was under the positive selection in the other primates, especially in the Old World monkey. TIM1 might undergo a selection pressure exerted by infectious disease and autoimmune disease.

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Supplemental information

Lineage-specific evolution of T-cell immunoglobulin and mucin domain 1 gene in the primates

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Supplemental figure: 2 figure

Supplemental Table S1. Primers used in the sequencing analyses of *TIM1*

Lineage*	Segment**	Forward primer (5'-3')	Reverse primer (5'-3')
H, O	Exon 2	CTGCTCATTTTCCTTCAGG	TATTCTGGTCCTGCTCACT
	Exon 3	TGCCTAGCCGAGAGGAAATA	GGAACCTCCTGTTTCCCTAT
	Exon 4	GGGCAATGACCAAGATTGAG	CTGTCCTTCTGCCTTGATGC
	Exon 5	AATGCCTGAAGGCCATCTTA	GGCACTCAAGTCGGATCTGT
	Exon 6	TTCAACTTTTCATCGCCAGA	AAAAATTCTGTGGGCTAGTCTTAC
	Exon 7	TTGGTCTTACCCTTATGTTCC	GGTAGATGGTATTACCATGTG
	Exon 8	TTAGGGAGGATATGTGGATGA	GCTACAAATATCACTGGAAGG
	Exon 9	GCTCCTGGAGTCTCGAATACC	TCATACAATGCTTCCAAATGAA
N	Exon 2	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
	Exon 3	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
	Exon 4	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
	Exon 5	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
	Exon 6	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
	Exon 7	CAGAAGTACCCAGGCACCAA	TTGGGGGATAAAGGGAAGTC
	Exon 8	CTTGTATGGGTCCTACAGAC	GGAGAGTTTAGGAGAGAGAC
	Exon 9	Same as the equivalent primer for H, O	Same as the equivalent primer for H, O
P	Exon 2	GCTCATTTTCCTTCAGGCTG	CTACTATCCTTCTACTCCCC
	Exon 3	TGAGTTTTCAAAGAGAATATGGA	GAAGGAACTTCCACTTTCCC
	Exon 4	GAGTTCTGCTTGGGGACAGA	CACCTGAGCCAGATTCACAC
	Exon 5	TTTCCTCCACAAATGGAACAC	AAGATTTGAATTATCCCAAGTGT
	Exon 6	GGCAATGTGAATATATTTAGTG	TCTGTGGGCTAGTCTTACA
	Exon 7	TTCCAGAAGTAGCTAAGGTCCA	TGAGGATTTGGGGACATAGG
	Exon 8	AGGGAAGGGTCCGAGTAACA	AACGGAGGTGCTCATTCATC
	Exon 9	CAATACTCAGGGCTACACTT	TCTGCAGTCATGGGCACAA

*; H: Homioid, O: Old World monkey, N: New World monkey, P: Prosimian

**; Each exon/equivalent region and its adjacent regions were amplified

Supplemental Table S2. Accession numbers for nucleotide sequences for *TIM1* deposited in DDBJ

Lineage	Primate species	Accession number
Homioid	human (*Homo sapiens*)	AB607987
	chimpanzee (*Pan troglodytes*)	AB607988
	bonobo (*Pan paniscus*)	AB607989
	western gorilla (*Gorilla gorilla*)	AB607990
	Bornean orangutan (*Pongo pygmaeus*)	AB607991
	western black-crested gibbon (*Nomascus concolor*)	AB607992
	lar gibbon (*Hylobates lar*)	AB607993
	siamang (*Symphalangus syndactylus*)	AB607994
Old World Monkey	rhesus macaque (*Macaca mulatta*)	AB607995
	long-tailed macaque (*Macaca fascicularis*)	AB607996
	Hamadryas baboon (*Papio hamadryas*)	AB607997
	mantled Guereza colobus (*Colobus guereza*)	AB607998
	dusky leaf monkey (*Trachypithecus obscures*)	AB607999
	silver leaf monkey (*Trachypithecus cristatus*)	AB608000
New World Monkey	Geoffroy's spider monkey (*Ateles geoffroyi*)	AB608001
	white-fronted spider monkey (*Ateles belzebuth*)	AB608002
	tufted capuchin (*Cebus apella*)	AB608003
	common squirrel monkey (*Saimiri sciureus*)	AB608004
	golden-handed tamarin (*Saguinus midas*)	AB608005
	white-lipped tamarin (*Saguinus labiatus*)	AB690311
	cotton-top tamarin (*Saguinus Oedipus*)	AB608006
	golden lion tamarin (*Leonthopithecus rosalia*)	AB608007
	common marmoset (*Callithrix jacchus*)	AB608008
Prosimian	Sunda slow loris (*Nycticebus coucang*)	AB608009

