A frequent functional toll-like receptor 7 polymorphism is associated with accelerated HIV-1 disease progression

Djin-Ye Oh\textsuperscript{a,\,*}, Konstantin Baumann\textsuperscript{a}, Osamah Hamouda\textsuperscript{b}, Jana K. Eckert\textsuperscript{a}, Konrad Neumann\textsuperscript{c}, Claudia Kücherer\textsuperscript{b}, Barbara Bartmeyer\textsuperscript{b}, Gabriele Poggensee\textsuperscript{b}, Nari Oh\textsuperscript{d,\,*}, Axel Pruss\textsuperscript{e}, Heiko Jessen\textsuperscript{d} and Ralf R. Schumann\textsuperscript{a}

Objectives: Toll-like receptors (TLRs) play an important role in the innate immune response to pathogens. TLR7 recognizes RNA of various viruses including HIV. The objective of this study was to examine the influence of individual genetic variations of TLR7 on the susceptibility to and progression of HIV disease.

Method: We genotyped a population of 734 HIV-positive adults and 545 healthy controls for three TLR7 single nucleotide polymorphisms. The frequency of TLR7 genetic variations was assessed and related to HIV disease progression. Furthermore, we analyzed peripheral blood mononuclear cells obtained from healthy individuals differing in their TLR7 genotype and assessed their response to a TLR7-specific ligand \textit{ex vivo}.

Results: Presence of the most frequent TLR7 polymorphism, TLR7 Gln11Leu, was associated with higher viral loads and accelerated progression to advanced immune suppression in HIV patients. Furthermore, in women this polymorphism may be associated with increased HIV-1 susceptibility as it was found more frequently among patients as compared with controls. Peripheral blood mononuclear cells from polymorphism carriers secreted significantly less IFN-\alpha following TLR7 activation, whereas IL-6 production remained unaltered.

Conclusion: This is the first report of a functional TLR7 variant to be associated with susceptibility to and a more severe clinical course of HIV-1 disease. These results may have implications for the risk assessment of individual patients as well as for HIV-1 therapy and vaccination strategies in the future.

Introduction

Toll-like receptors (TLRs) are pattern recognition receptors (PRRs) playing a key role in innate immune defense against a wide diversity of pathogens [1,2]. TLR-mediated recognition of highly conserved so-called ‘pathogen-associated molecular patterns (PAMPs)’ initiates complex signaling cascades, culminating in the activation of mediators and effector cells, including cytokines and cytotoxic T lymphocytes. This innate immune response includes a rapid inflammatory response that helps to clear the pathogen. Subsequently, TLRs contribute to adaptive immunity by stimulating the expression of MHC and costimulatory molecules, enhancing T-cell activation, and supporting the development of Th1-type responses that can be crucial for clearing infections. In the case of lentiviruses such as HIV, the ability of viral proteins to interfere with TLR signaling has been demonstrated [3].

\textsuperscript{a}Institute for Microbiology and Hygiene, Charité University Medical Center, \textsuperscript{b}Robert Koch Institute, \textsuperscript{c}Department of Medical Biometry and Clinical Epidemiology, Charité University Medical Center, \textsuperscript{d}The Berlin Trial on HIV and TLR SNPs, Gemeinschaftspraxis Jessen-Jessen-Stein, and \textsuperscript{e}Institute of Transfusion Medicine, Charité University Medical Center, Berlin, Germany.

Correspondence to Ralf R. Schumann, MD, PhD, Institute for Microbiology and Hygiene, Charité-Universitätsmedizin Berlin, Dorotheenstr. 96, 10117 Berlin, Germany.
Tel: +49 30 450 524141; fax: +49 30 450 524941; e-mail: ralf.schumann@charite.de
* Present address: The Floating Hospital for Children, Tufts Medical Center, Boston, Massachusetts, USA.
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of transcription factors such as nuclear factor (NF)κB and interferon regulatory factor (IRF)-7 [3,4]. The resulting differential gene expression, which varies depending on which TLR has been activated, leads to potent innate immune responses against the pathogen and modifies the adaptive immune response.

