

# IFN- $\gamma$ Genotype (874A>T) is associated with CCR5 Expression in HIV+ Patients

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## Introduction

- A number of G protein-coupled CC- and CXC-chemokine receptors have been shown to act as HIV-1 co-receptors *in vitro*, but CCR5 and CXCR4 are the major HIV-1 co-receptors *in vivo*<sup>1</sup>.
- Of the drugs used in HIV therapy protease inhibitors (PI) are known to be substrates for efflux transporters, ABCB1 (P-gp), ABCC1 (MRP1) and ABCC2 (MRP2)<sup>2</sup>
- We previously reported a positive correlation between expression of transporters and the HIV co-receptors CXCR4 and CCR5 in peripheral blood mononuclear cells (PBMC)<sup>3</sup>. This correlation may be of virological and pharmacological importance
- There are discordant reports in the literature as to the effects of cytokines on the expression of drug transporters and chemokine receptors in different cell types.
- Here we have examined a panel of cytokines for their effects on transporter and chemokine receptor expression in *ex-vivo* PBMC from healthy volunteers.
- In addition, we examined the impact of polymorphisms, previously reported to impact on expression of IL-2 (-330T>G)<sup>4</sup> and IFN $\gamma$  (874A>T)<sup>5</sup>, on expression of these proteins in PBMC from a cohort of HIV+ patients.