Supplemental Table S3. Primers used in the expression analysis of *TIM1*

Forward*	Reverse**	Forward primer (5'-3')	Reverse primer (5'-3')
within exon3	junction of exon 5-6	ACAGTTGTGTCTGACAGTGG	GGTGTCATTCCCATCTGTTG
junction of exon 5-6	within exon9	CAACAGATGGGAATGACACC	TGTCTTCTGCTTGGACTTCC

*; corresponding regions designed for forward primers

**; corresponding regions designed for reverse primers

Supplemental Table 4. Analysis of positive selection for TIM1 exon 4 by the Bn-Bs program

Lineage[#1]	ω[#2]	dn[#3]	ds[#4]	Z-score	p-value[#5]
Hum	0.57	0.011	0.020	0.0>	ns
Chi	1.13	0.032	0.029	0.1	ns
Bon	0.47	0.014	0.030	0.0>	ns
Gor	0.74	0.013	0.018	0.0>	ns
Ora	0.76	0.054	0.071	0.0>	ns
Gib	2.04	0.017	0.008	0.6	ns
Lar	8.66	0.003	0.000	0.7	ns
Sia	nc	0.005	0.000	1.1	ns
Rhe	nc	0.000	0.000	0.0	ns
Lot	nc	0.000	0.000	0.0	ns
Bab	0.42	0.004	0.009	0.0>	ns
Col	0.32	0.038	0.117	0.0>	ns
Dus	nc	0.000	0.000	0.0	ns
Sil	nc	0.000	0.000	0.0	ns
Lor	0.59	0.109	0.184	0.0>	ns
Hominoid ancestor	1.44	0.018	0.012	0.2	ns
Hum, Chi, Bon, Gor, Ora ancestor	6.37	0.017	0.003	0.7	ns
Hum, Chi, Bon, Gor ancestor	0.72	0.012	0.016	0.0>	ns
Hum, Chi, Bon ancestor	0.37	0.001	0.002	0.0>	ns
Chi, Bon ancestor	0.00	0.000	0.000	0.0>	ns
Gib, Lar, Sia ancestor	1.04	0.028	0.027	0.0	ns
Lar, Sia ancestor	0.00	0.000	0.013	0.0>	ns
Old World Monkey ancestor	5.22	0.087	0.017	1.9	0.031[#6]
Rhe, Lot, Bab ancestor	0.08	0.006	0.077	0.0>	ns
Rhe, Lot ancestor	0.44	0.020	0.046	0.0>	ns
Col, Dus, Sil ancestor	nc	0.001	0.000	0.1	ns
Dus, Sil ancestor	0.28	0.022	0.077	0.0>	ns

#1: Hum; human, Chi; chimpanzee, Bon; bonobo, Gor; gorilla, Ora; orangutan, Gib; black-crested gibbon, Lar; lar gibbon, Sia; siamang, Rhe; rhesus macaque, Lot; long-tailed macaque, Bab; baboon, Col; colobus, Dus; dusky leaf monkey, Sil; silver leaf monkey, Lor; Sunda loris.

#2; ω were not calculated (nc), because ds=0.

#3; number of reference sites for dn was 128.2

#4; number of reference sites for ds was 58.2

#5: ns; not significant, $p>0.05$

#6; chi-square obtained by the PAML program was 2.20 ($p>0.05$)

Supplemental Table S5. Length polymorphisms of TIM1 exon 4 in the Old World monkeys

Rhesus macaques (2n=32)			Long-tailed macaques (2n=20)		
Size of exon 4*	n	frequency	Size of exon 4*	n	frequency
384	3	0.09	330	2	0.10
402	2	0.06	408	4	0.20
408	2	0.06	414	2	0.10
426	6	0.19	426	3	0.15
438	1	0.03	456	6	0.30
456	2	0.06	459	1	0.05
462	2	0.06	462	1	0.05
474	2	0.06	498	1	0.05
498	6	0.19			
516	6	0.19			

*; size in base pairs.