Ten human TLRs have been identified [5]. Although TLR research initially concentrated on bacterial PAMPs, it is now firmly established that viruses also interact with the TLR system [6]. TLRs implicated in the response to viral stimulation include TLR7 and TLR8 sensing viral single-strand RNA [7–9]. The outcome of viral TLR activation exhibits wide individual variation ranging from effective antiviral response to viral replication and spread [10].

Individual variation of the host response is particularly important for HIV. HIV-1 RNA activates TLR7/8 [8,11,12]. For both, TLR7 stimulation and TLR8 stimulation, an anti-HIV effect has been demonstrated depending on an enhancement of the adaptive [13,14], and also the innate immune response [15,16]. In acute HIV-infection, TLR8-induced HIV-restriction depends on unknown soluble factors [15], whereas TLR7-induced HIV-constraint is governed mainly by IFNα [16]. HIV-1 RNA induces the release of IFNα in plasmacytoid dendritic cells (pDCs) via TLR7 activation [11]; type I interferons induce a wide range of antiretroviral factors and potently restrict HIV-1 replication in vitro [17–23] and in vivo [24–26].

Conversely, TLR ligands have been demonstrated to mediate a continuous activation of the immune system driving viral replication and progression to AIDS [27–29]. As the HIV-long terminal repeat (LTR) contains NFκB transcription sites, TLR-triggered NFκB activation leading to LTR-dependent transcription is viewed as one of the mechanisms for increased HIV-1 replication [15,27,29–31]. Furthermore, immune activation has been implicated with natural killer (NK)-cell activation in vivo [39]. The study group comprised a total of 1279 individuals of White or African ethnicity, 734 HIV-1-positive individuals of either White or African ethnicity, 734 HIV-1-positive individuals.

Material and methods

Sample genotyping

Three coding nonsynonymous TLR7 SNPs were determined in the study group by real-time PCR assays. Of these, two (Gln11Leu, Ala448Val) are coding nonsynonymous SNPs reported at an allelic frequency of more than 1% in the Entrez dSNP database (http://www.ncbi.nlm.nih.gov/sites/entrez?db=snp and http://www.ensembl.org/); the third (T -120G) was previously identified to be related to another RNA-viral disease, HCV infection [51]. Whereas Gln11Leu and Ala448Val are located to exon 3 of the TLR7 gene, T-120G is an intronic polymorphism. The detailed PCR curve and melting curve analysis methodology is available online in the Supplementary Methods section.

Study population

The study group comprised a total of 1279 individuals of either White or African ethnicity, 734 HIV-1-positive individuals.
adults [men 671 (91%); women 63 (9%); Whites 708 (96.5%); Africans 26 (3.5%); median age, 39 years (interquartile range, IQR, 34–43 years)], and 545 seronegative controls [men 489 (90%); women 56 (10%); Whites 510 (94%); Africans 35 (6%); median age 38 years (IQR, 30–43 years)].

Seropositive individuals are HIV-1 patients either enrolled in the German HIV-1 Seroconverter Study (n = 646) or in the Berlin Trial on HIV and TLR SNPs (n = 88). The German HIV-1 Seroconverter Study is a nationwide study based on a cohort of HIV-infected individuals for whom the date of seroconversion can be reliably estimated as the midpoint between the last negative and the first positive HIV-1 antibody test within a maximum interval of 3 years, or the date of the first reactive test if acute seroconversion was documented [52–54]. The Berlin Trial on HIV and TLR SNPs was established for the purpose of this study and enrolled seroconverters (n = 24) and seroprevalent patients (n = 64) for whom the date of seroconversion has not been documented. Further details on both study cohorts are provided online in the Supplementary Methods section.

