Supplementary Data

Supplementary Table S1. Sequence Polymorphisms in HIV-1 Nef Functional Domains Potentially Associated with HIV-Associated Pulmonary Arterial Hypertension

Specific variation or functional domain	*Nef residue*	*Subtype consensus*	*New residue*	*France/Italy*					*San Francisco, California*			
				% of subjects with polymorphisms		*% of clones with polymorphisms*			*% of subjects with polymorphisms*		*% of clones with polymorphisms*	
				HIV-PH n=*11*	*Control* n=*7*	*HIV-PH* c=*207*	*Control* c=*88*	*G-value*[a]	*HIV-PH* n=*11*	*Control* n=*22*	*HIV-PH* c=*135*	*Control* c=*333*
Around M_{20}	19	R	K	9%	0%	7.7%	0	10.81	18.2%	13.6%	2.2%	8.7%
(MHC-1/AP-1 binding site)	***21***	***R***	***Q***	***18%***	***0%***	***5.8%***	***0%***	***7.31***	***9.1%***	***9.1%***	***3.0%***	***4.5%***
CD4 downregulation (WL)	***58***	***L***	***V***	***27%***	***0%***	***17.4%***	***0%***	***23.18***	***9.1%***	***9.1%***	***8.9%***	
Acidic cluster	***63***	***E***	***G***	***18%***	***0%***	***14.5%***	***0%***	***20.95***	***9.1%***	***0%***	***2.2%***	***0%***
Phosphorylation site for PKC	79	M	I	9%	0%	4.4%	0%	5.36	0%	0%	0%	0%
	80	T	N	18%	0%	9.2%	0%	12.58	9.1%	4.5%	2.2%	1.2%
	81	***Y***	***F***	***55%***	***15%***	***35.3%***	***11.4%***	***14.23***	***54.5%***	***27.3%***	***3.0%***	***19.5%***
Proline-rich area (PxxP)	***150***	***P***	***A,R,S,K,Q***	***46%***	***0%***	***25.%***	***0%***	***16.06***	***36.4%***	***9.1%***	***14.8%***	***6.3%***
$H_{40}Y$ (SHIV*nef* mutation #2)	***40***	***H***	***Y***	***64%***	***29%***	***25.1%***	***13.6%***	***0.01***	***9.1%***	***9.1%***	***4.4%***	***7.5%***
$A_{53}P$ (SHIV*nef* mutation #3)	***53***	***A***	***P***	***27%***	***0%***	***23.2%***	***0%***	***31.98***	***27.3%***	***4.5%***	***28.1%***	***2.1%***

[a]G-value for ranking purposes only.

Nef has highly conserved protein domains essential for its function, which are mapped to specific locations in the polypeptide.[21] The frequency of Nef amino acid residues per position was determined for each molecular clone. The consensus residue per position was deduced from HIV-1 Nef subtype B sequences.[7] Polymorphisms were ranked using G tests for independence with William's correction. Note that these values are a heuristic for ranking the polymorphisms of interest rather than measures of significance, as they do not correct for the relationships among sequences intrasubject or for multiple comparisons. The polymorphisms that were overrepresented in the HIV-PH subjects and confirmed in the second cohort are in italics.

MHC, major histocompatibility complex; AP-1, adaptor protein- 1; PKC, protein kinase C.

Supplementary Table S2. Sensitivity and Specificity from the ROC Analysis of the Initial and Validation Cohorts

Cohort	*Cutoff*	*Sensitivity %*	*95% CI*	*Specificity %*	*95% CI*
European	>1	90.9	58.7% to 99.8%	57.1	18.4% to 90.1%
	>2	*72.7*	*39.0% to 94.0%*	*100*	*59.0% to 100%*
	>3	54.6	23.4% to 83.8	100	59.0% to 100%
	>4	27.3	6.0% to 61.0%	100	59.0% to 100%
	>5	9.1	0.2% to 41.3%	100	59.0% to 100%
San Francisco	>1	90.9	58.7% to 99.8%	40.9	20.7% to 63.7%
	>2	*63.6*	*30.8% to 89.1%*	*77.3*	*54.6% to 92.2%*
	>3	9.1	0.23% to 41.3%	95.5	77.2% to 99.9%
	>4	9.1	0.23% to 41.3%	100	84.6% to 100%

We generated receiver-operator characteristic (ROC) curves for samples received from Europe and UCSF to establish a threshold in terms of number of Nef functional domains with genotypic changes. French subjects with only one variant Nef functional domain are 2.12 times more likely to develop HIV-PH than a subject with zero changes. Area under the ROC curve, 0.8961; SE, 0.07641; 95% CI, 0.7463 to 1.046; p value=0.005761. Subjects from San Francisco with one, two, and three variant Nef functional domains are 1.54, 2.8, and 2 times more likely to develop HIV-PH, respectively, than a subject with zero variations. Area under the ROC curve, 0.7417; SE, 0.09098; 95% CI, 0.5634 to 0.9201; p value=0.02552. Cutoff values with higher sensitivity (true positive rate) and specificity (true negative rate) are in italics.

Supplementary References

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