# A 6-amino-acid insertion/deletion polymorphism in the mucin domain of TIM1 confers protections against HIV-1 infection

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## Abstract

**Background:** TIM-1 (T-cell immunoglobulin and mucin domain 1), a cell surface glycoprotein, facilitates the entry of enveloped virus including HIV, into host cells. Because the length of the mucin domain of TIM-1 is a critical factor in modulating viral entry, we assessed whether the TIM-1 18-bp insertion/deletion polymorphism associates with susceptibility to HIV-1 infection in three independent cohorts of HIV-exposed seronegative (HESN) individuals.

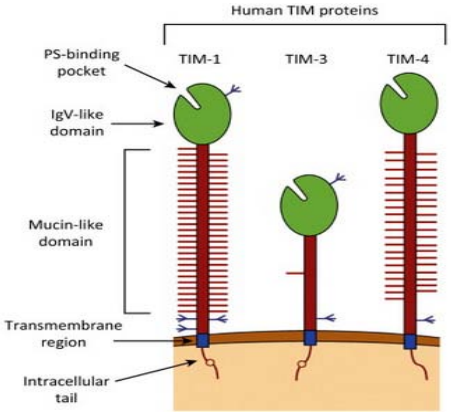
**Methods:** The Tim-1 18-bp insertion/deletion polymorphism was genotyped in three case/control cohorts of HIV sexually-exposed HESN and their HIV-1-infected partners with different geographic origin (Italy, Peru and Colombia); data from an additional cohort were retrieved from a previous study conducted in Thailand. CD4+ T lymphocytes purified from 34 healthy controls (HC) grouped according to their TIM-1 genotype were infected in vitro with HIV-1Ba-L and viral replication was assessed after 5 days by measuring viral p24 levels

**Results:** Homozygosity for the short TIM-1 allele was more common in HESN than in HIV-1 infected subjects in all cohorts. A meta-analysis of the four association analyses, revealing no heterogeneity among samples, yielded a p value of 0.005. These results were reinforced by data showing a significant reduction of HIV replication in CD4+ T lymphocytes of HC that were homozygous for the short TIM-1 allele compared to those carrying at least one long allele (t-test, p= 0.042)

**Conclusions:** The TIM-1 deletion allele protects from infection with a recessive effect. In vitro infection assays of CD4+ T lymphocytes support this conclusion and underscore a complex interaction between enveloped viruses and TIM molecules that need further investigation.

## Background

- Infection of cells by enveloped viruses is a multi-step process requiring both the binding of viral glycoproteins to specific cellular receptors/coreceptors and interactions with accessory molecules whose main function is to locate the virus closer to its receptor(s) [1].
- Virus internalization occurs when TIM binds phosphatidylserine (PtdSer) on the viral envelope; [2, 3].
- Structurally, all TIM proteins have a conserved ectodomain consisting of an immunoglobulin (IgV)-like domain and a heavily glycosylated mucin-like domain, anchored to the cell through a transmembrane domain followed by a cytoplasmic tail [3].
- An 18-bp insertion/deletion polymorphism in the exon, causing a six amino acid insertion/deletion variant (157ins/delMTTTVP), was associated with the risk of developing acute liver failure following HAV infection [4] and to modulate AIDS progression in HIV-1 infected subjects [4].
- The length of the mucin-like domain is critical for enhancing enveloped virus entry [3].
- TIM-1 molecules with a short mucin-like domain (157delMTTTVP) bind HAV less efficiently than those with a long domain (157insMTTTVP) [5]




## Aim of the study

To assess whether the *HAVCR1* 18-bp insertion/deletion polymorphism modulates susceptibility to HIV-1 infection in independent cohorts of HESN

## Materials and Methods

• The HAVCR1 18-bp insertion/deletion polymorphism was genotyped in three independent cohorts of HIV-1 exposed seronegative individuals (HESN) repeatedly exposed to the virus through unprotected sexual intercourse

• CD4+ T cells isolated by magnetic selection from PBMC of 34 healthy control (HC) volunteers, were divided according to their 18-bp insertion/deletion HAVCR1 genotype and :

- Analysed for TIM-1 mRNA expression levels (RT-Real Time PCR)
- Infected with HIV-$1_{Ba-l}$ and analysed for viral replication 5 days post-infection (p24 ELISA)

