Predictive tools in the risk assessment of new proteins in GMOs: the case of Celiac Disease

Frits Koning
LUMC, Leiden, The Netherlands
Celiac Disease Consortium
Gluten proteins in wheat

HLA-DQ2/8

T-cells
Gluten specific T cell response in the small intestine

Gluten peptide

HLA-DQ2(8)

Tissue damage
It takes three to tango!!!!

Tissue damage: release of intracellular tTG

tTG

deamidation

Q → E

tissue repair

gluten peptides generated by pepsin

T Cells

HLA-DQ2(8)

- Enhanced binding
- Amplification of the immune response

APC
The specificity of tTG is determined by proline, the 2\textsuperscript{nd} most abundant aa in gluten.

Characteristic gluten sequences:

- QP: no modification
- QXP: yes
- QXXP: no
- QXPY or QXPF: yes

LGQQQPFPQPQQPYPQPQPFPSQLPYLQLQPFQPQPL
LGQEQPFPPEQPYPQPQPFPSELPYLQLQPFQPQPL
Predict toxic gluten sequences?

<table>
<thead>
<tr>
<th>Gluten</th>
<th>Hordein</th>
<th>Secalin</th>
<th>Avenin</th>
<th>Tcells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>Barley</td>
<td>Rye</td>
<td>Oats</td>
<td></td>
</tr>
</tbody>
</table>

Search Algorithm

| 46 | 60 | 33 | 2 |

Specificity of tissue transglutaminase explains cereal toxicity in celiac disease.

Identification of T cell stimulatory peptides in cereals

*Gliadin* (wheat):
QLQPFQPQQLPYQPQ
PFPQPQLPY
PQPQLPYPQ

*Secalin* (rye):
PQQPFQPQQPFQPSQ
PFPQPQQF
PQPQQF
PQPQFPQ
DQ2-glia-α2 recognition

TRAV26-01
TRBV7-02^+

T cell receptor
Peptide
DQ2

Conserved β-chain footprint

Petersen et al, NMSB 2014
DQ2-glia-a2 recognition: PQPQLYPQP

Broughton Immunity 2012; Petersen et al, NMSB 2014; Petersen JI 2015
Bona fide toxicity of gluten for patients with celiac disease

• Well defined
• Mechanism underlying toxicity clear
RA of (novel) proteins: celiac disease

Fig 2. Search for sequence identity

- 100% match with T-cell stimulatory epitope
  - Hazard identified

- Concerns raised from a partial match* with T-cell stimulatory epitope
  - Further investigations are necessary

- No concerns raised from a partial match* with T-cell stimulatory epitope
  - No hazard identified

* A partial match with a known T cell-stimulatory peptide raises concern because of the position and nature of the identical amino acids.
### Celiac disease — DQ2 T-cell epitopes

