# Role of IL18 in HIV Associated Lipodystrophy

Poster No. 92

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## Introduction

- Lipodystrophy caused by highly active antiretroviral therapy (HAART) is associated with an increased risk of metabolic disturbances and ischaemic heart disease [1] .
- Antiretroviral (ARV) induced adipose toxicity is central to HIVLD pathogenesis resulting in impaired adipogenesis and dysregulated secretion of adipokines from the adipose tissue[2].
- Plasma levels of IL18, an adipokine associated with insulin resistance is elevated in HIVLD patients[3].
- Variants in IL18 gene also predispose to the development of HIVLD[4].
- Interleukin-18 suppresses adiponectin expression in adipocytes via NFATc4 phosphorylation[5].
- We utilised *in vitro* (adipocytes) and *in vivo* (gene association) studies to characterise the role of IL18 in HIVLD.

## Aims and Objectives

- To investigate whether ARVs modulate IL18 and NFATc4 expression *in vitro* in adipocytes
- To utilise *in vitro* model to identify candidate molecules to target IL18 and NFATc4
- To investigate whether genetic variants in IL18 predispose to the development of HIVLD by HAART

## Methods

- Differentiating 3T3-F442A murine adipocytes were incubated with ARVs (Lopinavir [LPV], ritonavir [RTV], atazanavir [ATV] and efavirenz [EFV]) in the presence and absence of telmisartan (TEL; 1-5µM).
- Secreted IL18 protein levels were assessed by ELISA.
- Real-Time PCR was used to study gene expression of IL18 and NFATc4.

**Genetic Association study**

- DNA samples were obtained from ARV-treated patients with (HIVLD+; n=115) or without LD (HIVLD-; n=51).
- Diagnosis of HIVLD was carried out by clinician's confirmation of patient self-report
- SNP selection: Haplotype tag SNP approach; functional SNPs also selected; total 14 SNPs selected
- Sequenom MALDI-TOF was used for genotyping

**Statistical Analysis**

- *In vitro* studies were analysed by paired t-test and data are presented as mean ±SD for 20µM incubation of ARVs and 5µM for TEL.
- SNP analysis was performed using Haploview software.

**Table 1. Patients characteristics**

| Characteristics | Study participants (n = 180) HIVLD+; n = 124, HIVLD-; n = 56 | |
|---|---|---|
| Age in years, median (range) | 40 (24-68) | |
| Gender | | |
| Men | 146 (81.5%) | |
| Women | 34 (18.5%) | |
| Duration of HIV infection, median (range) | 7.6 years (1.4 -19.3 years) | |
| CD4 cell count, mean (range) | 330.5 (3-1218) | |
| PIs | LPV, ATV, RTV | |
| NRTI backbone | d4T, 3TC, AZT | |
| Other drugs | ddI, ddc, ABC, EFV, NVP | |
| | HIVLD+ | HIVLD- |
| Time of exposure to PIs, median | 24.3 months | 24.5 months |
| Time of exposure to NRTIs, median | 39 months | 39 months |

