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Molecular evolution of $\alpha4$ integrin binding site to lentiviral envelope proteins in new world primates

Mirela Darc [a,b], Carlos G. Schrago [a], Esmeralda A. Soares [b], Alcides Pissinatti [c], Albert N. Menezes [b], Marcelo A. Soares [a,b], Héctor N. Seuánez [a,b,*]

[a] *Departamento de Genética, Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil*
[b] *Programa de Genética, Instituto Nacional de Câncer, Rio de Janeiro, Brazil*
[c] *Centro de Primatologia do Rio de Janeiro, Rio de Janeiro, Brazil*

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ABSTRACT

Integrin epitopes encoded by *ITGA4* exons 5 and 6 encompass the $\alpha4\beta7$ binding site to natural ligands and HIV-1 gp120. Functional assays of $\alpha4$ variants of new world primates (NWP) showed reduced binding of several ligands, including the HIV-1 envelope, probably accounting for restriction phenotypes conferring resistance to lentiviral infection (Darc et al., 2011). In this paper, we have analyzed, by cloning and sequencing, the $\alpha4$ domain polymorphisms present in 10 NWP species and four old world primates (including human). Analyses of differential selection at codon sites and along evolutionary lineages were carried out. We identified codons under positive selection, including polymorphic variations at codon 201, presumably convergent during NWP radiation and significant positive selection leading to a single allele (SagVar2).

1. Introduction

Early stages of infection by primate lentiviruses are characterized by substantial depletion of activated CD4$^+$ T cells in hosts that succumb to immunodeficiency, as is the case of rhesus monkeys, although the mechanisms underlying CD4$^+$ T cell depletion in the gut of these primates remain controversial (Ho et al., 1995; Mehandru et al., 2004; Veazey et al., 2000, 2001). Conversely, natural reservoirs of lentiviruses, like sooty mangabeys and African green monkeys, exhibit limited disease and pathogenesis upon infection (Hazenberg et al., 2003; Hirsch et al., 1995; LaBonte et al., 2002; Lederer et al., 2009; Silvestri, 2009), while New World primates (NWP) are not apparently infected *in natura* or in captivity by lentiviruses (McClure et al., 1989; Spertzel, 1989), probably protected by factors that prevent infection (LaBonte et al., 2002; Ribeiro et al., 2005; Sawyer et al., 2004; Sayah et al., 2004; Sebastian and Luban, 2005; Wong et al., 2009).

Integrins are a family of heterodimeric glycoproteins composed of α and β subunits playing a relevant role in cell-to-cell and cell-to-extracellular matrix interactions and defining specific patterns of cell migration to different tissues in vertebrates (Hemler, 1990; Hynes, 1992). Integrin $\alpha4\beta7$, encoded by *ITGA4* and *ITGB7*, is essential for cellular trafficking to gut-associated lymphoid tissue (GALT), the principal reservoir of CD4$^+$ T cells, and a marker of cellular activation (Agace, 2008; Erle et al., 1994; Hamann et al., 1994; Holzmann et al., 1989; Springer, 1994). In 2008, $\alpha4\beta7$ was also recognized as an HIV-1 receptor (Arthos et al., 2008).

Recently, we reported that the $\alpha4\beta7$ binding site to natural ligands and HIV-1 gp120 protein was located at epitopes encoded by *ITGA4* exons 5 and 6 (Darc et al., 2011). In addition, analyses of functional binding of NWP $\alpha4$ variants showed that some species-specific amino acid substitutions reduced binding of some ligands, including the HIV-1 envelope. We postulated that some of these $\alpha4$ variants might have resulted from positive selection at different residues of the binding site and were relevant in determining restriction phenotypes rendering NWP resistant to lentiviral infection. Most of these changes were lineage-specific, characteristic of different species, genera or families, apparently following the evolutionary NWP radiation, and their emergence could be traced in the phylogeny of this group (Darc et al., 2011).

In this study we analyzed *ITGA4* exons 5 and 6 of 10 NWP species and the presence of polymorphisms with the purpose of identifying sites under positive selection of potential functional significance to lentiviral binding. Positive and negative selection was tested using codon substitution models including data from African and Asian primates. These studies provided evidence of the evolutionary pressures at definite codon sites and branches of the $\alpha4\beta7$ lineage in new world primates.

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[*] Corresponding author at: Programa de Genética, Instituto Nacional de Câncer, Rua André Cavalcante, 37 – 4° andar, 20231-050 Rio de Janeiro, RJ, Brazil. Tel.: +55 21 3207 6582.

E-mail addresses: genetics@inca.gov.br, hseuanez@inca.gov.br (H.N. Seuánez).

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2. Materials and methods

2.1. Samples and genomic DNA

Genomic DNA of 151 different NWP primate specimens, belonging to all three families and 51 different species, has been isolated and analyzed in a previous report (Darc et al., 2011) (Table 1). We selected 10 species (*Aotus azarae, Callithrix geoffroyi, Callithrix jacchus, Callithrix kuhlii, Callithrix penicillata, Callithrix [Cebuella] pygmaea, Mico [Callithrix] argentata, Mico [Callithrix] emiliae, Mico [Callithrix] humeralifer, Mico [Callithrix] melanura*) whose α4 domain electropherograms indicated heterozygosity for cloning and sequencing.

Table 1
Specimens analyzed in this study.