For disease progression analyses, set point viral loads (available for 302 HIV-1 infected individuals; see Statistical Methods for definition) and, in Kaplan–Meier analysis of 464 seroconverters, the time from seroconversion to advanced immunosuppression as defined by a CD4+ T-cell count less than 350 μl were assessed. To preclude confounding effects, CD4+ T-cell count and viral load values gathered before the start of any antiretroviral treatment were considered only. Furthermore, carriers of the established HIV-protective CCR5Δ32 deletion [43,55–60] were excluded in a second step of both viral load and survival analysis.

The HIV-negative control collective consisted of 425 anonymized seronegative White blood donors and 120 healthy, unrelated volunteers. This group has been previously described [50]; for the survey reported here, 53 individuals (48 cases, five controls) were excluded from analysis, either because TLR7 genotyping was not feasible or because they were of rare or unknown ethnicity. Informed consent has been obtained, and written approval from the relevant ethical boards has been granted for all studied participants.

Statistical methods
Genotype frequencies of seropositive and seronegative individuals were compared using χ2 tests. Viral set points, defined as the median of all values of log10-transformed copies of HIV-1 RNA measured in plasma between 100 days and 2 years postseroconversion [47,61], were available for 302 therapy-naive patients. To evaluate the correlation between TLR7 genotypes and viral load, individual set points in each genetic group were compared by the nonparametric Mann–Whitney test. Survival analysis was conducted using the Kaplan–Meier method with an endpoint of less than 350 CD4+ T cells/μl. Differences between genotypes were analyzed by the log-rank test. P values less than 0.05 were considered statistically significant. We used SPSS 14.01 software (SPSS Inc., Chicago, Illinois, USA) for data management and statistical analyses, and PRISM 4 for figures.

Isolation and stimulation of peripheral blood mononuclear cells
Whole blood was obtained from age-matched pairs of healthy volunteers differing in their TLR7Gln11Leu genotype. All donors were healthy White males negative for the TLR7 T-120G, TLR7 Ala448Val and TIRAP Ser180Leu polymorphisms who had given written informed consent before donation. Detailed protocols for isolation and cultivation of peripheral blood mononuclear cells (PBMCs) are provided online in the Supplementary Methods section.

After seeding, cells were stimulated with 100 μl supplemented medium per well containing the respective ligands Imiquimod (Invivogen, Toulouse, France) and lipopolysaccharide (LPS) (Re595; Sigma, Deisenhofen, Germany) at the indicated concentrations. IFNα and IL-6 protein levels were measured from cell culture supernatants in a sandwich enzyme-linked immunosorbent assay (ELISA) procedure, which is supplied in detail in the Supplementary Methods section.

Results
To test the hypothesis that TLR7 genetic variation is related to the risk for, or the course of HIV-1 infection, distribution of three polymorphisms was analyzed in a total study population of 1279 participants. Of these, 734 were HIV-1 positive individuals and 545 were seronegative controls, matched according to age, ethnicity and sex.

Distribution of toll-like receptor 7 mutant alleles in HIV-1 patients and controls
If presence of a certain gene variant modifies the susceptibility to HIV-1, its frequency should vary between seropositive and seronegative populations. Thus, we compared TLR7 polymorphism frequencies for HIV-1-infected patients and controls. Because of the X-chromosomal localization of the TLR7 gene, analyses were conducted separately according to sex (Table 1).

The TLR7 T-120G polymorphism was found in White participants only and was absent in Africans. In White males, the proportion of hemizygotes ranged between 4.9% in seronegatives and 5.8% in seropositives; this difference was not significant. In women, the frequency of heterozygotes among HIV patients (12.7%) was similar as observed for healthy controls (10.7%).
The TLR7 Ala448Val alteration was found to be rare, with a total of eight SNP carriers detected in the entire study collective. All of these were White males. Two of them were HIV-infected, whereas the other six hemizygotes belonged to the seronegative control group corresponding to proportions of 0.3 and 1.3%, respectively.