## Results

**Fig 1. Effect of ARVs and Telmisartan on IL18 protein expression**

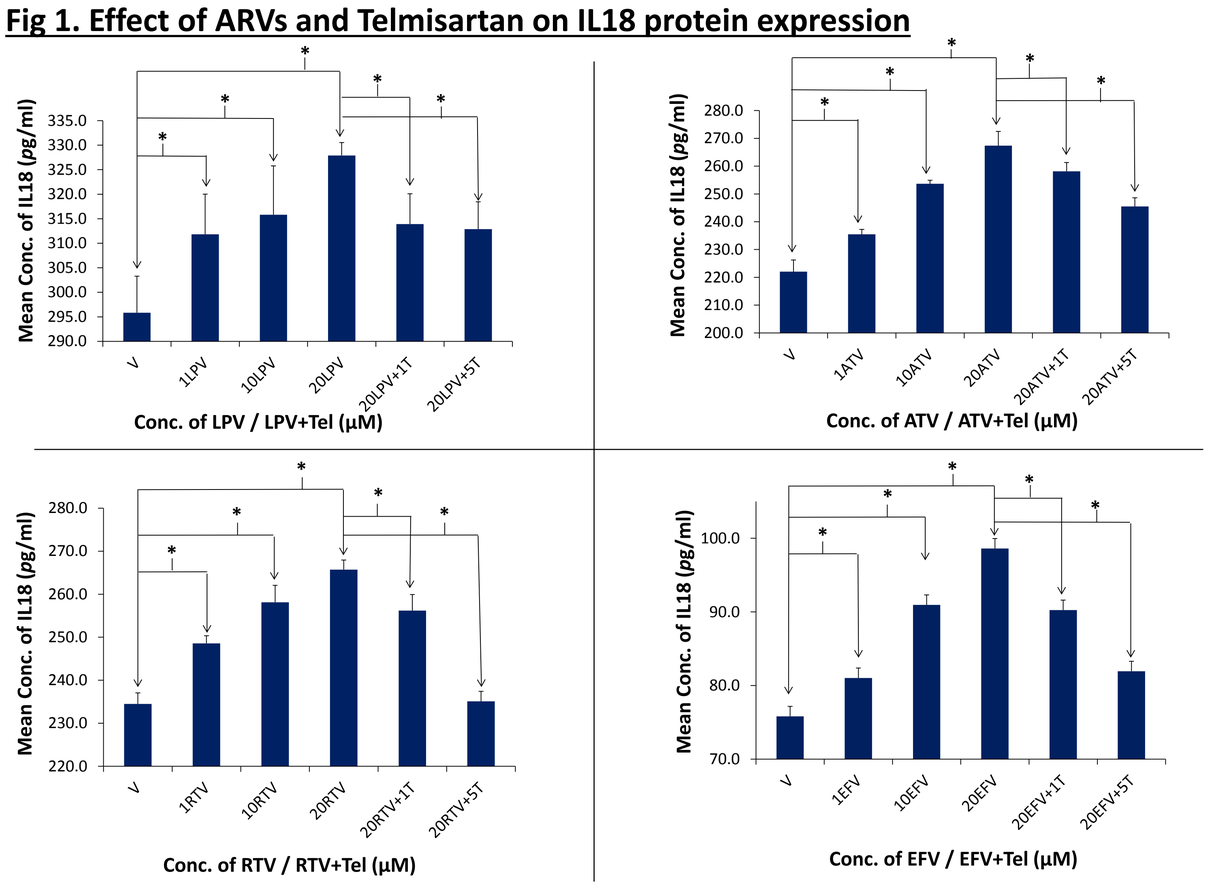


**Fig 1.** All of the ARVs (LPV, ATV, RTV, EFV) up-regulated secretion of IL18 in adipocytes. Telmisartan reversed the effect of ARVs when co-incubated with ARVs. Similar pattern was observed in gene expression study of IL18 (Data not shown) Note: Mean values were obtained from 4 independent repeats. *= *p* value < 0.05

**Fig 2. Effect of ARVs & Telmisartan on NFATc4 gene expression**

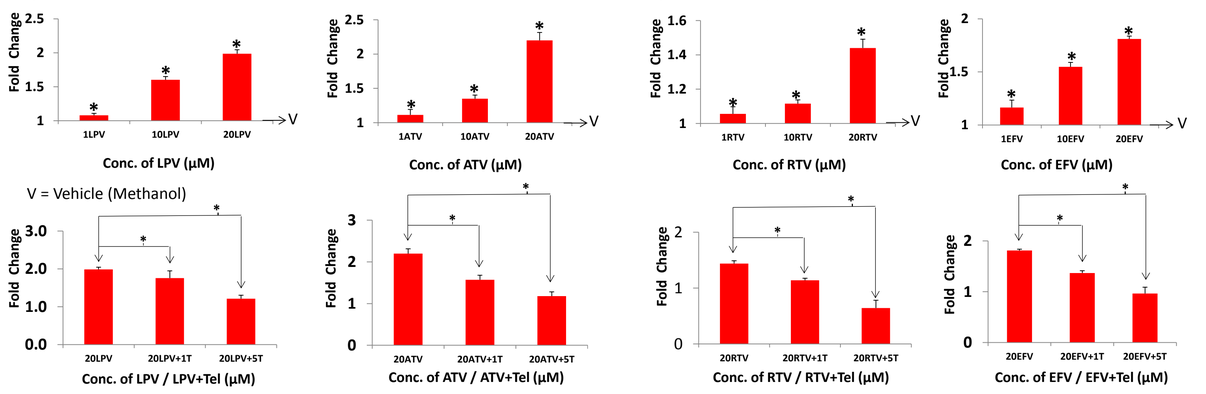


**Fig 2.** All of the ARVs (LPV, ATV, RTV, EFV) up-regulated gene expression of NFATc4 in adipocytes. Telmisartan reversed the effect of ARVs when co-incubated with ARVs. Note: Mean values were obtained from 4 independent repeats. *= *p* value < 0.05

**Fig 3. Genotyping Results**

| Table 2.Single marker Associations-haploview analysis | | |
|---|---|---|
| Sl. NO. | SNP ID. No. | P - Values |
| 1 | rs2115763 | 0.6742 |
| 2 | rs360719 | 0.5845 |
| 3 | rs1946518 | 0.2353 |
| 4 | rs1834481 | 0.5956 |
| 5 | rs549908 | 0.4461 |
| 6 | rs5744280 | 0.6525 |
| 7 | rs5744290 | 0.1828 |
| 8 | rs5744292 | 0.2101 |
| 9 | rs543810 | 0.4873 |

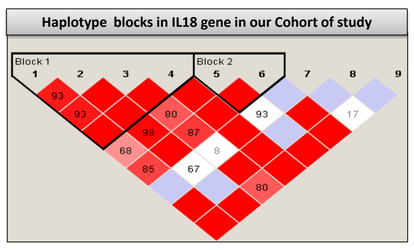


**Fig 3.** Linkage disequilibrium patterns within the IL18 gene haplotype blocks are represented here. Red boxes indicate strong linkage disequilibrium (LD), while grey boxes indicate weaker LD. Haplotype block 1 is comprised of rs215763, rs360719, rs1946518 and rs1834481. Haplotype block 2 consists of rs549908 and rs5744280.

## Summary of Results

- ARVs up-regulate secretion of IL18 in adipocytes and up-regulate the expression of NFATc4
- Effect of EFV on IL18 was lesser as compared to LPV, ATV and RTV
- NFATc4 gene expression was higher with ATV treatment as compared to LPV, RTV and EFV
- Telmisartan reverses ARV-induced increase in IL18 secretion and NFATc4 up-regulation
- IL18 SNPs did not show association with HIVLD in our cohort of study

## Conclusion

- ARV-mediated upregulation in IL18 could play a role in the development of HIVLD
- ARV-induced upregulation of NFATc4, a transcription factor through which IL18 causes inhibition of adiponectin (a marker of insulin sensitivity), could be mechanistically important in the development of insulin resistance.
- Telmisartan offers a potential strategy to treat ARV-induced adverse effects

## Future Work

- Blockade of NFATc4 using siRNA, an on going experimental strategy, may result to reduce adverse effect of ARVs
- Validation of murine adipocyte results in primary human adipocytes
- IL18 gene variants do not predispose to HIVLD; however further studies in well-phenotyped patients with adequate sample size are required to confirm this.

## References

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