Plant and food technological approaches to reduce the incidence of coeliac disease

Luud Gilissen

(no Conflicts of Interest)
Cereal-related disorders

- Allergy; Intolerance; Sensitivity

Plant and food technological approaches

- Plant related strategies
  - Selection; Chromosome deletions; New synthetic hexaploids; RNAi (GM); Mutation breeding; Genome editing (GM?)

- Food technological approaches
  - Reduction of vital gluten; Elimination of gliadin from gluten; Sourdough; The gluten contamination elimination diet (GCED)

- Alternative grains
  - Minor wheat species; Oat

Conclusions
Major cereal allergies

- Wheat allergy (world-wide; 0.25%)
- Maize allergy (S-EU, Mexico, USA; <<)
- Rice allergy (Asia; <<)

Cereal allergy is rare

Wheat sensitization is high (2%)

→ Wheat contains ~30 IgE-responding antigens from different protein families
→ No clinical symptoms
Wheat allergy

More U.S. Children Have Food Allergies
Prevalence of food allergies has been rapidly increasing

Prevalence 7/4470 (0.25%)

 Symptoms in Children:
• Eczema
• Vomiting

 Symptoms in Adults:
• Anaphylaxis (rare)
• Bakers asthma
Non-celiac wheat sensitivity (ncWS)

- A new health threat?
- No diagnostic tools (biomarkers) available (elimination diet is the only diagnostic tool)
- Increasing demand during the last decade for gluten-free diet may reflect the impact
- Correlation with Irritable Bowel Syndrome (IBS) → prevalence ncWS of 5-10% (Carroccio et al., 2012; Brouns et al., 2013)

—200 million restaurant visits include a gluten-free order

Chicago, March 6, 2013 — As of this January about a third of U.S. adults say they want to cut down or be free of gluten in their diets, the highest percentage making this claim since The NPD Group, a leading global information company, began asking the question in 2005. NPD’s Dieting Monitor, which continually tracks on a bi-weekly basis top-of-mind dieting and nutrition-related issues facing consumers, reports that 30 percent of adults, one in every three adults, claimed to cut down on or avoid gluten completely in January 2013.

Genetic/immune Relationship Unknown

lost the wheat, lose the weight, and find your path back to health

WHEAT BELLY
WILLIAM DAVIS, M.D.

De Voedselzandloper

De Voedselzandloper, deel 2: Wheat, Dairy and Gluten Intolerance, David Perlmutter, M.D.

Grain Brain
David Perlmutter, M.D.

Google Trends: Interest Over Time
Which compounds involved in ncWS?

- Gluten? ATIs? FODMaPs? Combi? None?

→ Functional bowel complaints
  - Rapidly rising (~5-10% in USA and UK)
  - Genetic predisposition still unknown
  - No biomarkers known

- In IBS, 30% improves on ‘gluten-free’ and ‘FODMaP’-low diet (including wheat free)

- Health Grain Forum: intervention study under construction regarding cereal and gluten avoidance
  - ‘Analysis of food processing effects on wheat species compounds and their impact on bowel symptoms and wellness complaints’
Coeliac disease (CD)

- Chronic inflammation of the small intestine
  - Increased 4x during the last 50 years (current prevalence: 0.5-2%)
  - Genetic predisposition (HLA-DQ2/8)
  - Gluten (seed storage proteins) from wheat, rye and barley

Major symptoms of CD in children
- Chronic bowel ache and diarrhoea
- Growth retardation

Major symptoms of CD in adults
- Chronic fatigue, headache, bowel complaints
- Reduced fertility; miscarriage
- Dermatitis herpetiformis
- Osteoporosis
- Deafness
- Neuropathy
- Intestinal cancer (lymphoma)
The gluten/wheat challenge: wheat is everywhere
Wheat is a major food crop
Whole grain wheat is healthy (fibre)
Increase of ‘vital’ gluten application as major food industrial protein

Wheat components are applied in >30% of super market food items
Strategies for prevention of CD and ncWS should aim at:

- **Diagnosed individuals**
  
  (only 10-20% of estimated CD patient population)
  
  - Gluten-free, Wheat-free, FODMAP-low

- **Undiagnosed and potential patients**
  
  - What to do for this group?

→ **Plant and Food technological approaches**
Plant related strategies

- Selection of low-CD-immunogenic wheat lines
- Deletion of specific chromosome parts
- New synthetic hexaploids
- RNAi (GM)
- Mutation breeding (non GM) and Genome editing (GM?)
Genetics and evolution of wheat

- **Triticum urartu** (AA)
- **Triticum speltoides** (BB)
- **Triticum tauschii** (DD)

**Wild species** (diploid)

- **Triticum turgidum** (AABB)
  - ~500,000 years ago

**Durum wheat** (tetraploid)

- **Triticum aestivum** (AABBDD)
  - ~9,000-12,000 years ago

**Bread wheat** (hexaploid)
Gluten proteins in wheat

Variety 1 2 3 4 5 6

SDS-PAGE (CBB) kDa

200.0
116.3
97.4
66.2
45.0
31.0
21.5

~70% starch
8-15% protein, mainly gluten

HMW-GS: high molecular weight glutenin subunit; LMW-GS: low molecular weight glutenin subunit
Epitopes mainly in gliadins