## Results

### Demographic table

| Characteristics | Italy HESN (n=121) | Italy SP (n=110) | Colombia HESN (n=63) | Colombia SP (n=51) | Peru HESN (n=133) | Peru SP (n=92) |
|---|---|---|---|---|---|---|
| Age, mean yrs. ± SD | 40.7±9.1 | 41.4±8.8 | 35.1±10.6 | 33.9±7.5 | 31.2±10.7 | 30.8±6.7 |
| Males, n (%) | 51 (42.3) | 71 (65) | 27 (44.2) | 26 (50) | 123 (90) | 94(99) |
| Viral load, median copies/mL (interquartile range) | nd | 10,250 (399–27,410) | nd | 2,569 (488–25,075) | nd | 29,694 (11,162–63,381) |
| CD4+ T cell/µL count, median (interquartile range) | nd | 374 (239–553) | nd | 366 (190–568) | nd | 417[n] (331-544) |
| Monthly unprotected sexual episodes, mean (range)[b] | 3 (1.5-10) | | 8 (1 – 30) | | 7 (1-25) | |
| Previous history of sexually transmitted diseases and/or AIDS-defining illnesses (%) | nd | 39 | 22[c] | 40 | 29 | nd |
| Heterosexual orientation (%) | 100 | 100 | 90.5 | 79.7 | 17 | nd |
| Homosexual orientation (%) | 0 | 0 | 2.5 | 3 | 44 | nd |
| Bisexual orientation (%) | 0 | 0 | 7 | 17.3 | 39 | nd |
| Ethnicity – Ancestry[d], % | European (Tuscan): 100 | European (Tuscan): 100 | Afr: 22.6 Amer: 41.9 Eur: 35.5 | Afr: 25.5 Amer: 40.3 Eur: 34.1 | Mestizo: 89 Indigenous: 5 others: 6 | Mestizo: 100 |

**Clinical status of the populations.**
**SP: Seropositives, HESN: HIV-1 exposed seronegative; SD: Standard deviation; nd: is not determined; yrs: Years; Afr: African; Amer: Amerindian; Eur: European**
**[a] Cohort inclusion criteria was CD4 count of above 250 (requested by ethics board)**
**[b] In Peru, this refers to number of partners, not sexual episodes**
**[c] HESNs have presented sexually transmitted diseases but no AIDS-defining illnesses**
**[d] Ancestry of the Colombian cohort was previously reported in (14)**

### Association of Tim-1 18-bp insertion/deletion polymorphism with HIV-1 infection susceptibility

| Origin | Genotype counts (LL/SL/SS) HESN | SP | Genotype counts (recessive) (LL+SL/SS) HESN | SP | $p_{recessive}$[a] | OR (95 CI)[b] | Meta-analysis ($P_{recessive}$ and OR[c]) | Meta-analysis with Thai sample ($P_{recessive}$ and OR[c]) |
|---|---|---|---|---|---|---|---|---|
| Italy | 19/57/45 | 20/63/27 | 76/45 | 83/27 | 0.0393 | 0.549 (0.31-0.97) | | |
| Colombia | 7/24/32 | 5/25/21 | 31/32 | 30/21 | 0.3068 | 0.68 (0.32-1.43) | 0.0088, OR: 0.62 | 0.0050, OR: 0.65 |
| Peru | 7/28/98 | 3/29/60 | 35/98 | 32/60 | 0.1732 | 0.67 (0.38-1.36) | | |

**SP: Seropositives, HESN: HIV-1 exposed seronegative**
**[a] Logistic regression *p* value for a recessive model**
**[b] Odds ratio (OR) for a recessive model with 95% confidence intervals**
**[c] Random-effect meta-analysis *p* value (recessive model) and OR**

### HIV-1 infection Assay

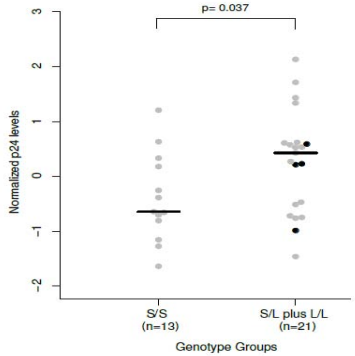



**Figure 1. p24 concentration in the supernatants of HIV-infected CD4+ T cells of subjects with different *TIM1 genotypes* 5 days post HIV infection. Mean values and S.E. are shown.**

### TIM1 mRNA expression

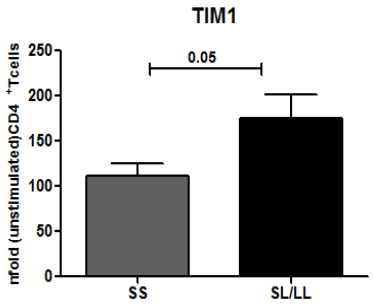



**Figure 2. Basal mRNA expression of TIM1 in CD4+ T cells of subjects with SS genotype (grey bars) and heteroSL plus LL genotypes (black bars)**

## Conclusions

- **The S (short ) allele of the 6-amino acid insertion/deletion polymorphism protects from HIV-1 infection with a recessive effect; the protective effect is independent from the route of exposure and ethnic origin**
- **CD4 T cells isolated from subjects carrying the S allele sustain lower viral replication compared to L/L S/L genotypes.**
- **The protection conferred by the S allele is correlated with a reduction of TIM1 mRNA expression level**
- **These results underscore a complex interaction between enveloped viruses and TIM molecules that need further investigation.**

## References

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