.0-fold increased odds of having NCI (75% vs 33%, 2-tail FET p=0.04). The effect size was large and the association between DKK1 and NCI was highly specific (92%), although the sensitivity was poor (35%). In comparison, recursive partitioning failed to identify a statistically significant threshold value for MCP-1. Multivariable analysis among HIV+ subjects identified that the association between higher DKK1 levels and NCI remained statistically significant after accounting for the effects of nadir CD4+ T-cell counts, drugs of abuse, and ART use**.**

Conclusions: These findings underscore the potential specificity of DKK1 as a biomarker for NCI in HIV+ adults. Based on our prior work, the mechanism driving this relationship is via HIV and inflammatory responses that mediate elevation in DKK1 which in turn reduces Wnt/β-catenin signaling in astrocytes and diminish glutamate/glutamine cycling by astrocytes, leading to neurotoxicity.

BACKGROUND

The introduction of combined antiretroviral therapy (cART) transformed HIV from a death sentence to a chronic disease with several co-morbid complications, the most notable of which is HIV Associated Neurocognitive Disorders (HAND). Despite cART, HAND affects approximately 50% of HIV infected individuals [1, 2] and its prevalence is expected to increase as the HIV-infected population ages. This underscores the need to define biomarkers that can accurately predict the chance for developing HAND, reveal mechanisms that drive HAND, and ultimately devise novel strategies to prevent and/or treat HAND.

We are focusing on defining whether molecules related to Wnt/β-catenin signaling **(Fig.1)** are clinical and neuropathologic biomarkers of HAND. Wnt/β-catenin regulates cell structure, synaptic activity, cell proliferation, and survival. We established that Wnt/β-catenin is a restriction factor for HIV in astrocytes, monocytes, and T cells and determined the mechanism by which it does so [3-7]. We also established that inhibition of β-catenin increases the expression of the neuroinflammatory transcription factor CAAT/enhancer-binding proteins (C/EBP) and has a dramatic effect on the ability of astrocytes to scavenge for glutamate by inhibiting the expression of Excitatory Amino Acid Transporter 2 (EAAT-2/GLT-1 in rodents) and glutamine synthetase. Our working model is that signals that diminish Wnt/β-catenin will compromise brain homeostasis and will contribute to HAND.

Because β-catenin is an intracellular protein, it is not a useful biomarker. For this reason, we turned to Dickkopf-related protein 1 (DKK1). DKK1 is a soluble antagonist of the Wnt/β-catenin pathway. It binds to LRP5/6 (a co-receptor of Wnts) and sequesters it away from Wnts (**Fig.1**). We showed that an inflammatory signal (e.g., IFNγ) inhibits Wnt/ β-catenin signaling in astrocytes through induction of DKK1 in a STAT3-dependent manner [8]. Recent studies demonstrate that Wnt blockade with DKK1 induces the rapid disassembly of synapses in mature neurons. DKK1 also mediates synaptic loss induced by Amyloid-β and it is elevated in drug-abusing psychotic patients [9]. These emerging studies point to a relationship between DKK1 and neurodegenerative and psychotic disorders. We assessed here whether plasma DKK-1 level are associated with worse global neurocognitive functioning among people living with HIV.

