



# An *IL7RA* exon 5 polymorphism is associated with impaired IL-7R $\alpha$ splicing and protection against tuberculosis in Ghana

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## Abstract

Functional interleukin-7 receptor  $\alpha$ -chain (IL-7R $\alpha$ ) genetic variants, which affect alternative splicing and expression of the soluble IL-7R $\alpha$ , are associated with susceptibility to autoimmunity. We previously described aberrant IL-7R $\alpha$  expression and impaired IL-7-mediated T-cell functions in tuberculosis patients. In the present study, we investigated a possible role of *IL7RA* gene variants. Six exonic *IL7RA* polymorphisms were genotyped and two minor alleles were found at lower frequencies in tuberculosis patients as compared to healthy contacts from Ghana (rs11567764,  $p = 0.002$ ; rs1494558,  $p = 0.01$ ). The rs11567764 polymorphism tags an *IL7RA* haplotype exclusively found in African populations and was predicted to affect splicing of exon 5. Reduced mRNA expression of the  $\Delta$ exon\_5-6 variant was found in T-cells from carriers of the protective rs11567764 allele. Although we were not able to demonstrate the causative effect of rs11567764, our findings suggested functional implications of genetic variants on IL-7R $\alpha$  splicing and with potential impact on T-cell protection against tuberculosis.

## Introduction

Tuberculosis is a life-threatening mycobacterial disease of global relevance. The vast majority of *Mycobacterium tuberculosis*-infected individuals, however, does not develop tuberculosis but rather controls infection by

immune surveillance [1]. An important role of genetic factors for immune protection against tuberculosis has been demonstrated in animal models [2] and in humans [3]. Previous studies indicated the capacity of IL-7 to improve protection against *M. tuberculosis* [4] and found impaired IL-7-promoted T-cell response during acute tuberculosis [4, 5]. IL-7 promotes several functions of T-cell immunity including proliferation, cell survival, and cytokine expression [6].

The T-cell response to IL-7 is critically regulated on the IL-7R $\alpha$  (CD127, gene: *IL7RA*) level, and membrane-associated (m)IL-7R $\alpha$  as well as a soluble (s)IL-7R $\alpha$  expression are of central relevance for IL-7-response and availability [7]. The sIL-7R $\alpha$  variant is generated by alternative splicing and has been described to exert IL-7 reservoir functions for T cells [8]. A single nucleotide polymorphism (SNP) (C>T) [9, 10] located in *IL7RA* exon 6 affects alternative splicing, thereby altering the sIL-7R $\alpha$  plasma concentration [9, 11]. Alternative splicing events also generate *IL7RA* variants lacking exon 5-6, but the biological relevance hereof remains elusive [12]. Exonic *IL7RA* SNPs were previously found to be associated with susceptibility to develop autoimmune diseases, like multiple sclerosis [9].

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Own previous studies revealed reduced mIL-7R $\alpha$  and sIL-7R $\alpha$  expression during tuberculosis. Minor differences in the frequency of the rs6897932T *IL7RA* allele between the study groups contributed to decreased sIL-7R $\alpha$  plasma concentrations in tuberculosis patients [5]. Here, we investigated a possible role of exonic *IL7RA* SNPs in tuberculosis and evaluated functional effects of the rs11567764 polymorphism.

## Methods

### Study participants

Patients with clinically confirmed tuberculosis ( $n = 114$ ) and exposed, healthy contacts ( $n = 170$ ) were recruited from 2011 to 2016 in Kumasi/Ghana. Diagnosis of tuberculosis was based on patient's history, symptoms (i.e., persistent cough and fever, weight loss) and chest X-ray. For adults, tuberculosis was additionally confirmed by sputum smear test and, in case of a negative sputum test, by *M. tuberculosis* sputum culture. Healthy contacts were recruited from households of patients with confirmed tuberculosis. Venous blood was collected into heparinised Vacutainer Tubes (BD Biosciences). Anti-tuberculous medication according to the Ghanaian guidelines was initiated immediately after the first blood sample was taken. Analysis of mRNA from enriched CD4<sup>+</sup> T cells was performed on a subset of samples. Part of this patient group has been used previously for the analysis of IL-7 and IL-7R $\alpha$  and results are published elsewhere [5].