**DQ2 restricted epitopes**

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Motif</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQ2.5-glia-α1a</td>
<td>PFPQ PQLY</td>
<td>Arentz-Hansen et al. (2000)</td>
</tr>
<tr>
<td>DQ2.5-glia-α1b</td>
<td>PYPQPQLY</td>
<td>Arentz-Hansen et al. (2002)</td>
</tr>
<tr>
<td>DQ2.5-glia-α2</td>
<td>PQPQPQLY</td>
<td>Arentz-Hansen et al. (2000)</td>
</tr>
<tr>
<td>DQ2.5-glia-α3</td>
<td></td>
<td>Vader et al. (2002b)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ1</td>
<td></td>
<td>Sjöström et al. (1998)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ2</td>
<td></td>
<td>Qiao et al. (2005), Vader et al. (2002b)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ3</td>
<td></td>
<td>Arentz-Hansen et al. (2002)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ4a</td>
<td></td>
<td>Arentz-Hansen et al. (2002)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ4b</td>
<td></td>
<td>Qiao et al. (2005)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ4c</td>
<td></td>
<td>Arentz-Hansen et al. (2002)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ4d</td>
<td></td>
<td>Qiao (unpublished)</td>
</tr>
<tr>
<td>DQ2.5-glia-γ5</td>
<td></td>
<td>Arentz-Hansen et al. (2002)</td>
</tr>
<tr>
<td>DQ2.5-glia-ω1</td>
<td></td>
<td>Tye-Din et al. (2010)</td>
</tr>
<tr>
<td>DQ2.5-glia-ω2</td>
<td></td>
<td>Tye-Din et al. (2010)</td>
</tr>
<tr>
<td>DQ2.2-glut-L1</td>
<td></td>
<td>Vader et al. (2002b)</td>
</tr>
<tr>
<td>DQ2.2-glut-L2</td>
<td></td>
<td>Stepniak et al. (2005), Vader et al. (2002b)</td>
</tr>
<tr>
<td>DQ2.5-hor-1</td>
<td></td>
<td>Tye-Din et al. (2010), Vader et al. (2003)</td>
</tr>
<tr>
<td>DQ2.5-hor-2</td>
<td></td>
<td>Vader et al. (2003)</td>
</tr>
<tr>
<td>DQ2.5-sec-1</td>
<td></td>
<td>Tye-Din et al. (2010), Vader et al. (2003)</td>
</tr>
<tr>
<td>DQ2.5-sec-2</td>
<td>PQQPQFPQ</td>
<td>Vader et al. (2003)</td>
</tr>
<tr>
<td>DQ2.5-ave-1</td>
<td>PYPEQQEPF</td>
<td>Arentz-Hansen et al. (2004), Vader et al. (2003)</td>
</tr>
<tr>
<td>DQ2.5-ave-1b</td>
<td>PYPEQQFPF</td>
<td>Arentz-Hansen et al. (2004), Vader et al. (2003)</td>
</tr>
</tbody>
</table>

**Q/E-X1-P-X2**

*Sollid et al., 2012. Immunogenetics, 64, 455-460*
Q-X-P-X

- PFPQPQLPYP
- PQPQLPYPQ

- PXP in addition to QXPX is associated with the most immunogenic epitopes
- In contrast: PP is never found in T cell epitopes
- Positively charged amino acids in general diminish likelihood of DQ-binding and T cell recognition. Positive charge at p1, p4, p6, p7 and p9 bad for DQ-binding.
Celiac disease — DQ8 T-cell epitopes

Sollid et al., 2012. Immunogenetics, 64, 455-460

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Motif</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQ8-glia-α1</td>
<td>QGSFQPSQQ</td>
<td>van de Wal et al. (1998b)</td>
</tr>
<tr>
<td>DQ8-glia-γ1a</td>
<td>QQPQQPFQP</td>
<td>Tolleyfson et al. (2006)</td>
</tr>
<tr>
<td>DQ8-glia-γ1b</td>
<td>QQPQQPYQP</td>
<td>Tolleyfson et al. (2006)</td>
</tr>
<tr>
<td>DQ8-glut-H1</td>
<td>QGYYPTSPQ</td>
<td>van de Wal et al. (1999)</td>
</tr>
</tbody>
</table>

Partial matches without the Q/E-X1-P-X2 to be investigated.
Partial matches: Q/E-X1-P-X2 motif is present

\[\text{PFQPQLPY and } \text{ALPLQLPA}\]

4 identical, two invisible, one conservative: \text{POTENTIAL HAZARD}

\[\text{PQPQLYPQ and } \text{PLTQLPASR}\]

4 identical, one conservative BUT \(Y > A, P > S\) and \(Q > R\) prohibit recognition: \text{NO HAZARD}
Partial matches: Q/E-X1-P-X2 motif is NOT present

QGSFQPSQQ and
EGSIQAGQQ

5 identical, one conservative, one enhances binding:
POTENTIAL HAZARD

QGSFQPSQQ and
QGLFSPSAQ

6 identical BUT
Critical T cell receptor contact residues differ:
NO HAZARD
Peptide binding and Modelling
Potential antigenicity can be predicted