Regardless of serostatus, ethnicity, and sex TLR7 Gln11Leu represented the single most common genetic variation in all subgroups examined. In men, frequencies of hemizygotes were roughly similar in HIV-patients (23.4%) and in HIV-negative controls (20.9%; \( P = 0.305 \)). By contrast, in HIV-infected women the mutant allele carriage frequency was significantly higher than that observed in healthy controls (\( P = 0.002 \)). This distinction remained significant when analysis was restricted to women of either White or African ethnic background. The association might represent a stochastic event, given the low number of women in our study collective. However, it may also indicate that female carriers of the TLR7 Gln11Leu variant allele are at an increased risk of being infected with HIV-1.

### Table 1. Prevalence and genotype frequencies of toll-like receptor 7 polymorphisms in HIV-positive individuals and controls.

<table>
<thead>
<tr>
<th>TLR7 polymorphism</th>
<th>Gender</th>
<th>Ethnicity</th>
<th>Status</th>
<th>Number of individuals in each genotype (%)</th>
<th>( P^b )</th>
<th>Prevalence, %</th>
<th>( P^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR7 T-120G</td>
<td></td>
<td>Combined</td>
<td>Case</td>
<td>T/G or T/- ( ^d ) T/G</td>
<td></td>
<td>632 (94.2)</td>
<td>39 (5.8)</td>
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<tr>
<td></td>
<td>Male</td>
<td>White</td>
<td>Case</td>
<td>-</td>
<td></td>
<td>465 (95.1)</td>
<td>24 (4.9)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Control</td>
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<td></td>
<td>623 (94.1)</td>
<td>39 (5.9)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>Control</td>
<td>-</td>
<td></td>
<td>453 (95.0)</td>
<td>24 (5.0)</td>
</tr>
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<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>9 (100)</td>
<td></td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>12 (100)</td>
<td>-</td>
<td></td>
<td>0 (0.0)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Combined</td>
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<td>55 (87.3)</td>
<td>8 (12.7)</td>
<td>0.737</td>
<td>12.7</td>
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<td>50 (89.3)</td>
<td>6 (10.7)</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Case</td>
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<td>38 (82.6)</td>
<td>8 (17.4)</td>
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<td>17.4</td>
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<td>27 (81.8)</td>
<td>6 (18.2)</td>
<td>18.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>17 (100)</td>
<td>0 (0.0)</td>
<td>0.0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>0 (0.0)</td>
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<tr>
<td></td>
<td></td>
<td>Gln11Leu</td>
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</tr>
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<td></td>
<td>Male</td>
<td>Combined</td>
<td>Case</td>
<td>Gln/Gln or Gln/- ( ^d ) Gln/Leu</td>
<td>157 (23.4)</td>
<td>0.305</td>
<td>23.4</td>
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<td></td>
<td></td>
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<td>Case</td>
<td>-</td>
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<td>102 (20.9)</td>
<td>19.0</td>
</tr>
<tr>
<td></td>
<td></td>
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<td>Control</td>
<td>-</td>
<td></td>
<td>155 (23.4)</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>77 (77.8)</td>
<td>22 (22.2)</td>
<td>0.748</td>
<td>22.2</td>
</tr>
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<td></td>
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<td>Control</td>
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<td>-</td>
<td></td>
<td>2 (16.7)</td>
<td>16.7</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Combined</td>
<td>Case</td>
<td>35 (55.6)</td>
<td>24 (38.1)</td>
<td>4 (6.3)</td>
<td>0.006*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>Case</td>
<td>46 (82.1)</td>
<td>9 (16.1)</td>
<td>1 (1.8)</td>
<td>17.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td>25 (54.3)</td>
<td>17 (37.0)</td>
<td>4 (8.7)</td>
<td>0.050*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>26 (78.8)</td>
<td>7 (21.2)</td>
<td>0 (0.0)</td>
<td>21.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10 (58.8)</td>
<td>7 (41.2)</td>
<td>0 (0.0)</td>
<td>0.023*</td>
<td>41.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gln/Leu</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Combined</td>
<td>Case</td>
<td>Gln/Leu or Leu/- ( ^d ) Leu/Leu</td>
<td>19 (24.1)</td>
<td>0.006*</td>
<td>24.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>Case</td>
<td>-</td>
<td></td>
<td>6 (1.2)</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td>-</td>
<td></td>
<td>2 (0.3)</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>9 (10.0)</td>
<td>0 (0.0)</td>
<td>NS</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>12 (10.0)</td>
<td>-</td>
<td></td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>Combined</td>
<td>Case</td>
<td>62 (100)</td>
<td>0 (0.0)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>White</td>
<td>Case</td>
<td>46 (100)</td>
<td>0 (0.0)</td>
<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
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<td>0</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>African</td>
<td>Case</td>
<td>17 (100)</td>
<td>0 (0.0)</td>
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<td></td>
<td>Control</td>
<td>23 (100)</td>
<td>0 (0.0)</td>
<td></td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