The study was approved by the Committee on Human Research, Publication and Ethics (CHRPE) at the School of Medical Sciences (SMS) at the Kwame Nkrumah University of Science and technology (KNUST) in Kumasi, Ghana (Ref.: CHRPE/AP/275/14, CHRPE/AP/221/14, CHPRE/AP/328/15). The aims and procedures of the study were explained to participants aged  $\geq 18$  years, and all adult participants gave written informed consent. For children, the aims and procedures of the study were explained to participants (if appropriate) and to their parents or legal guardians. Assent of children and written informed consent was obtained from parents/guardians. In some cases, consent was confirmed by thumbprint, a procedure approved by the review board. All participants were free to drop out at any time of the study.

### Genotyping of *IL7RA* single nucleotide polymorphisms

DNA was isolated from peripheral blood mononuclear cells (Biocoll, Biochrom) or whole blood using QIAamp DNA Mini kit (Qiagen) or GenoType DNA Isolation Kit (Hain

Lifescience). The sample size was chosen based on previous studies that identified associations of the rs6897932 SNP with other immune pathologies and immune interventions. Genotyping was performed with predesigned TaqMan SNP Genotyping Assays (Applied Biosystems) and analysed on a 7500 Real-Time PCR machine (Applied Biosystems). A call rate  $\geq 97\%$  was found for all SNPs, and none of the SNPs deviated from the Hardy–Weinberg equilibrium.

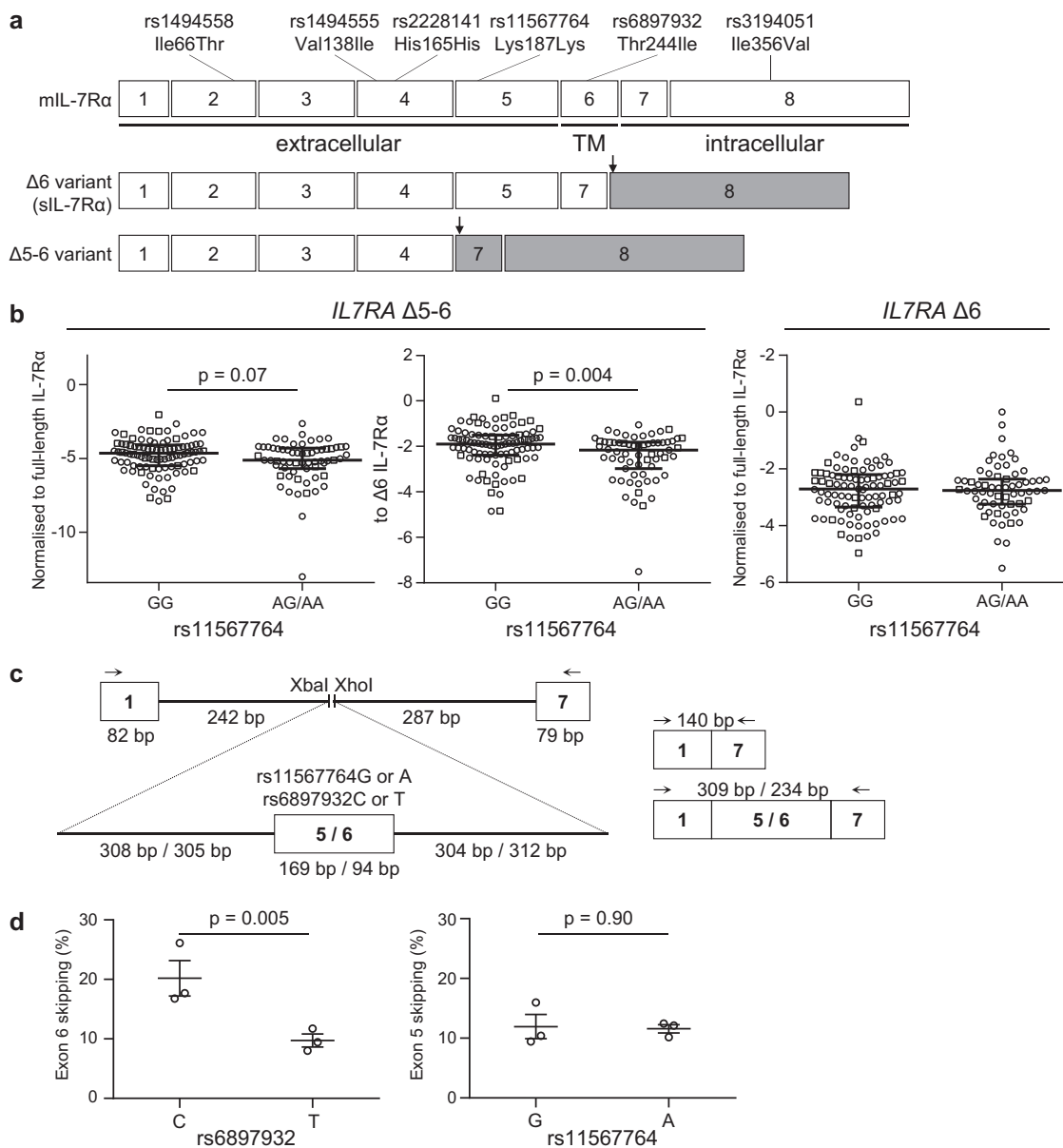
### Real-time PCR of IL-7R $\alpha$ variants