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## Methods

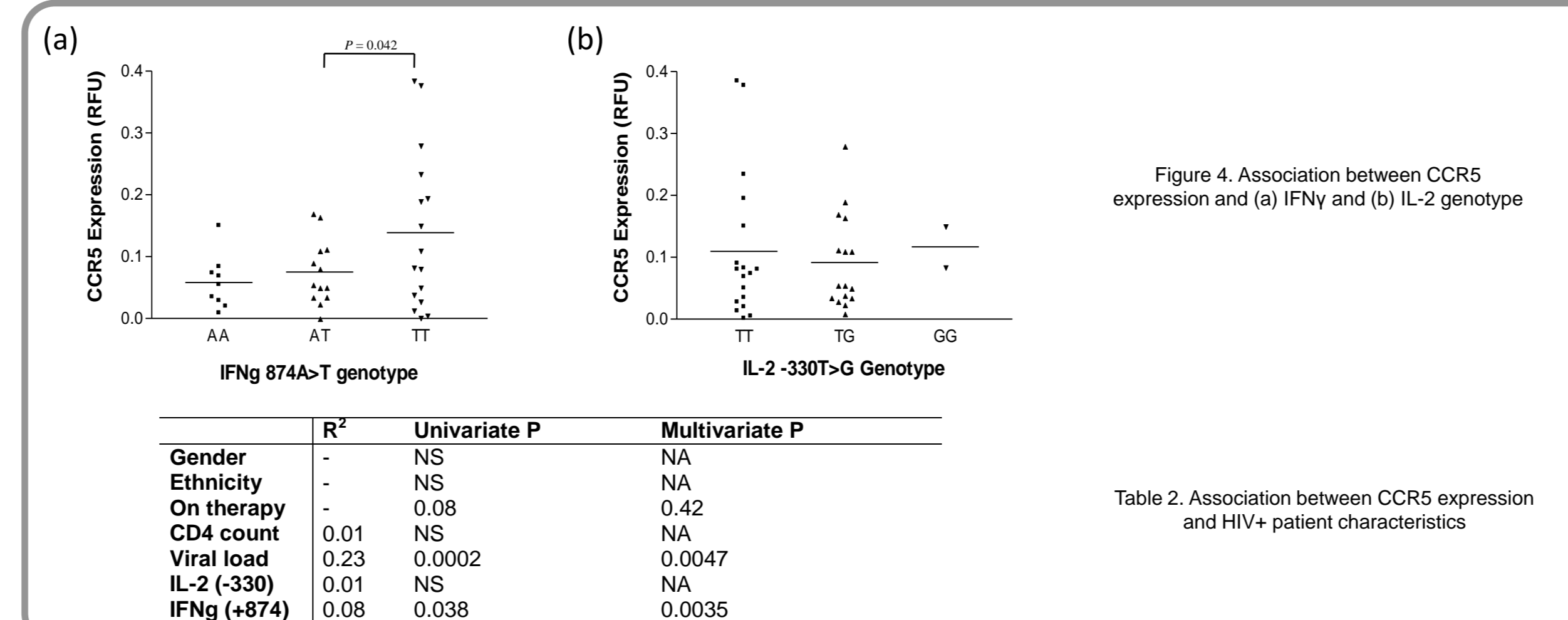
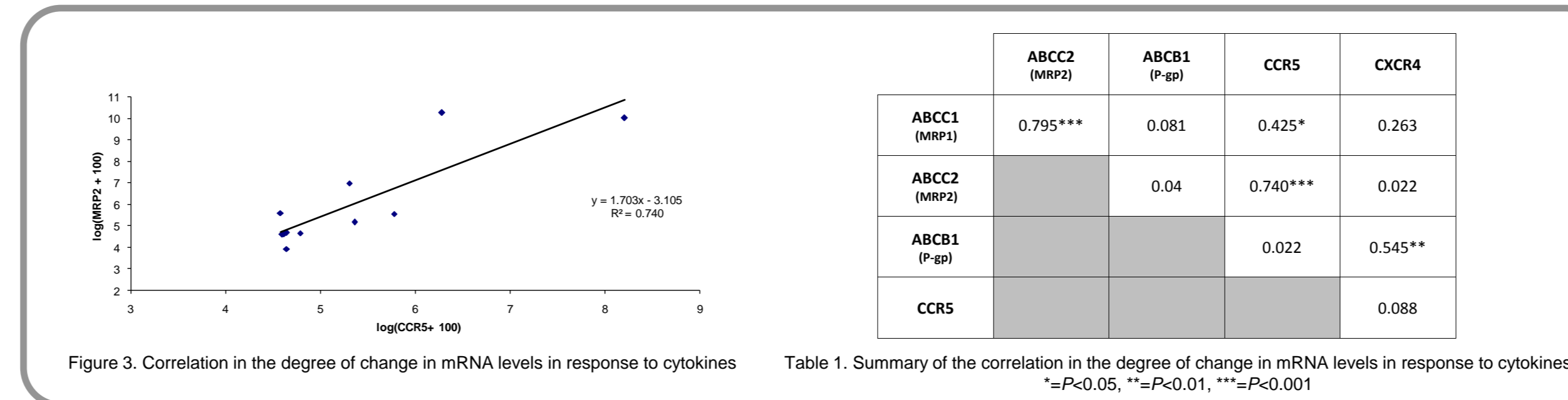
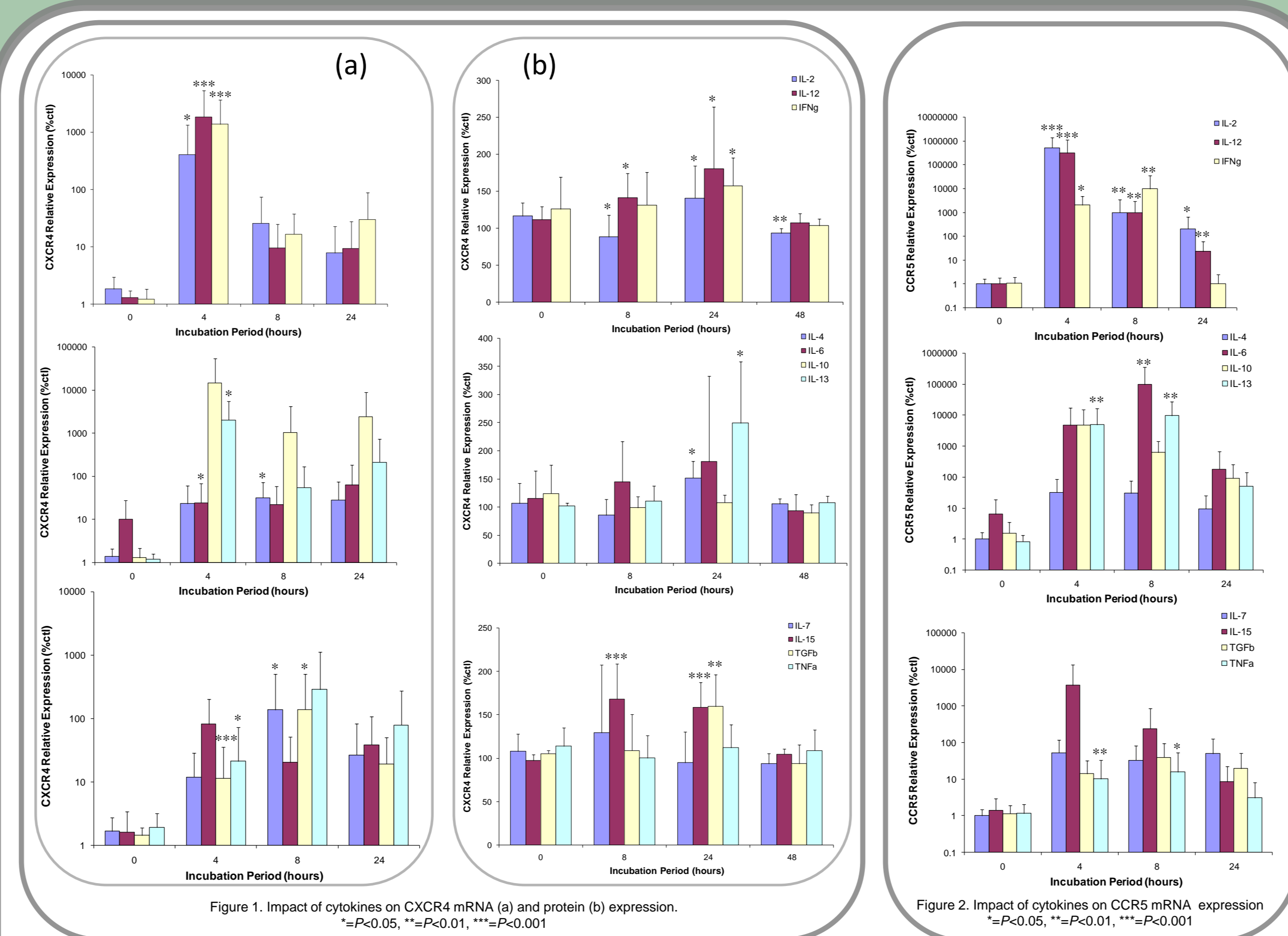
- PBMC were isolated from healthy volunteer whole blood (n=8) and expression of P-gp, MRP1, MRP2, CXCR4 and CCR5 measured by qPCR (mRNA) and flow cytometry (protein) after 0, 2, 4, 8, 24 and 48h incubation with cytokines IL-2, IL-4, IL-6 IL-7, IL-10, IL-12, IL-13, IL-15, TGF $\beta$ , TNF $\alpha$  and IFN $\gamma$  (10 ng.ml<sup>-1</sup>).
- Genotyping for 874T>A and -330T>G was conducted in HIV+ patients (n=66) by allelic discrimination and P-gp, MRP1, CXCR4 and CCR5 quantified by flow cytometry.
- Median (range) viral load (VL) and CD4 counts were 249 (<50, 646000) and 327 (8, 1318), respectively.
- 44 patients were receiving therapy, 42 were male and 53 were Caucasian.
- Statistical analysis was conducted by Mann-Whitney U test (impact of cytokines), linear regression (relationship between changes in expression) and multiple linear regression using best subset selection (differences between genotypes).