\[ ^a \text{Comparisons were conducted separately according to ethnicity and gender.} \]
\[ ^b \text{2 \times 2 or 3 \times 2 \chi^2 \text{ comparisons, depending on the presence or absence of heterozygous and mutant hemizygotes/homozygotes in the respective subgroup.} \]
\[ ^c \text{2 \times 2 \chi^2 \text{ comparisons.} \]
\[ ^d \text{Denotes absence of the particular allele in hemizygous males.} \]

The TLR7 Ala448Val alteration was found to be rare, with a total of eight SNP carriers detected in the entire study collective. All of these were White males. Two of them were HIV-infected, whereas the other six hemizygotes belonged to the seronegative control group corresponding to proportions of 0.3 and 1.3%, respectively.

Regardless of serostatus, ethnicity, and sex TLR7 Gln11Leu represented the single most common genetic variation in all subgroups examined. In men, frequencies of hemizygotes were roughly similar in HIV-patients (23.4%) and in HIV-negative controls (20.9%; \( P = 0.305 \)). By contrast, in HIV-infected women the mutant allele carriage frequency was significantly higher than that observed in healthy controls (\( P = 0.002 \)). This distinction remained significant when analysis was restricted to women of either White or African ethnic background. The association might represent a stochastic event, given the low number of women in our study collective. However, it may also indicate that female carriers of the TLR7 Gln11Leu variant allele are at an increased risk of being infected with HIV-1.

**Association of toll-like receptor 7 polymorphisms with HIV-1 disease progression**

We next examined whether either of the two common TLR7 SNPs examined here, TLR7 T-120G and TLR7 Gln11Leu, were related to the severity of HIV-1 disease. To this end, we first analyzed baseline CD4\(^+\) T-cell counts and observed lower CD4\(^+\) T-cell counts in male TLR7 Gln11Leu mutation carriers as compared with carriers of the common allele (Supplementary Table S1). To assess
the influence of these polymorphisms on disease progression, we examined their association with set point viral loads and with the endpoint of less than 350 CD4+ T cells/μl, which signifies immunologically advanced HIV-1 infection. Because of the low number of women with these parameters available, analysis was confined to male seropositives.

Impact of toll-like receptor 7 genotype on HIV-1 set point viral load

We observed no association between TLR7 T-120G genotype and set point plasma viral loads (see Material and Methods for a definition), implying that this polymorphism does not play a role in establishing steady-state levels of HIV-1 after seroconversion (Table 1).

Regarding TLR7 Gln11Leu genotype, however, males hemizygous for the rare ‘Leu’ alteration displayed significantly higher set point viral loads implying a deleterious effect of the minor gene variant ($P = 0.017$; Fig. 1a). To exclude confounding issues resulting from different ethnic backgrounds, the analysis was limited to Whites and this difference remained significant ($P = 0.013$; data not shown). When individuals heterozygous for the HIV-1 protective CCR5Δ32 deletion were excluded from analysis, the distinction between genotypes was more pronounced ($P = 0.006$; Fig. 1b).