Species name	Common name[a]	No. of individuals and cloned samples	Amino acid sequence (AAS)	Nucleotide sequence (NS)
Homo sapiens	Human	1	AAS1	NS1
Pan troglodytes	Common chimpanzee	1	AAS1	NS1
Pongo pygmaeus	Oranguntan	1	AAS1	NS1
Macaca mulatta	Rhesus monkey	1	AAS2	NS2
Alouatta belzebul	Red-handed howler	6	AAS3	NS3
Alouatta caraya	Black howler	4	AAS3	NS3
Alouatta guariba	Brown howler	1	AAS3	NS3
Alouatta seniculus	Venezuelan red howler	1	AAS3	NS3
Ateles paniscus paniscus	Red-faced spider monkey	1	AAS4	NS4
Brachyteles arachnoides	Southern muriqui	3	AAS4	NS4
Aotus sp.	Night monkey	4	AAS5	NS5
Aotus azarae (*A1*)	Azara's night monkey		AAS5	NS5
Aotus azarae (*A2*)	Azara's night monkey	18 (13)	AAS5	NS6
Aotus a. boliviensis	Azara's night monkey	3	AAS5	NS5
Aotus a. infulatus	Azara's night monkey	1	AAS5	NS5
Aotus infulatus	Azara's night monkey	1	AAS5	NS5
Callimico sp.	Goeldi's marmoset	1	AAS6	NS7
Callimico goeldii	Goeldi's marmoset	1	AAS6	NS7
Callithrix aurita	Buffy-tufted marmoset	2	AAS7	NS8
Callithrix geoffroyi	White-headed marmoset	3 (1)	AAS8	NS9
Callithrix jacchus	Common marmoset	2 (1)	AAS7	NS10
Callithrix kuhlii (*A1*)	Wied's marmoset		AAS7	NS10
Callithrix kuhlii (*A2*)	Wied's marmoset	2 (1)	AAS9	NS11
Callithrix penicillata (*A1*)	Black-tufted marmoset		AAS7	NS10
Callithrix penicillata (*A2*)	Black-tufted marmoset	3 (1)	AAS8	NS9
Callithrix (*Cebuella*) *pygmaea*	Pygmy marmoset	2 (1)	AAS7	NS12
Cebus sp.	Capuchin	6	AAS10	NS13
Cebus albifrons	White-fronted capuchin	5	AAS10	NS13
Cebus apella	Tufted capuchin	11	AAS10	NS13
Cebus apella paraguayanus (*A1*)	Tufted capuchin from Paraguay		AAS10	NS13
Cebus a. paraguayanus (*A2*)	Tufted capuchin from Paraguay	8	AAS10	NS14
Cebus capucinus	White-headed capucnhin	1	AAS10	NS13
Cebus olivaceus	Weeper capuchin	1	AAS10	NS13
Cebus o. nigrivittatus	Weeper capuchin	5	AAS10	NS13
Cebus xanthosternos	Golded-bellied capuchin	4	AAS10	NS13
Leontophitecus chrysomelas	Golden-headed lion tamarin	4	AAS6	NS15
Leontophitecus chrysopygus	Black lion tamarin	2	AAS6	NS15
Leontophitecus rosalia	Golden lion tamarin	2	AAS6	NS15
Mico (*Callithrix*) *argentata*	Silvery marmoset	5 (1)	AAS7	NS10
Mico (*Callithrix*) *emiliae*	Emilia's marmoset	6 (1)	AAS7	NS10
Mico (*Callithrix*) *humeralifer*	Santarem marmoset	3 (1)	AAS7	NS10
Mico (*Callithrix*) *melanura*	Black-tailed marmoset	2 (1)	AAS7	NS10
Saguinus bicolor bicolor	Pied tamarin	1	AAS11	NS16
Saguinus imperator	Emperor tamarin	3	AAS6	NS17
Saguinus martinsi	Martins' tamarin	1	AAS11	NS16
Saguinus midas midas	Red-handed tamarin	3	AAS11	NS16
Saguinus mystax	Moustached tamarin	2	AAS6	NS17
Saguinus niger	Black tamarin	1	AAS11	NS16
Saimiri sp.	Squirrel monkey	4	AAS12	NS18
Cacajao melanocephalus	Black-handed uacari	3	AAS13	NS19
Callicebus sp. (*A1*)	Titi		AAS14	NS20
Callicebus sp. (*A2*)	Titi	2	AAS14	NS21
Callicebus coimbrae	Coimbra Filho's titi	1	AAS14	NS20
Callicebus moloch	Red-bellied titi	1	AAS14	NS21
Callicebus nigrifrons	Black-fronted titi	3	AAS14	NS20
Callicebus personatus	Atlantic titi	1	AAS14	NS21
Callicebus p. personatus	Atlantic titi	2	AAS14	NS20
Chiropotes sp.	Saki	3	AAS13	NS19
Chiropotes albinasus	White-nosed saki	1	AAS13	NS19
Chiropotes israelita	Brown-backed bearded saki	1	AAS13	NS19
Pithecia irrorata	Rio Tapajos saki	1	AAS13	NS19

Numbers in parentheses indicate number of cloned samples (one per individual). All other sequences were previously reported by Darc et al. (2011), except for *Homo sapiens* and *Pan troglodytes* which were retrieved from GenBank. *A1*, *A2*: alleles.

[a] Common names follow Groves (2005).