Supplemental Table S6. Analysis of positive selection for *TIM3* and *TIM4* in the primates by the Bn-Bs Program

a) *TIM3*

Lineage[#1]	ω	dn[#2]	ds[#3]	Z-score	p-value[#4]
Hum	0.48	0.005	0.01	<0.0	ns
Chi	1.07	0.010	0.01	0.1	ns
Hum, Chi ancestor	0.69	0.008	0.01	<0.0	ns
Ora	0.74	0.013	0.02	<0.0	ns
Hum, Chi, Ora ancestor	0.16	0.003	0.02	<0.0	ns
Rhe	0.87	0.038	0.04	<0.0	ns
Mar	0.43	0.061	0.14	<0.0	ns

b) *TIM4*

Lineage[#1]	ω	dn[#5]	ds[#6]	Z-score	p-value
Hum	0.81	0.006	0.01	<0.0	ns
Chi	29.43	0.006	<0.001	2.1	0.017[#7]
Hum, Chi ancestor	0.30	0.011	0.04	<0.0	ns
Ora	0.67	0.015	0.02	<0.0	ns
Hum, Chi, Ora ancestor	0.61	0.010	0.02	<0.0	ns
Rhe	0.46	0.023	0.05	<0.0	ns
Mar	0.79	0.072	0.09	<0.0	ns

#1: Hum; human, Chi; chimpanzee, Ora; orangutan, Rhe; rhesus, Mar; marmoset.

#2: number of reference sites for dn was 616.9.

#3: number of reference sites for ds was 205.1.

#4: ns; not significant, $p>0.05$.

#5: number of reference sites for dn was 830.0.

#6: number of reference sites for ds was 271.0.

#7: chi-square obtained by the PAML program was 3.81 ($p>0.05$).

Legend to Supplemental Figures

Figure S1. Sequence alignment of *TIM1* exon2-equivalent region in New World monkeys

Nucleotide sequences of exon 2-equivalent region from New World monkeys are aligned with exon 2 sequences of human *TIM1* starting from the initiation codon ATG. Dashes indicate alignment gaps. Direct repeats flanking the insertion are indicated in yellow.

Figure S2. Sequence alignment of human *TIM1* and predictive coding molecule of marmoset *TIM1*-like gene

Marmoset *TIM1*-like gene in chromosome 13 was translated and aligned with human *TIM1*. Dashes indicate alignment gaps. Asterisks in yellow indicate in-frame termination codons.