Relation between toll-like receptor 7 genotype and advanced immunological stage of HIV-1 infection

A CD4+ T-cell count less than 350/μl is the immunological criterion for advanced HIV-1 disease in adults; it firmly indicates the need to initiate antiretroviral therapy according to the revised European [62] and American [63] guidelines. We hypothesize that the time until the CD4+ T-cell number reaches 350/μl depends on TLR7 genetic variation.

Although no such association was observed for the TLR7 T-120G SNP (data not shown), Kaplan–Meier analysis of 464 seroconverters revealed that TLR7 Gln11Leu hemizygotes took a median time of 17 [95% confidence interval (CI): 12–22] months to reach the study endpoint of less than 350 CD4+ T cells/μl, whereas carriers of the normal gene variant took a median 32 [24–40] months, indicating an accelerated disease course in Gln11Leu mutation carriers. This difference was significant ($P = 0.021$; Fig. 2a), remained so when only Whites were considered ($P = 0.020$; data not shown), and was more pronounced in the subgroup of individuals negative for the CCR5Δ32 deletion ($P = 0.011$; Fig. 2b).

We conclude that presence of the TLR7 Gln11Leu genetic variation is associated with a detrimental effect on HIV-1 disease progression as defined by viral set points and the time in reaching a stage of immunologically advanced infection. This prompted us to study the functional impact of the Gln11Leu polymorphism by use of ex-vivo cell stimulation assays.

Effect of the Gln11Leu polymorphism on toll-like receptor 7 stimulation ex vivo

This SNP is common in White populations providing a chance to examine its functional relevance ex vivo. We obtained PBMCs from healthy volunteers that had been genotyped for the TLR7 SNPs, stimulated the cells employing the TLR7-specific ligand Imiquimod and measured IFNα as well as IL-6 secretion in the supernatants. Donors hemizygous for the rare ‘Leu’-allele were compared with matched donors hemizygous for the common ‘Gln’ variant.

Fig. 1. Presence of the toll-like receptor 7 Gln11Leu polymorphism is related to higher set point viral loads in HIV-1 infected patients. Median levels of plasma HIV-1 RNA between 100 days and 2 years postseroconversion were computed for each of 302 therapy-naïve HIV-infected males. (a) The correlation between TLR7 Gln11Leu genotype and set point viral load is statistically significant ($P = 0.017$) and (b) distinct in 259 individuals with normal CCR5Δ32 genostatus ($P = 0.006$). Data shown represent the mean and SEM, transformed to a linear scale for ‘Gln’ and ‘Leu’ hemizygous individuals.
Following Imiquimod treatment at 5 μmol/l, a time-dependent IFN-α response was observed which was lower in ‘Leu’ hemizygotes than in ‘Gln’ hemizygotes (P = 0.05). Regarding IL-6 response, the difference was weak and did not reach statistical significance (Fig. 3a). Next, we assessed dose-response relationships following 8 and 24h of Imiquimod treatment. Again, presence of the ‘Leu’ allele was associated with decreased IFNα levels, whereas IL-6 levels were similar in donors of both genotypes (Fig. 3b,c). After normalization according to LPS-induced IL-6 levels, a TLR7-independent measure of cell activation, significance levels did not change (data not shown). These findings imply that TLR7 activation leads to a weakened IFNα response in TLR7 Gln11Leu SNP carriers as compared with wild type controls.

Of interest, the shapes of IFNα and IL-6 dose-response curves differed. Depending on TLR7 genotype and incubation time, maximum IFNα concentrations were induced by Imiquimod-doses between 5 and 10 μmol/l, whereas higher doses led to diminished IFNα levels; a cytopathic effect of the higher Imiquimod doses seems unlikely because for IL-6, at doses up to 100 μmol/l, a steadily increasing response was observed.