DKK-1 antagonizes Wnt signaling

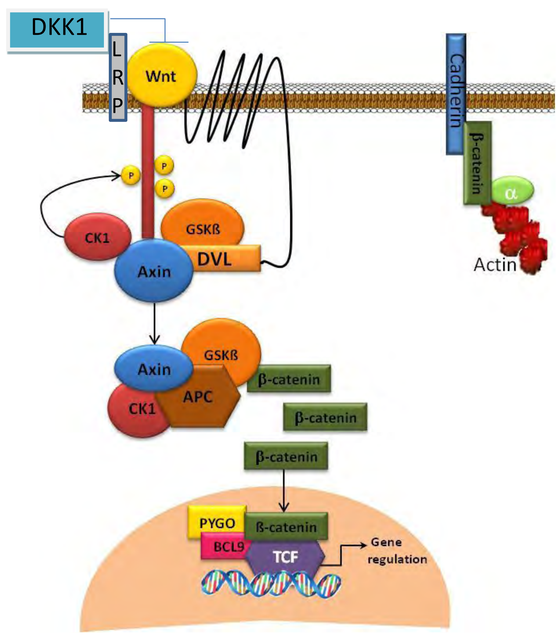


Fig.1: Wnt/b-catenin signaling pathway. Wnts are small secreted glycoproteins that bind to Frizzled receptors and the co-receptor low-density lipoprotein receptor related protein (LRP) 5/6 leading to destabilization of a b-catenin destruction complex (Axin, APC, CK1α GSK3β). Hypophosphorylated β-catenin translocates to the nucleus, where it interacts with TCF/LEF to displace its co-repressors and recruit either positive or negative transcription co-factors to regulate Wnt target genes. In the absence of Wnts cytosolic β-catenin is phosphorylated by the destruction complex and undergoes βTrcp-mediated ubiquitination and proteasomal degradation. *Further, Wnt signaling is inhibited when DKK-1 binds to LRP.*

Study Design: Using 84 samples from HIV+ (n=41) and HIV- (n=43) subjects with and without HAND from the UCSD TMARC cohort, we compared plasma DKK1 levels to HIV serostatus and, among HIV+ subjects, HAND diagnosis, using MCP-1 levels in plasma as a comparator biomarker. Conventional ELISAs for DKK-1 and MCP-1 were used.

RESULTS

Global NC Performance	HIV-	HIV+		Unimpaired	Impaired	
Sample Size	42	41	**P value**	58	25	**P value**
Age (Years)	38.8 (12.7)	42.6 (8.9)	0.12	41.3 (10.9)	39.3 (11.6)	0.45
Sex (Women)	9 (21.4%)	6 (14.6%)	0.42	8 (13.8%)	7 (28.0%)	0.13
Ethincity (non-White)	20 (47.6%)	13 (31.7%)	0.40	22 (37.9%)	11 (44.0%)	0.9
Education (Years)	12.9 (1.7)	13.2 (2.4)	0.56	13.0 (1.9)	13.3 (2.4)	0.61
Beck Depression Inventory-II	8.8 (9.4)	11.5 (10.5)	0.23	8.8 (9.8)	13.5 (10.0)	**0.05**
Methamphetamine Dependence	20 (47.6%)	22 (53.7%)	0.58	25 (43.1%)	17 (68.0%)	0.06
Urine Drug Screen Positive	1 (2.4%)	0 (0%)	0.25	1 (1.7%)	0 (0%)	1.00
HCV Seropositive	0 (0%)	0 (0%)	1.00	0 (0%)	0 (0%)	1.00
plasma MCP-1 (pg/mL)	173.1 (118.6)	203.5 (80.7)	**0.02**	189.8 (109.7)	184.1 (84.0)	0.62
plasma DKK-1 (pg/mL)	598.0 (399.8)	656.7 (446.2)	0.54	577.0 (391.6)	743.0 (472.6)	0.11
Among HIV+ Subjects	0	41		24	17	
AIDS Diagnosis	-	26 (63.4%)	-	14 (58.3%)	1 (5.9%)	**0.0008**
Est. Duration of HIV Disease (Months)	-	129.2 (97.4)	-	115.9 (95.1)	148.0 (100.5)	0.31
Current CD4+ T-cells (/µL)	-	422.6 (192.5)	-	445.9 (200.9)	387.6 (179.8)	0.34
Nadir CD4+ T-cells (/µL)	-	198.4 (148.0)	-	234.2 (155.0)	147.8 (124.8)	0.06
Current ART Use	-	29 (70.7%)	-	13 (54.2%)	16 (94.1%)	**0.006**
Plasma HIV RNA ≤ 50 c/mL Among Those Taking ART	-	25 (86.2%)	-	12 (92.3%)	13 (81.2%)	0.60

Table 1: Cohort demographics and characteristic

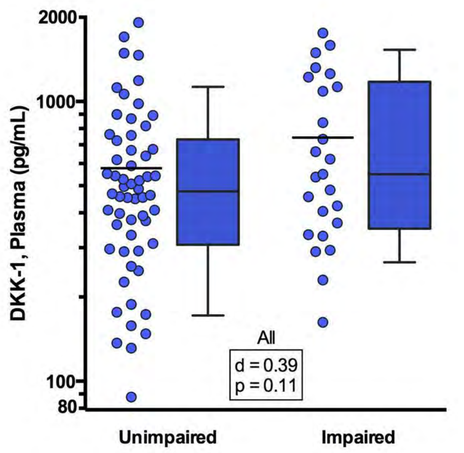


Fig. 2 Plasma DKK1 among ALL (HIV+ & HIV-) subjects classified as unimpaired or impaired.

