

Identification of a *IL-7RA* Risk Allele for Rapid Progression to AIDS: Results of a Genomic *IL-7/IL-7RA* Study in the GRIV Cohort.

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Abstract

Background : IL-7 is a critical factor for homeostasis of the memory T-cell pool and thymopoiesis. A dysregulation of IL-7RA expression has been described in HIV infection. IL-7 blood levels are elevated during the last stages of HIV infection. We investigated whether the polymorphisms of the *IL-7/IL-7RA* genes were associated with disease progression.

Material and Methods : We have exhaustively genotyped the *IL-7* and *IL-7RA* genes in the AIDS GRIV (Genetics of Resistance to Immunodeficiency Virus) cohort and in 370 healthy controls (CTR). The GRIV cohort involves 90 rapid (RP; pts with progression to CD4<300/mm³ less than 3 years following seroconversion) and 280 slow (SP; asymptomatic subjects with CD4>500/mm³ for more than 8 years after diagnosis of seropositivity) progressors. *IL-7* gene was analyzed by PCR/sequencing. *IL-7RA* gene was analyzed with the SNPlex method based on HapMap derived tagSNPs. Statistical analyses were performed by the Fisher's exact test by comparing either RP or SP with CTR.

Results : We identified 28 polymorphisms by genotyping the *IL-7* gene, and we determined 9 tagSNPs covering the 50 HapMap SNPs known for *IL-7RA*. No association was detected in SP neither at the level of individual SNPs nor at the level of haplotypes. In RP, weak positive signals (P comprised within 0.02 to 0.05) were found for 3 intronic SNPs of *IL-7* and a stronger signal was found for one intronic *IL-7RA* tagSNP (P=0.007). No signal was detected in the single promoter SNP found for *IL-7* nor in the single one present in *IL-7RA*. No exonic SNP was found for *IL-7*, however we could haplotype the combinations of tagSNPs corresponding to the 4 known exonic variants of *IL-7RA*. A strong signal (P=0.003) was found for the combination Ile244Thr and Val356Ile, i.e the haplotype 244Thr/356Ile was found more prevalent in the recessive mode among RP (32.1%) than in CTR (17.15%) or in SP (20.73%).

Conclusion : Our preliminary study points out a protein risk allele of *IL-7RA* for rapid progression to AIDS. Interestingly, the 244 variant identified here is a major risk factor for another immune-related disease, namely Multiple Sclerosis, as recently described in a genome-wide study [11]. Further studies in other HIV cohorts and functional analysis of these variants need to be performed in order to confirm this hypothesis and to understand their impact on immune regulation.

Background

IL-7/IL-7RA are major factors of T cell homeostasis both at the thymic and peripheral level. Numerous recent findings highlight the importance of the interleukin-7 pathway impairment in the pathogenesis of HIV infection. Notably, IL-7 levels increase with advancing CD4+ T cell-lymphopenia, whereas IL-7RA expression is downregulated mainly on CD8+ T cells [1]. We have undertaken a genomic approach to evaluate the influence of the IL-7/IL-7RA pathway in AIDS by exploring the polymorphisms of the *IL-7* and *IL-7RA* genes in the GRIV cohort and looking for associations with HIV progression.

Material & Methods

The GRIV cohort

The GRIV study is a case-control study comparing seropositive subjects with extreme profiles of progression with control subjects, all of them of European descent and living in France [2-6].

- 280 **Slow Progressors (SP):** asymptomatic individuals, seropositive for 8 or more years with CD4 > 500/mm³ in the absence of antiretroviral therapy
- 90 **Rapid Progressors (RP):** seropositive patients exhibiting a progression to CD4 < 300/mm³ less than 3 years after the last seronegative test
- 350 seronegative **Control subjects (CTR)**

Genotyping

IL-7 gene was genotyped by PCR-sequencing (Figure 1A).

IL-7RA gene was genotyped using the SNPlex method, a multiplexed genotyping system based on HapMap derived tagSNPs (Figure 1B). *IL-7RA* tagSNPs were chosen from the HapMap data in order to cover all the frequent SNPs (minor allele frequency>1%) of the region with a linkage disequilibrium > 0.95.