Discussion

TLRs are of crucial importance for the detection of viruses and the subsequent elicitation of an antiviral response [6]. Clearance of a variety of viral infections, for example, vesicular stomatitis virus, cytomegalovirus and hepatitis B virus is critically dependent on TLR activation [64–66].

TLR7/8 stimulation has been demonstrated to confer anti-HIV activity in vivo [13,14] and ex vivo [15,16]. On the other hand, TLR7/8 triggering has recently been implicated with persistent immune activation, a hallmark of progressive HIV-1 infection [32–34]. It therefore has been suggested by others that modulation of TLR7/8 signaling may represent a novel concept for future antiviral therapy [32–34]. We have recently identified a functional TLR8 variant associated with a restriction of HIV-1 disease [50] and in the present study present evidence for the association of a functional TLR7 variant with HIV-1 disease progression.

We detected a robust relationship between the TLR7 ‘Leu’ genetic variant and higher set point viral loads. Progression to an advanced stage of immune suppression was accelerated in TLR7 ‘Leu’ carriers. We observed that presence of the TLR7Gln11Leu SNP was associated with lower baseline CD4+ T-cell counts and possibly increased HIV-1 susceptibility in women; these results are suggestive of a strong effect on the initial stages of HIV infection. Assuming that the deleterious effect of the TLR7 Gln11Leu genetic alteration depends on a diminished IFNα induction, these findings would be in line with the fact that IFNα production is of importance for the control of HIV replication during early, rather than late disease stages [24–26].

We observed an overrepresentation of the rare TLR7-Gln11Leu genetic variant in HIV-1 infected females as compared with HIV-negative controls implying an increased risk for HIV-1 acquisition. This association was found in female but not male study participants; possible explanations may involve the significant gender-specific variation of IFNα levels induced by TLR7 ligands [67]. The number of women included in our study is less and the observed effect may well be due to a stochastic event only. Thus, confirmatory studies involving higher numbers of female participants are needed;
however, in a cohort of female patients with chronic HCV infection, a significantly increased frequency of this SNP was also reported [68]. Taken together with these findings, our results suggest that, in vivo, presence of the TLR7 ‘Leu’ variant is associated with a gender-dependent increase of susceptibility not only to one but to various RNA viral diseases.

In our stimulation assays, the presence of the TLR7Gln11Leu variant was associated with decreased IFNα production. The induction of IL-6 was only marginally impaired. Although these data do not allow for direct conclusions on how this SNP exacerbates the course of HIV-1 disease, they suggest a selective impairment of the IRF7-dependent signaling pathway in TLR7Gln11Leu variant carriers. As a consequence, these individuals might suffer from a restriction of the antiretroviral IFNα response, leading to higher viral loads and accelerated disease progression. Moreover, the NFκB-dependent pathway culminating in the secretion of proinflammatory cytokines appears to remain unaltered. Thus, the mutant TLR7 protein would still be able to recognize HIV-1 RNA and contribute to chronic activation of the immune system, which is of major importance in AIDS pathogenesis [27,33]. pDCs are of paramount importance in linking innate and adaptive immunity to HIV and many other viruses. They are also the PBMC sub-population responsible for the bulk of IFNα production following

Fig. 3. Peripheral blood mononuclear cells from individuals of distinct TLR7 Gln11Leu genotypes vary in their responsiveness to TLR7 stimulation. Peripheral blood mononuclear cells cultured at 3 x 10⁷ cells in 0.1 ml were treated with Imiquimod, a TLR7-specific stimulus at 37°C. IFNα and IL-6 levels in the supernatants were determined by enzyme-linked immunosorbent assay. (a) Kinetics for Imiquimod stimulation at 5 μmol/l. (b) Imiquimod dose-response curves after 8 h stimulation. (c) Imiquimod dose-response curves after 24 h stimulation. Three independent experiments involving three pairs of healthy matched volunteers were performed in duplicate, under blinded conditions. Values are expressed as ng/ml and represent the mean ± SEM. n.s., not significant; *P = 0.05 for the difference between mutant ‘Leu’ and wild type ‘Gln’ hemizygotes as tested by the Mann–Whitney test.
TLR7 stimulation [37]. Further studies are needed and currently underway in our laboratory to examine the effect of the Gln11Leu SNP on the IFNα and inflammatory cytokine response in pDCs.