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## Aims

The aims of this study were to:

- analyse the effects that cytokines have on the expression of drug transporters and HIV co-receptors in human peripheral blood mononuclear cells (PBMC) from healthy volunteers.
- investigate the association between cytokine polymorphisms and expression of these proteins in HIV+ patients.



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## Results

- Cytokines included in the current study altered the expression of HIV co-receptors at the mRNA level (fig. 1a & fig. 2). Some but not all of these changes were also observed at the protein level (fig. 1b).
- Impact on expression was dependant on incubation time and was cytokine specific .
- CCR5 mRNA expression was significantly affected after 4 hours incubation with the Th1 cytokines IL-2 (334.09, 0.23-1953386.2)  $P = 0.0006$  and IL-12 (364.1, 0.35-2192481.1,  $P = 0.0006$ ). IFN $\gamma$  had its greatest effect on CCR5 expression after 8 hours incubation (18.58, 1.23-67354.6,  $P = 0.0054$ ) (fig. 2).
- The degree of change in expression in response to cytokines was proportional among certain transcripts notably ABCC2 & CCR5 (fig. 3, summarised for all transcripts in table 1).
- An association between the T allele of the IFN $\gamma$  874A>T genotype and increased CCR5 expression in HIV+ patients was observed (fig. 4a & table 2).
- Viral load was also associated with CCR5 expression (table 2).
- No association was found between CCR5 expression and IL-2 genotype or other patient data (table 2).

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## Conclusions

- Cytokines included in this study had a number of significant effects on the expression of drug transporters and chemokine receptors. The degree to which expression was affected supported the hypothesis of co-regulation of these genes.
- The combined effects of cytokines on transporter and chemokine receptor expression may have important virological and pharmacological implications.
- The T allele of the IFN $\gamma$ 874 genotype, previously shown to increase IFN $\gamma$  production, was associated with increased CCR5 expression in HIV+ patients.
- Further studies are now warranted to determine the impact of this polymorphism on disease progression.

## References

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