Results

Analysis of *IL-7* polymorphisms and progression to AIDS

Only weak associations were found in the *IL-7* gene at the level of intronic polymorphisms, by comparing RP with CTR. The signals were positive under both the allelic frequency calculation mode (not shown) and the dominant mode (Table 1).

Analysis of *IL-7RA* polymorphisms and progression to AIDS (Table 2)

An association involving an intronic tagSNP (IL7RA_18514) in linkage disequilibrium (LD) with a 3' gene region SNP was found by comparing the RP and CTR groups (p=0.041 and 0.0073 in allelic frequency and dominant modes respectively). A 3' gene region tagSNP (IL7RA_20835) in LD with the exonic SNP (Val356Ile) exhibits a significant association when comparing the RP and CTR groups (p=0.0086 and 0.021 in allelic frequency and dominant modes respectively).

Analysis of *IL-7RA* haplotypes and progression to AIDS

Four exonic SNPs are known in the *IL-7RA* gene: Thr66Ile, Ile138Val, Thr244Ile and Val356Ile (Figure 2), Thr66Ile and Ile138Val being in full LD. Only the haplotypes derived from the two exonic SNPs Thr244Ile and Val356Ile yielded a positive signal by comparing the RP and CTR groups (Table 3). The H0 haplotype (Thr244Ile/356Ile) leads to positive signals in the allelic (p=0.023) and recessive (p=0.0034) modes, and the H1 haplotype (Thr244Ile/356Ile) leads to positive signal in the allelic (p=0.0079) and dominant (p=0.019) modes. The genotype H0/H0 promotes rapid progression to AIDS (Figure 3). The association found for the H1 haplotype, which seems to prevent rapid progression (Figure 3), is explained by the single SNP IL7RA_19195 (Val356Ile) which is in full LD with the tagSNP IL7RA_20835 (Table 2).

Discussion & Conclusion

We present here the first genomic study dealing with the role of IL-7/IL-7RA pathway in AIDS progression. The most significant signals were found in the *IL-7RA* gene, with the intronic SNP IL7RA_18514 and the exonic SNP IL7RA_19195 (p<0.02). Interestingly, these two very SNPs have been implicated in the progression of Multiple Sclerosis (MS) by previous genomic studies [9, 10]. We also found signals for the haplotypes derived from the exonic SNPs Thr244Ile and Val356Ile (Table 3 and Figure 3), which corresponds to a transmembrane mutation (Figure 2), has also been recently implicated in the development of MS by a genome-wide study [11] and it influences the ratio of soluble to membrane-bound isoforms of IL-7RA by putatively disrupting the splicing of exon 6 [12]. Val356Ile is a mutation located in the intracytoplasmic tail (Figure 2). A bioinformatics analysis reveals a putative phosphorylation site in Thr357 [13] and Val356Ile could thus affect signal transduction. It could also alter interaction with the common IL2Rg subunit.

Our results suggest an effective role of the IL-7/IL-7RA pathway in the development of HIV-1 infection. As for any genomic study, the results found in this study will have to be extended by the analysis of other AIDS cohorts. Previous works have investigated the role of the *IL-7RA* gene in MS, focussing in particular on the exonic SNP Thr244Ile. We point out in addition a putative role for the Val356Ile SNP and for the 2-exonic SNP haplotype: both will have to be investigated more thoroughly in genomic studies in HIV infection as well as in MS. The exploration of the biological function of these variants is under investigation. Their significance in disease progression is important as IL-7 therapy is already used in phase I clinical trials during HIV infection [14].

Material & Methods

Bioinformatics and Statistical analysis

We used the Ishape2 software [7] to infer the haplotypes obtained from the SNPs of each candidate gene. For selected polymorphisms (located in 5' gene region or exons), we used the SUBHAP method [8] to infer the subhaplotypes. Differences in the allelic distributions between either RP or SP and CTR were examined using Fisher's exact tests. Three modes of calculation were performed for the genetic analysis: allelic frequency, dominant mode and recessive mode.