CD4<sup>+</sup> T cells from freshly isolated peripheral blood mononuclear cells (at least  $1.5 \times 10^7$  cells) were isolated using anti-human CD4 magnetic particles (BD Biosciences) obtaining a cell purity generally  $>95\%$  as evaluated by flow cytometry. RNA was isolated and cDNA was generated as described previously [13]. RT-PCR was performed with the QuantiTect SYBR Green PCR kit (Qiagen) as described before [5].

### *IL7RA* minigenes: generation and analyses in HEK293T cells

*IL7RA* minigenes were generated as shown in Fig. 1c by PCR of human DNA from exon 1 including 242 bp of the downstream intron, and exon 7 including 287 bp of the upstream intron. A complementary overhang containing *XbaI/XhoI* restriction enzyme sites was included downstream and upstream of the introns, respectively. The construct was inserted into the LeGO iB2 vector [14] using *BamHI-BglIII/NotI* restriction enzyme sites. Exon 5 and exon 6 of *IL7RA*—including part of the upstream and downstream introns—were inserted into the minigene vector using *XbaI/XhoI* restriction enzyme sites. To generate mutants, PCR was performed with Phusion High-Fidelity PCR Master Mix with GC Buffer (New England BioLabs) and appropriate primers (sequences are given in Supplementary Table 2). The reactions were run for 30 s at 98 °C followed by 18 cycles of 7 s at 98 °C, 30 s at 60 °C, and 8 min at 72 °C, followed by 10 min at 72 °C. Plasmid of bacterial origin was removed by DpnI digestion prior to transformation. Successful mutation was verified by DNA sequencing.

HEK293T cells were kindly provided by the group of B. Fehse (University Hospital Eppendorf, Hamburg) and are regularly tested for mycoplasma contamination. HEK293T cells were transfected with minigene vectors, and after 24 h incubation, mRNA was isolated from cells using the NucleoSpin RNA kit (Macherey-Nagel) followed by generation of cDNA using Maxima H Minus First Strand cDNA Synthesis kit (Thermo Fisher Scientific). PCR was performed with Phusion High-Fidelity PCR Master Mix with GC Buffer (New England BioLabs) and primers



