A functional toll-like receptor 8 variant is associated with HIV disease restriction.


K30 Journal Club
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Two stages of the immune response

**Innate Response**
- Rapid Response
- Pattern recognition receptors - germ-line encoded
  - TLR, mannose and scavenger
- ↑ Cytokines, costimulatory molecules - instructive role for adaptive response
- Direct Response for host defense
  - Phagocytosis
  - Antimicrobial activity

**Adaptive Response**
- Slow response
- Recognition - initially low affinity receptors
- Gene rearrangement
- Clonal expansion
- Response T- and B-cells with high affinity, very specific receptors and antibodies
- Memory

ADJUVANT
Drosophila Toll receptor mediates primitive immune system

Figure 5. Germinating Hyphes of *A. fumigatus* on a Dead Drosophila. Scanning electron micrograph of a Drosophila adult that succumbed to infection by *A. fumigatus* and is covered with germinating hyphae (200× magnification).

*Lemaitre et al.* 1996
Toll-like receptors

Modlin and Cheng, 2004
Figure 1a&b. TLR8 responsiveness
TLR8 SNPs

- 3 non-synonymous TLR8 SNPs.
  - Arg715Gln (Isoform A and B, exclusive to African background)
  - Met10Val (Isoform A and B)
  - A1G (Isoform B, altered start ATG site)
Study Group

- The study group consisted of 1332 individuals:
  - 782 HIV-1–positive adults (male, 711 [91%]; female, 71 [9%])
    - white, 712 [91%]
    - African, 26 [3%]
    - other, 44 [6%]
  - 550 seronegative control subjects (male, 494 [90%]; female, 56 [10%])
    - white, 515 [94%]
    - African, 35 [6%]
- Seropositive individuals were HIV-infected patients enrolled in either:
  - the German HIV-1 Seroconverter Study (n=684)
  - Berlin Trial on HIV and TLR SNPs (n=98).
TLR8 SNPs in controls vs seropositive

- **Arg715Gln**
  - not found in study group
- **Met10Val** (found in 17% African controls)
  - No significant differences detected between controls and patients (possibly due to small sample size)
- **A1G**
  - Most abundant mutant allele (nearly 25% in both case and control populations)

• Take home message – no correlation between any SNP genotype and HIV infection (in white persons)
## TLR8 SNPs in HIV disease progression

<table>
<thead>
<tr>
<th>Sex, ethnicity, TLR8 A1G genotype</th>
<th>No. (% of subgroup)</th>
<th>Mean (SE)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/A or A/−</td>
<td>180 (76.3)</td>
<td>−7.26 (0.84)</td>
<td>.008</td>
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<tr>
<td>A/G</td>
<td>4 (1.7)</td>
<td>−4.70 (1.32)</td>
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<tr>
<td>G/G or G/−</td>
<td>52 (22.0)</td>
<td>−1.78 (1.55)</td>
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<tr>
<td>White</td>
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<td></td>
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<tr>
<td>A/A or A/−</td>
<td>172 (77.5)</td>
<td>−7.50 (0.87)</td>
<td>.005</td>
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<tr>
<td>A/G</td>
<td>3 (1.4)</td>
<td>−5.49 (1.49)</td>
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</tr>
<tr>
<td>G/G or G/−</td>
<td>47 (21.2)</td>
<td>−1.73 (1.61)</td>
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<tr>
<td>Male</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Combined</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A/−</td>
<td>170 (76.9)</td>
<td>−7.28 (0.88)</td>
<td>.004</td>
</tr>
<tr>
<td>G/−</td>
<td>51 (23.1)</td>
<td>−2.21 (1.52)</td>
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<tr>
<td>White</td>
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<td></td>
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<tr>
<td>A/−</td>
<td>164 (77.7)</td>
<td>−7.42 (0.91)</td>
<td>.002</td>
</tr>
<tr>
<td>G/−</td>
<td>47 (22.3)</td>
<td>−1.73 (1.61)</td>
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</tbody>
</table>
Figure 2. SNP and disease progression (<200 CD4 cells/µl)

<table>
<thead>
<tr>
<th></th>
<th>TLR8A1G mutant</th>
<th>TLR8A1G wild type</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time since Seroconversion [years]</td>
<td>124 39 15 8 3 1</td>
<td>363 114 38 9 1 1</td>
<td>487 153 53 17 4 2</td>
</tr>
</tbody>
</table>
Figure 1c. TLR8 SNP function

![Graph showing relative light units vs. Resiquimod concentration for different TLR8 isoforms.](image)
Figure 3a. A1G mutant function

<table>
<thead>
<tr>
<th>Group</th>
<th>Method</th>
<th>Concentration</th>
<th>P-value</th>
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<tbody>
<tr>
<td>TLR7/8</td>
<td>Resiquimod</td>
<td>1 μM</td>
<td>0.012</td>
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<tr>
<td>TLR8</td>
<td>RNA-40</td>
<td>10 μg/ml</td>
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<tr>
<td>Neg</td>
<td>RNA-41</td>
<td>10 μg/ml</td>
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</tr>
<tr>
<td>TLR4</td>
<td>LPS</td>
<td>50 ng/ml</td>
<td></td>
</tr>
</tbody>
</table>

**A1G Mutant Function**

- **TNF-α (ng/ml)**
  - WT: [scatter plot]
  - Mut: [scatter plot]
  - P-value: 0.012

- **IL-6 (ng/ml)**
  - WT: [scatter plot]
  - Mut: [scatter plot]
Figure 3b-d. A1G function timecourse and dose response

B

[Graph showing timecourse of TNF-α and IL-6 levels with different concentrations of Resiquimod over 24 hours.]

C

[Graph showing timecourse of TNF-α and IL-6 levels with different concentrations of Resiquimod over 16 hours.]

D

[Graph showing timecourse of IL-10 levels with different concentrations of Resiquimod over 24 hours, with statistical significance indicated by p = .028.]
Figure 4. PBMC TLR7 response
Figure 5. PBMCs WT and A1G pure TLR8 response

A

![Graph showing TNF-α levels for TLR8A1G wild type and mutant at different CL075 concentrations. The y-axis represents TNF-α levels in pg/ml, and the x-axis represents CL075 concentration in µM. The graph shows a significant increase in TNF-α levels for the TLR8A1G mutant compared to the wild type at 0.50 µM CL075.]

B

![Graph showing IL-10 levels for TLR8A1G wild type and mutant at different CL075 concentrations. The y-axis represents IL-10 levels in pg/ml, and the x-axis represents CL075 concentration in µM. The graph shows a notable increase in IL-10 levels for the TLR8A1G mutant compared to the wild type at 0.50 µM CL075.]

Authors have previously shown that TLR8 activation of neutrophils primes the cells to respond to both arachidonic acid or f-MLP stimulation.
Conclusion

• TLR8 SNP A1G is associated with delayed disease progression in HIV, but not susceptibility to infection.
Strengths

• Correlation of a functional TLR polymorphism to disease or disease outcome.

• Clear data supporting the protective effects of A1G mutants.

• This study should instigate both clinical and basic research into both TLR polymorphisms and the mechanism of A1G protection in HIV.
Weaknesses

• Lack of a clear and definitive hypothesis for the cellular or molecular mechanism.
  – Authors conclusion and data contradict, A1G has lower NF-κB activation but higher TNF-α.
  – Cell lines, monocytes, PBMCs, PMNs.

• Study group restricts the ability to examine the other SNPs.
Other thoughts

• Protection from one, susceptible to another.