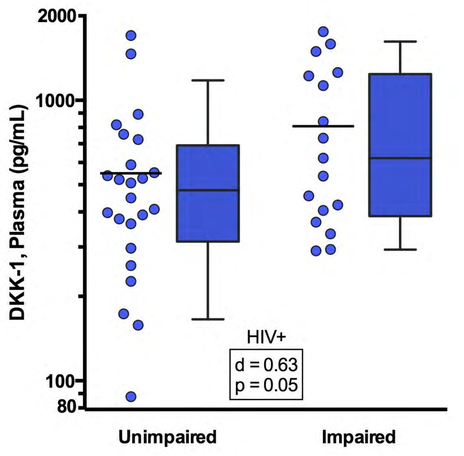


Fig. 3 Plasma DKK1 among HIV+ subjects only classified as unimpaired or impaired.

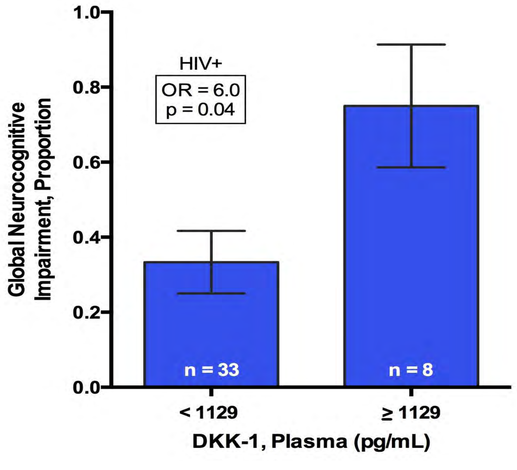


Fig. 4 Plasma DKK1 level association with Global Neurocognitive Impairment score among HIV+ subjects. Multivariable analysis among HIV+ subjects identified that the association between higher DKK-1 levels and HAND remained even after accounting for nadir CD4+ T-cell counts, drugs of abuse, and ART use

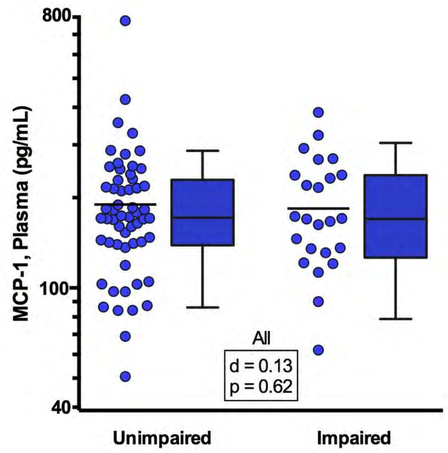


Fig. 5 Plasma MCP-1 level among ALL (HIV+ and HIV-) subjects classified as unimpaired or impaired. Recursive partitioning failed to identify a statistically significant threshold value for MCP-1.

CONCLUSIONS

- Levels of MCP-1 (p = 0.02) but not DKK1 (p = 0.65) were higher in HIV+ subjects.
- Higher levels of DKK-1 (p = 0.05) but not MCP-1 (p = 0.59) were associated with HAND among the HIV+ subjects. The difference in DKK-1 between HIV+ subjects with or without HAND had at least a medium effect size (d = 0.63). This strengthened when recursive partitioning was used to identify an informative threshold value in DKK-1. Among the 41 HIV+ subjects, those who had plasma DKK-1 levels of at least 1,129 pg/ml had a 6.0-fold increased odds of having HAND (75% vs 33%, 2-tail FET p=0.04). The effect size was large and the specificity of DKK-1 (92%) was superior to its sensitivity (35%).
- These findings are promising for DKK-1, particularly considering the small sample size. Collectively, these findings underscore the potential specificity of DKK1 as a biomarker for HAND.

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