**Fig. 1** *IL7RA* single nucleotide polymorphisms (SNPs) and influence of genetic variants on *IL7RA* splicing. **a** Position of six selected SNPs in the exons of *IL7RA*. The full-length *IL-7R* including the transmembrane domain (TM) as well as two alternative splice variants lacking exon 6 ( $\Delta 6$ , coding for sIL-7R $\alpha$ ) or exon 5-6 ( $\Delta 5-6$ ) are shown. Premature stop codons caused by a change in the reading frame are indicated by arrows for the  $\Delta 6$  and the  $\Delta 5-6$  variants. **b** Expression of *IL7RA* transcripts coding for IL-7R $\alpha$  lacking exon 6 ( $\Delta 6$ ) or exon 5-6 ( $\Delta 5-6$ ) in CD4<sup>+</sup> cells from tuberculosis patients (squares) and households contacts (circles) carrying rs11567764GG ( $n = 93$ ) or rs11567764AG/AA ( $n = 63$ ). Expression level is normalised to full-length IL-7R $\alpha$  or  $\Delta 6$ . Median and interquartile range for cycle threshold differences ( $2^{-\Delta C_t}$ ) are indicated. Exact Mann-Whitney  $U$ -tests were used for comparison of groups. rs11567764AG and rs11567764AA carriers. **c**, **d** Effects of *IL7RA* rs11567764 alleles on

alternative splicing were determined using a minigene-based approach. **c** Schematic depiction of *IL7RA* minigene generation. Exon 5 or exon 6 of the *IL7RA* (including ~300 bp of the introns upstream and downstream of the exons) were inserted into a construct generated from *IL7RA* exon 1 and exon 7 including part of the downstream and upstream introns, respectively. Exon 5 and exon 6 were mutated to obtain rs11567764G/A and rs6897932C/T variants. On the right side, primer positions for PCR are indicated by arrows and expected product lengths are shown. **d** HEK293T cells were transfected in triplicate with minigene constructs. The proportion of transcript with exon5/exon6 skipped is indicated in the plots with mean  $\pm$  SEM. One representative experiment of four (rs11567764G/A) and two (rs6897932C/T) is shown. Data analysed by exact Mann-Whitney test stratifying for experiment, and  $p$ -values are based on data from all experiments

covering exon 1 to exon 7 (forward 5'-ATGACAATTCTA GGTACAACCTTTTGG-3', reverse 5'-AAGATGTTCCAG

AGTCTTCTTATG-3'). The reactions were run for 30 s at 98 °C followed by 30 cycles of 7 s at 98 °C, 15 s at 60 °C,

**Table 1** Association between *IL7RA* SNPs in tuberculosis patients and healthy household contacts

| SNP        | Genotype | Frequency (%) |            | Codominant       |                 | Dominant         |                 | Recessive        |                 |
|------------|----------|---------------|------------|------------------|-----------------|------------------|-----------------|------------------|-----------------|
|            |          | Contacts      | TB         | OR               | <i>p</i> -Value | OR               | <i>p</i> -Value | OR               | <i>p</i> -Value |
| rs1494558  | CC       | 39.9          | 45.0       | –                |                 | –                |                 | –                |                 |
|            | CT       | 44.0          | 48.6       | 0.98 (0.59–1.62) | <b>0.04</b>     | 0.81 (0.50–1.31) | 0.39            |                  | <b>0.01</b>     |
|            | TT       | <b>16.1</b>   | <b>6.3</b> | 0.35 (0.14–0.86) |                 |                  |                 | 0.35 (0.15–0.84) |                 |
| rs1494555  | AA       | 79.2          | 77.7       | –                |                 | –                |                 | –                |                 |
|            | AG       | 17.9          | 21.4       | 1.22 (0.67–2.23) | 0.37            | 1.09 (0.61–1.95) | 0.77            |                  | 0.21            |
|            | GG       | 3.0           | 0.9        | 0.31 (0.04–2.66) |                 |                  |                 | 0.29 (0.03–2.55) |                 |
| rs2228141  | CC       | 80.6          | 75.0       | –                |                 | –                |                 | –                |                 |
|            | CT       | 19.4          | 24.1       | 1.33 (0.75–2.38) | 0.26            | 1.38 (0.78–2.45) | 0.27            |                  | 0.40            |
|            | TT       | 0.0           | 0.9        | NA               |                 |                  |                 | NA               |                 |
| rs11567764 | GG       | 56.5          | 64.0       | –                |                 | –                |                 | –                |                 |
|            | AG       | 35.1          | 35.1       | 0.88 (0.53–1.46) | <b>0.008</b>    | 0.73 (0.45–1.19) | 0.21            |                  | <b>0.002</b>    |
|            | AA       | <b>8.3</b>    | <b>0.9</b> | 0.09 (0.01–0.72) |                 |                  |                 | 0.10 (0.01–0.75) |                 |
| rs6897932  | CC       | 87.6          | 83.0       | –                |                 | –                |                 | –                |                 |
|            | CT       | 12.4          | 17.0       | 1.45 (0.74–2.84) | 0.28            | NA               | NA              |                  | NA              |
|            | TT       | 0.0           | 0.0        | NA               |                 |                  |                 | NA               |                 |
| rs3194051  | AA       | 50.0          | 43.5       | –                |                 | –                |                 | –                |                 |
|            | AG       | 39.2          | 48.1       | 1.41 (0.85–2.35) | 0.33            | 1.30 (0.80–2.11) | 0.29            |                  | 0.49            |
|            | GG       | 10.8          | 8.3        | 0.88 (0.37–2.12) |                 |                  |                 | 0.75 (0.32–1.73) |                 |