Supplemental Figure S1

```
human:                          ATGCATCCTC AAGTGGTCAT CTTAAGCCTC ATCCTAC--- ---------- ---------- ---------- ---------- ----------
common marmoset:                CAGCATCCTC AAGTGGTCAT CTTAAGCCTC ATCCTAC--- ---TTTTTTT TTAATTGCAT TTTAGGTTTT GGGGTACATG TGAAGAACAT
golden lion tamarin:            ATGCATCCTT AAGTGGTCAT CTTAAGCCTC ATTCTACTTC TTTTTTTTTT TTTATTGCCT TTTAGGTTTT GGGGTACATG TGAAGAACAT
cotton-top tamarin:             ATGCATCCTC AAGTGGTTGT CTTAAGCCTT ATCCTAC--- -----TTTTT TTAATTGCCT TTTAGGCTTT GGGGTACATG TGAAGAACAT
white-lipped tamarin:           ATGCATCCTC AAGTGGTTGT CTTAAGCCTT ATCCTAC--- ----TTTTTT TTTATTGCCT TTTAGGCTTT GGGGTACATG TGAAGAACAT
golden-handed tamarin:          ATGCATCCTC AAGTGGTTGT CTTAAGCCTT ATCCTAC--- -TTTTTTTTT TTTATTGCCT TTTAGGCTTT GGGGTACATG TGAAGAACAT
common squirrel monkey:         ATGCCTCCTC AAGGGGTCGT CTTAAGCCTC ATCCTAC--- ---------- ---------- ---------- ---------- ----------
tufted capuchin:                ATGCATCCTC AGGTGGTCGT CTTAAGCCTC ATGCTAC--- ---------- ---------- ---------- ---------- ----------
white-fronted spider monkey:    ATGCATCCTC AAGTGGTCGT CTTAAGCCTC ATCCTAC--- ---------- ---------- ---------- ---------- ----------
Geoffroy's spider monkey:       ATGCATCCTC AAGTGGTTGT CTTAAGCCTC ATCCTAC--- ---------- ---------- ---------- ---------- ----------

human:                          ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
common marmoset:                GCAAGATAGT TGCATAGGTA CACATGTGGC AGTGTGATTT GCTGCCTTCC TCCCCTTCAC CTATA-CTGG CATTTTCCCC CATGCTATCT
golden lion tamarin:            GCAAGATAGT TGCATAGGTA CACACGCGGC AGTGTGATTT GCTGC-TTCC TCCCCTTCAC CTATATCTGG CATTTTTCCC CATGCTCTCT
cotton-top tamarin:             GCAAGATAGT TGCATAGATA CACACGTGGC AATGTGATTT GCTGCCTTCC TCCCCTTCAC CTATATCTGG CATTTTTCCC CATGCTCTCT
white-lipped tamarin:           GCAAGATAGT TGCATAGATA CACACGTGGC AGTGTGATTT GCTGCCTTCC TCCCCTTCAC CTATATCTGG CATTTTTCCC CATGCTCTCT
golden-handed tamarin:          GCAAGATAGT TGCATAGATA CACACGTGGC AATGTGATTT GCTGCCTTCC TCCCCTTCAC CTATATCTGG CATTTTTCCC CATGCTCTCT
common squirrel monkey:         ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
tufted capuchin:                ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
white-fronted spider monkey:    ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------
Geoffroy's spider monkey:       ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- ----------

human:                          ---------- ---------- ---------- ---------- ---------- ---------- ---------- -ATCTGGCAG
common marmoset:                CT-CCCCAAC TACCCACCCC CGCTGTCCC- TCCCCATCAT TTTCAGCAAA CTGACACAAG CCTCATCCTA CTTCTAGCAG
golden lion tamarin:            CTCCCCAACT CCCCGCCCCC CGCTGTCCC- TCCCCATCAT TCTCAGCAAA CTGACACAAG CCTCATCCTA CTTCTAGCAG
cotton-top tamarin:             TTCCCCAACT CCCAGCCCCC CGCTGTCCCC TCCCCATCAT TCTCAGCAAA CTGACACGAG CCTCATCCTA CTTCTAGCAA
white-lipped tamarin:           CTCCCCAACT CCCAACCCCC CGCTGTCCCC TCCCCATCAT TCTCAGCAAA CTGACAGGAG CCTTATCCTA CTTCTAGCAG
red-handed tamarin:             CTCCCCAACT CCCAACCCCC CGCTGTCCCC TCCCCATCAT TCTCAGCAAA CTGACAGGAG CCTTATCCTA CTTCTAGCAG
common squirrel monkey:         ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TTCTAGCAG
tufted capuchin:                ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TTCTAGCAG
white-fronted spider monkey:    ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TTCTAGCAG
Geoffroy's spider monkey:       ---------- ---------- ---------- ---------- ---------- ---------- ---------- -TTCTAGCAG
```

Supplemental Figure S2

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Human TIM1:          MHPQVVILSL ILHLADSVAG SVKVGGEAGP SVTLPCHYSG AVTSMCWNRG SCSLFTCQNG IVWTNGTHVT YRKDTRYKL-
Marmoset TIM1-like: MPPQVVILSL ILLLAD-ALV SLQVGGVAGP STMLPCSYSG DVTSMC*NRD RCSLLRCPNS IIWTNGTHVT YHCAVNYML*

Human TIM1:          -LGDLSRRDV SLTIENTAVS DSGVYCCRVE HRGWFNDMKI TVSLEI---- -----VP--- ---------- ----------
Marmoset TIM1-like: TMGDLSKRDV SLTLGALWEA EVGGSQGQEI ETSLGNIVKT LSLLKI*KLS QAWWHVPVVQ LLGRLRQENC LNPRGRACSK

Human TIM1:          ---PKVTTTP IVTTVPTVTT VRTSTTVPTT TTVPMTTVPT TTVPTTMSIP TTTTVLTTMT VSTT---TSV PTTTSIPTTT
Marmoset TIM1-like: PRSHHCTPTW QQSETPSPTK KRKENTSLSD SGLYCCHVGH KV*FNDMKI- TVSLAMVPPR VTTTPIVTIV PTFTTVRMST