At this point, we can only speculate about the molecular mechanism underlying the effect of the TLR7 Gln11Leu SNP on cytokine and interferon responses; possible explanations for its relevance include linkage disequilibrium with another genetic variation accounting for functional implications. However, this SNP is located in the signal sequence of TLR7, adjacent to the typical basic residues in the N-terminal part of this sequence. Signal peptide degeneracy, even when subtle, is known to modulate posttranslational modification, localization, quantity and thus the functionality of the affected protein [69]. The IRF-7 dependent pathway is characterized by a higher signaling threshold than the NF-κB dependent pathway [39]. Thus, lower quantities or impaired functionality of the variant TLR7 protein would indeed disturb IFNα rather than interleukin production.

The substantial impact of the host's genetic variation on the individual course of HIV-1 infection has been established during the last years and is subject of ongoing major studies [43,47,70,71]. The important role of TLRs, in particular TLR7, in HIV pathogenesis is currently being elucidated [11,12,15,16,34]. The data presented here demonstrates for the first time that TLR7 genetic variability influences HIV-1 progression. The whole genome association studies [71] that are currently underway will be needed to assess the relative contribution of genetic variation of this and other TLRs to the total genetic impact.

In summary, our results demonstrate the effect of a TLR7 genetic polymorphism on HIV disease progression in a German seroconverter cohort. Furthermore, presence of this polymorphism might lead to a gender-specific increase of the risk to acquire HIV-1. In TLR7-specific stimulation assays, we show *ex vivo* that presence of this mutation is associated with decreased production of IFNα but normal production of IL-6. This combination of limited antiretroviral IFNα activity with an unaltered degree of immune activation is a potential underlying mechanism for the exacerbated disease course in SNP carriers. The data presented here may help define an individual patient’s HIV-1 risk profile. They are of interest with regard to the potential of TLR7 agonists and antagonists as immunomodulatory antiretroviral drugs.

**Author contributions**

D.-Y.O. performed TLR7 genotyping, data analysis, recruitment of PBMC donors, obtained the ethical vote for PBMC stimulation experiments, and wrote the manuscript; K.B. performed TLR7 genotyping, data analysis, and PBMC stimulation assays; O.H. designed, supervised and conducted the German HIV-1 Seroconverter Study; J.K.E. performed PBMC stimulation assays; C.K., B.B. and G.P. provided DNA samples of the German HIV-1 Seroconverter Study patients, collected patient's data, and obtained the ethical vote for this collective; K.N. performed statistical analysis; H.J. and N.O. recruited patients of the Berlin Trial on HIV and TLR SNPs, provided EDTA blood samples and clinical data of these patients, and obtained the ethical vote of the Berlin Medical Association for this collective; A.P. recruited healthy controls; R.R.S. designed and supervised the project, and wrote the manuscript.

**Participating collaborators**

Aachen: Dres. Habets and Knechten; Augsburg: Dr Hammond (Klinikum Augsburg); Berlin: Dres. Mayr, Schmidt, Speidel, and Strohbach (Medizinisches Versorgungszentrum, Ärztforum Seestraße), PD Dr Arasteh (Auguste-Viktoria-Krankenhaus/Vivantes), Dres. Bieniek and Cordes, Dr Claus, Dres. Baumgarten, Carganico, and Dupke, Dres. Freiwald and Rausch, Dres. Gölz, Klausen, Moll, and Schleehauff, Dr Hintsche, Dres. Jessen and Jessen, Dres. Köppe and Krauthausen, Dr Reuter; Bielefeld: Dr Pfaff (Krankenhaus MARA II); Bochum:
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