TB tuberculosis patients, OR odds ratio, NA not applicable

and 45 s at 72 °C, followed by 8 min at 72 °C. PCR products were mixed with Hi-Density TBE Sample Buffer and separated on a 10% Novex TBE gel in Novex TBE Running Buffer (Thermo Fisher Scientific) at 200 V. Subsequently, the gel was stained with GelRed (Biotium) and analysed on a ChemiDoc Touch Imaging System (Bio-Rad).

### Statistical analysis

Haplotypes of selected SNPs in *IL7RA* were predicted from 1000 Genomes data set using HaploView version 4.2. Statistical analyses were performed in R version 3.3.0. Due to non-normality of the data (Shapiro–Wilk test of normality), exact Mann–Whitney *U* test from the package *coin* was applied for group comparisons. SNP associations were evaluated by logistic regression using the package *SNPassoc* [15]. Plots were generated in GraphPad Prism version 6.07. Two-tailed *p*-values <0.05 were considered significant.

### Results and discussion

We genotyped six exonic *IL7RA* SNPs (Fig. 1a) in tuberculosis patients ( $n = 114$ ) and healthy contacts ( $n = 170$ ). For two SNPs, i.e., rs1494558C>T and rs11567764G>A, minor alleles were present at higher frequencies among healthy contacts as compared to tuberculosis patients

(Table 1). The major effects were seen for recessive models (rs11567764: OR 0.10, 95% CI: 0.01–0.75; rs1494558: OR 0.35, 95% CI: 0.15–0.84). Only 0.9% of tuberculosis patients were homozygous for the minor rs11567764A allele whereas 8.3% of contacts had this genotype (Table 1). These differences are comparable to heterozygous hemoglobin S variant carrier frequencies, a well-known example for a protective allele against malaria [16]. rs11567764 is a synonymous SNP located in exon 5 (Fig. 1a), and its minor allele is almost exclusively found in African populations (Supplementary Table 1). rs1494558 is located in exon 2 of *IL7RA* and causes a nonsynonymous substitution (i.e., 197C>T, Thr66Ile; Fig. 1a). In combination, the associated minor alleles of both SNPs, i.e., rs11567764A and rs1494558T tag a haplotype frequently present in African populations (Supplementary Table 1). Genome-wide association studies (GWAS) in tuberculosis patients and healthy controls have been performed previously [3, 17]. However, both associated SNPs were not included in these studies of tuberculosis patients. This instance, and the restricted sensitivity of applied statistical methods for detection of recessive allele effects [18], may explain that the *IL7RA* genomic region on chromosome 5 was not identified as a candidate [3].