Human TIM1:          SVPVTTTVST FVPPMPLPRQ NHEPVATSPS SPQPAETHPT TLQGAIRREP TSSPLYSYTT DGNDTVTESS DGLWNNNQTQ
Marmoset TIM1-like: TVPTTMTVSS FAPPTPSPTQ NHGP-ATPPS SPQPTETHPA MLQEATRTQR AGSPLHSYTT NGNDTVTESS DGLWNNDQTQ

Human TIM1:          LFLEHSLLTA NTTKGIYAGV CISVLVLLAL LGVIIAKKYF FKKEVQQLSV SFSSLQIKAL QNAVEKEVQA EDNIYIENSL YATD-
Marmoset TIM1-like: LSPAQSPQMA TPTKGICAGV CMPVLVPLAL LGVIIARKYF FRNKI*QLSF SFRRLQIKAL QNAVKKEVQA EDSVYVENNL YATDS
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Status of TIM-1 exon 4 haplotypes and CD4+T cell counts in HIV-1 seroprevalent North Indians

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ABSTRACT

The *TIM* (T cell/transmembrane, immunoglobulin and mucin) proteins are crucial regulators of Th1/Th2 immune responses and have been implicated in several diseases including HIV-1/AIDS. The *TIM1* exon 4 that codes for mucin domain is highly diverse, with sequence variants associated with varying phenotypes. In this study, *TIM1* exon 4 was sequenced among 227 HIV-1 seroprevalent and 288 healthy non infected individuals from North Indian population and haplotypes established. A novel but rare haplotype D1* was identified among the healthy and differed from D1 by a synonymous substitution G>T at Thr208Thr. The *TIM1* haplotype diversity showed no association with susceptibility to HIV-1 infection. The seroprevalent individuals carrying D3A had relatively higher median CD4+T cell counts (368/μl) than those without (313/μl; $p = 0.02$). A comparison of CD4+T counts between D3-A individuals on ART or ART naïve did not show any significant difference plausibly due to confounding nature of ART and other factors.

1. Introduction

The human TIM (T cell/transmembrane, immunoglobulin and mucin) family consists of three type I cell surface glycoproteins (TIM1, TIM3 and TIM4) with N terminal immunoglobulin (Ig) like domains at distal end and highly polymorphic proximal mucin domains with N- and O-linked glycosylation sites [1–3]. The TIM family genes are located on chromosome 5q33.2 [4], and have been reported to be associated with asthma [5], atopy [6], autoimmunity [1], malaria [7] and viral infections [8].

The *TIM1* mucin domain, in particular, has been a hotspot of both positive and over-dominant selection with numerous non-synonymous substitutions and phenotypic variations [9]. It appears to have undergone a selective sweep in Chimpanzees caused by pathogens like SIV and is thus an important candidate gene for evaluating human immune responses against HIV-1 [8,9].

The TIM-1 receptor is preferentially expressed on Th2 cells and acts as a potent co-stimulatory activator of Th2 responses [3]. An imbalance of Th1/Th2 cell responses has been linked to poor prognosis and increased viral replication during HIV-1 infection [10]. The role of TIM gene family products and their variability on Th1/Th2 immune responses in HIV-1 infection, disease progression and immunopathogenesis are largely unknown. There has been only one study reported so far that has shown an association of *TIM1* haplotype (D3-A) with delayed progression to AIDS and better CD4 counts among HIV-1 infected Thai female cohort [8]. Therefore, the present study was planned with the aim of evaluating *TIM1* mucin domain polymorphisms among North Indian population infected with HIV-1.

2. Materials and methods

The study was conducted using materials collected from 227 HIV-1 positive (+ve) patients, enrolled from Department of Microbiology and antiretroviral treatment (ART) clinic of All India Institute of Medical Sciences, New Delhi and a control group of 288 healthy individuals, all unrelated and evenly distributed within the North Indian states of Delhi, Punjab, Haryana, Himachal Pradesh, Uttarakhand and Uttar Pradesh. The healthy and patient groups were age and gender matched; with median age of 28 and 32 years and male/female ratios of 1.28 and 1.7, respectively. Among patients, 121 were asymptomatic ART naïve while 106 were on ART. The patients were followed for clinical and immunological details including CD4 counts for more than 2 years from the date of enrollment.

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Table 1
Distribution of TIM-1 haplotypes in healthy and HIV +ve subjects ('ART naïve' and 'on ART') in North Indian population.