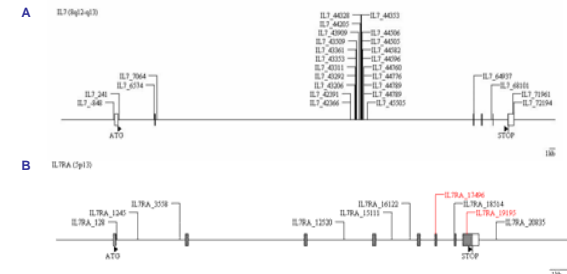


Figure 1: Genetic maps of *IL-7* (A) and *IL-7RA* (B) genes. Exons and UnTranslated Regions (UTR) are symbolized respectively by full boxes and empty boxes.

| Allele | Location | Dominant frequency | | | p values for dominant mode | | |
|-------------|----------|--------------------|-----|-----|----------------------------|-----------|----------|
| | | CTR | RP | SP | SP vs CTR | RP vs CTR | SP vs RP |
| IL7_42366-- | intron | 10% | 23% | 13% | 0.57 | 0.027 | 0.11 |
| IL7_44205-C | intron | 18% | 37% | 30% | 0.075 | 0.040 | 0.43 |
| IL7_44353-G | intron | 18% | 34% | 27% | 0.14 | 0.042 | 0.41 |

Table 1: Weak intronic associations found in the *IL-7* gene.

| Allele | Location | LD (r>0.95) | Dominant frequency | | | p values for dominant mode | | |
|---------------|----------------|--|--------------------|-----|-----|----------------------------|-----------|----------|
| | | | CTR | RP | SP | SP vs CTR | RP vs CTR | SP vs RP |
| IL7RA_18514-T | intron | 3' gene region (1) | 83% | 66% | 80% | 0.40 | 0.0073 | 0.057 |
| IL7RA_20835-G | 3' gene region | intron (6) exon (Val356Ile) 3' gene region (1) | 53% | 39% | 52% | 0.85 | 0.021 | 0.060 |

Table 2: Two associations with rapid progression to AIDS found in the *IL-7RA* gene. SNPs number (n) in strong LD with tagSNPs studied and their location are informed

| Allele | Dominant/Recessive frequency | | | p values for dominant/recessive mode | | | |
|--------|------------------------------|-----|-----|--------------------------------------|-----------|-----------|----------|
| | CTR | RP | SP | Mode | SP vs CTR | RP vs CTR | SP vs RP |
| H0 | 17% | 32% | 21% | R | 0.33 | 0.0034 | 0.059 |
| H1 | 53% | 38% | 52% | D | 0.85 | 0.019 | 0.057 |

Table 3: Two *IL-7RA* haplotypes associated with rapid progression to AIDS. R for recessive mode and D for dominant mode

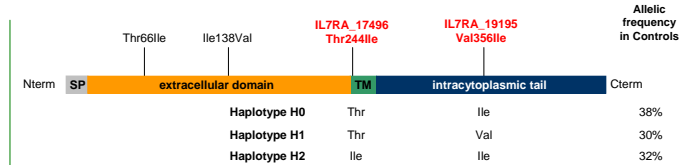


Figure 2: *IL-7RA* protein & Haplotypes definition. SP for Signal Peptide ; TM for transmembrane

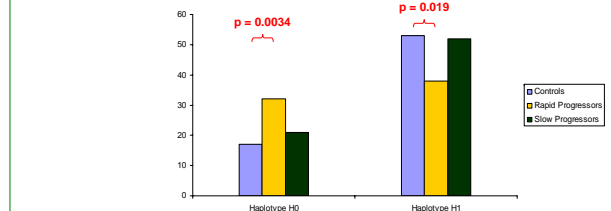


Figure 3: Haplotypes H0 and H1 associated with rapid progression to AIDS: H0/H0 promotes rapid progression to AIDS, whilst H1 prevents rapid progression

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