*IL7RA* transcripts lacking exon 6 ( $\Delta 6$ , coding for sIL-7R $\alpha$ ) or exon 5–6 ( $\Delta 5-6$ ) are generated by alternative splicing [12, 19]. Since the SNP rs6897932 located in exon 6 has been shown to influence exon 6 splicing [9], we

predicted the potential effect of the exon 5 SNP rs11567764 on alternative splicing using HEXplorer [20]. Marked differences in HEXplorer scores indicated a potential negative effect of the rs11567764A allele on exon 5 splicing (Supplementary Figure 1). Consequently, we measured IL-7R $\alpha$  mRNA of enriched CD4<sup>+</sup> T cells from study group donors for  $\Delta$ 5-6,  $\Delta$ 6 (sIL-7R $\alpha$ ), and full-length (mIL-7R $\alpha$ ) IL-7R $\alpha$  and compared relative amounts of variants between different rs11567764 allele carriers. Donors with the minor rs11567764A allele had a tendency of lower  $\Delta$ 5-6 mRNA levels in comparison to full-length IL-7R $\alpha$  mRNA ( $p = 0.07$ ; Fig. 1b, left graph) and significantly lower  $\Delta$ 5-6 levels as compared to  $\Delta$ 6 IL-7R $\alpha$  mRNA ( $p = 0.004$ ; Fig. 1b, middle graph). No effect was seen when comparing  $\Delta$ 6 mRNA with full-length IL-7R $\alpha$  mRNA ( $p = 0.92$ ; Fig. 1b, right graph). Because of a low number of homozygous rs11567764A carriers, subgroup analysis of tuberculosis patients and healthy contacts for the recessive effect of rs11567764A on  $\Delta$ 5-6 mRNA expression was not possible. To investigate if differential  $\Delta$ 5-6 mRNA expression could be ascribed to the rs11567764 SNP, we generated exon 5 minigenes containing either rs11567764A or rs11567764G (Fig. 1c). The rs6897932 exon 6 variants were also included since these have been proven to affect IL-7R $\alpha$  exon 6 alternative splicing [9] (Fig. 1c). In accordance, we detected reduced levels of exon 6 skipping for the minigene including the rs6897932T variant (Fig. 1d, left graph). However, no differences of exon 5 splicing were seen between rs11567764G and rs11567764A variants (Fig. 1d, right graph). Therefore, we could not assign differential  $\Delta$ 5-6 mRNA expression specifically to the rs11567764 SNP. However, linkage of rs11567764 to another functional SNP or regulatory elements not covered by the minigene could potentially account for this. The function of the  $\Delta$ 5-6 variant remains unclear. It is predicted to be a target for nonsense-mediated decay due to a premature stop codon located upstream of the exon 7/exon 8 junction [21] (Fig. 1a). In addition, the  $\Delta$ 5-6 variant lacks several structural parts of the D<sub>2</sub> domain coded by exon 5 [22]. Therefore, we assume that  $\Delta$ 5-6 variant effects are indirect, e.g., by increasing expression of the other IL-7R $\alpha$  variants. Further studies are needed to elucidate the role of the  $\Delta$ 5-6 variant.

These results indicated protective effects of *IL7RA* rs1494558T/rs11567764A alleles against the development of tuberculosis. Genetic polymorphisms in *IL7RA* were shown to affect several immune pathologies including chronic viral [23, 24], autoimmune [9, 10], and inflammatory diseases [25] as well as immune interventions (e.g., graft-versus-host disease after transplantation [26]). Our results may form the basis for further investigations to address the question how different *IL7RA* polymorphisms and combinations affect T-cell function and immune pathologies.

There is a strong evidence that IL-7 has a decisive role in the generation of protective T-cell memory against chronic infections [27, 28], and the protective capacity of IL-7-mediated effects on T cells against tuberculosis has also been shown [4]. IL-7 can revert T-cell exhaustion [29] and is considered an important candidate for immunomodulatory therapy against chronic infectious diseases [30]. However, we demonstrated previously that acute tuberculosis is accompanied by impaired IL-7 responses of antigen-specific T-cells and aberrant low sIL-7R $\alpha$  plasma levels [5]. How genetic variants promote protective IL-7R $\alpha$  functions against the development of tuberculosis needs further clarification. Beyond the field of tuberculosis, this study may generally add to our understanding of crucial processes involving IL-7 in T-cell response of autoimmune and infectious diseases.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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