TIM1 haplotype[#]	T>C (Thr158Met)	3bp deletion (Thr160del)	18bp deletion (6AA 161-166 del)	C>T (Pro180Leu)	3bp deletion (Thr201del)	1bp deletion (frameshift fs207)	A>G (Thr208Ala)	G>T (Thr208Thr)	Haplotype frequency			
									Healthy controls($2n = 576$) ($n = 288$) Number (frequency)	Total HIV +ve ($2n = 454$) ($n = 227$) Number (frequency)	ART Naïve HIV +ve ($2n = 242$) ($n = 121$) Number (frequency)	On ART HIV +ve ($2n = 212$) ($n = 106$) Number (fFrequency)
D1	T	del	w	T	w	w	A	T	12 (0.021)	10 (0.022)	6 (0.025)	4 (0.019)
D1*	T	del	w	T	w	w	A	G	4 (0.007)	0 (0)	0 (0)	0 (0)
D3-A	C	w	del	C	w	w	A	G	274 (0.475)	211 (0.465)	112 (0.463)	99 (0.467)
D3-C	C	w	del	C	w	w	G	G	126 (0.219)	109 (0.240)	61 (0.252)	48 (0.226)
D4	T	w	w	C	del	w	A	G	123 (0.214)	99 (0.218)	49 (0.202)	50 (0.236)
W-A	T	w	w	C	w	w	G	G	33 (0.057)	23 (0.051)	12 (0.050)	11 (0.052)
W-C	T	w	w	C	w	w	A	G	4 (0.007)	2 (0.004)	2 (0.008)	0 (0)

[#] Haplotypes as described by Nakajima et al. [9].

Ethical approval for the study was obtained from the Institutional Ethical Committee of All India Institute of Medical Sciences and study subjects were enrolled following their informed consent. The study protocol was also approved by the Ethics Reviewing Committee of Medical Research Institute, Tokyo Medical and Dental University. Ten ml of peripheral blood was collected by venipuncture into Na_2-EDTA coated vacutainers for DNA extraction and plasma isolation. Ammonium acetate salting out procedure was used for extraction of DNA from blood samples [11] and used for *TIM1* exon 4 sequencing.

Polymorphisms in the mucin domain of *TIM-1* encoded by exon 4 were analyzed by direct sequencing, as described previously [8]. Briefly, primers 5′-GGGCAATGACCAAGATTGAC-3′ and 5′-ACCTTGATACAATGCCCTGG-3′ were used to amplify a 470 bp fragment containing exon 4 of *TIM1*. The PCR products were sequenced by using the PCR primers and BigDye Terminator v 3.1 cycle sequencing kit (Applied Biosystems, California, USA) in ABI Prism 3130xl genetic analyzer. The *TIM-1* haplotypes were determined using Haploview software based on the previous report [8,9]. Haplotypes were compared among the healthy and HIV-1 infected groups by the chi square test or Fisher's exact test wherever applicable. Continuous variables (CD4+T cell counts) were stratified based on the haplotypic background and compared by non-parametric Wilcoxon Rank Sum (Mann Whitney *U*) test.

3. Results

Of the known eleven *TIM-1* haplotypes [9], six haplotypes namely W-A, W-C, D1, D3-A, D3-C and D4 were observed in the North Indian population (Table 1). Among these, D3-A was found to be the most prevalent haplotype and occurred with comparable frequencies in healthy (47.5%) as well as HIV +ve subjects (46.5%). The next most frequent haplotype was D3-C (21.9%) followed by D4 (21.4%), W-A (5.7%), D1 (2.1%) and W-C (0.7%). In addition, a novel haplotype D1* which differed from D1 by a synonymous G to T transversion (Thr 208 Thr) was found in 4 healthy individuals (0.7%).

A comparison of distribution of *TIM-1* haplotypes between healthy controls and HIV +ve subjects is shown in Table 1. There was no significant difference in *TIM-1* haplotypic distribution among these groups. In addition, the haplotypic distribution of *TIM-1* did not show any significant difference in asymptomatic ART naïve HIV +ve subjects versus symptomatic patients on ART (Table 1).

The median CD4+T cell counts of HIV +ve subjects were analyzed by stratifying the individuals according to the presence or absence of various *TIM-1* haplotypes (D1, D3-A, D3-C, D4 and W-A) as shown in Table 2. The patients carrying the D3-A haplotype had relatively higher CD4+T counts/μl (median 368/μl) as compared to those without it (median 313/μl; $p = 0.02$). However, when patients on ART or ART naïve were compared among each other, the level of significance of this difference was abolished. This could plausibly be attributed to the confounding nature of ART and other factors and could be further established among a larger cohort size.

4. Discussion

This is a first preliminary study on the role of *TIM1* sequence variations during HIV-1 infection in the North Indian population. The study has shown the presence of six *TIM1* haplotypes W-A, W-C, D1, D3-A, D3-C and D4, similar to those as described earlier [8,9] plus an additional novel D1* haplotype. The haplotype D3-A was found to be the most predominant one and showed a modest association with higher CD4+T cell counts among HIV +ve individ-

Table 2
Comparison of median CD4+T cell counts/μl blood in HIV +ve subjects (with or without symptoms/antiretroviral treatment) stratified on the basis of presence or absence of various TIM-1 haplotypes.

Haplotype	D1		D3-A		D3-C		D4		W-A	
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	Present	Absent
ART naïve (n = 99)	n = 5	n = 94	n = 77	n = 22	n = 43	n = 56	n = 34	n = 65	n = 8	n = 91
Median CD4+T counts	448	439	448	400	425	457	410	468	451	440
p Value	0.96		0.44		0.76		0.1		0.81	
On ART (n = 101)	n = 4	n = 97	n = 66	n = 35	n = 41	n = 60	n = 41	n = 60	n = 11	n = 90
Median CD4+T counts	284	239	262	211	219	253	222	261	225	250
p Value	0.99		0.07		0.39		0.33		0.96	
Total HIV cohort (n = 200)	n = 9	n = 191	n = 143	n = 57	n = 84	n = 116	n = 75	n = 125	n = 19	n = 181
Median CD4+T counts	324	355	368	313	358	344	324	364	355	355
p Value	0.89		0.02		0.82		0.07		0.96	

Note: Haplotypes W-C and D1* were not evaluated since these were either absent or present in very low frequencies in the patients.

uals. Similar difference was also observed amongst ART naïve and on ART subgroups but could not reach statistical significance. This could plausibly be attributed to confounding nature of ART plus other factors and needs to be explored further among a larger cohort size. Recently, studies have shown that mucin 1 secreted in milk [12] and mucin 6 in seminal plasma [13] could bind to DC-SIGN and block viral transfer to CD4 cells and ultimately skew the mounted immune responses.

A similar study in a HIV-1 infected female cohort in Thailand also showed an association of *TIM1* D3-A haplotype with higher CD4+T cell counts and delayed disease progression to AIDS [8]. A possible link of these haplotypes has been suggested with relatively low levels of TIM-1 expression [8] and hence lower Th2 promotion and enhanced Th1 responses thereby facilitating enhanced CTL responses and better prognosis or delayed disease progression. On the contrary, enhanced Th1 could also favor proliferation of CCR5+CD4+ T cells and support viral replication.

The present study suffers from a major limitation of unavailability of dates of HIV-1 infection/seroconversion and regular viral load data. Hence, we could not assess the rates of progression among individuals with different *TIM1* haplotypes. A comparison of haplotype frequencies did not reveal any significant difference among healthy and HIV +ve subjects, suggesting a lack of direct association of *TIM1* haplotypes with the susceptibility to HIV infection in the Indian population, although their indirect effect via interaction with other genes cannot be ruled out.

The D3-A haplotype (and D3-C) of *TIM1* contain an 18 bp deletion due to which a 6 amino acid long stretch of MTTTVP is excluded and a shorter form of protein is expressed. It has been hypothesized that this shorter form evolved as a protective mechanism against the hepatitis A virus (HAV) since this receptor form does not bind to the virus as efficiently as long forms. It was shown that HAV-induced liver damage was associated with the insertion polymorphism [14], earlier shown to be associated with protection against asthma and allergic diseases. A relative analysis of presence of this deletion/insertion in the present study, however, did not reveal any significant correlation with the susceptibility to HIV-1 or to CD4+T cell counts (data not shown).

In conclusion, we report a possible influence of *TIM1* D3-A haplotype on HIV-1 infection in North Indians. Further studies are required to explore the influence of circulating *TIM1* and other TIM haplotypes on specific CD4+T cell subsets during HIV-1 infection and in the development of AIDS.

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