High in proline (P) and glutamine (Q)

\[ Q \rightarrow E \] (deamidation)
α-gliadin genes can be distinguished according to genome

Van Herpen et al. 2006 BMC Genomics 7: 1

gDNA
Selection of low-CD-immunogenic wheat

Selection from hundreds of varieties: mAbs

Line B is promising
• Further quantification of CD-toxicity
• Exploration of agronomic and baking qualities
• Testing in intervention study

Diversity in T-cell-response of wheat accessions:

Glia

LMW
Deletion lines – characterisation (Van den Broeck et al. 2009)

\[ \alpha-9 \]

\[ \alpha-20 \]

\[ \gamma-1 \]
Deletion lines – crossing  (Van den Broeck et al 2011)
CD-immunogenic gluten/peptide quantification

- Improved quantification of CD toxicity of wheat and foods
  - Using genomics, transcriptomics, proteomics (no mAb test kits; no T cells)
  - Proper quantitative product labeling

Salentijn et al 2013
Van den Broeck et al 2015
RNA-interference (RNAi): construct (GM)

Fig. 51. Constructs for RNAi-targeting gliadin genes. The vectors contain a γ-gliadin (pGhp-ω/α) or D-hordein promoter (pDhp-ω/α and pghp8.1), the nos terminator sequence (NOS), and the triggered sequences in sense and antisense orientations separated by the Ubiquitin 1 intron (Ubi1 intron). Vectors pGhp-ω/α and pDhp-ω/α include an inverted repeat (IR) chimeric 361-bp fragment from ω- and α-gliadins. Vector pghp8.1 includes a 169-bp IR fragment from γ-gliadin. Numbers indicate length in base pairs.
RNAi: effects

Gil-Humanes et al 2014
RNAi: baking quality

Figure 1. Reduced-gliadin bread: physical properties. (A) Loaves and slices of wild-type BW208, reduced-gliadin line D793, and rice. (B) Physical properties of bread loaves obtained from wild-type lines, reduced-gliadin lines, and rice. Lines with the same letter indicate that no significant differences exist among them as determined by the Tukey HSD post hoc all-pairwise comparison test (P<0.05).

doi:10.1371/journal.pone.0090898.g001

Gil-Humanes et al 2014
New synthetic hexaploids

- Bread wheat developed from a single AABB + DD hybridization (12,000 y ago)
- D-genome introduced many CD-epitopes
- Looking for low-CD-immunogenic
  - AABB varieties, e.g. line B
  - and D-genome diversity
    (Wang et al 2013)
- Create new low-immunogenic synthetic hybrid (non GM)
  - Cooperation with NIAB - UK
Mutation breeding: gliadin mutagenesis

- New collaborative project of PRI with NIAB (UK) (Jouanin, PhD)

- Objectives
  - Gliadin epitope point mutations → Prevention of HLA-DQ receptor binding
  - DNA fragment loss in gliadin gene family → Decrease gene copy number

- Approaches
  - Chemical mutagenesis (EMS): Gliadin sequences available for epitope testing (with UC Davis, CA) [Non GM]
  - Gamma-ray mutagenesis: Population of lines available for gliadin testing (with John Innes, UK) [Non GM]
  - Targeted mutagenesis (CRISPR/Cas9 method): Gene construct → Transformation of embryonic cells → Specific targeting of gliadins → Mutation → Deletion of construct [GM status pending]
Food related strategies

- Reduction of vital gluten
- Elimination of gliadin from gluten
- Sourdough
- The gluten contamination elimination diet (GCED)
Reduction of vital gluten

- Vital gluten: by-product in starch industry

- Large-scale application as bread improver: ...gives whole grain loaves a ‘boost’ ...

- Other factors, such as per capita vital gluten intake, variations in individual diets with regard to the amount and types of wheat consumed, wheat genetics, and agronomic practices (such as nitrogen fertilization), that affect protein content might contribute to determining the “toxicity” of wheat for people with the appropriate genetic susceptibility for celiac disease ... (Kasarda 2013)
Elimination of gliadin from gluten

- Labscale (Van den Broeck et al, pers. comm):
  - Will industrial separation be possible?
  - Will technological quality be maintained?
Sourdough bread

- Sourdough bread seems safe to CD patients?
  - Breakdown of resistant peptides (e.g. 33-mer) (Greco et al., 2011)
  - More research is needed to confirm this claim

- Low prevalence of CD in Germany: due to high consumption of sourdough bread?
  - 0.3% in Germany; 2.4% in Finland (Mustalahti et al., 2010)
Adjustment of gluten epitope profile to patient sensitivity

Vader et al., Gastroenterol 2002

Camarca et al., J Immunol 2009

<table>
<thead>
<tr>
<th>Epitopes</th>
<th>Novel DQ2 gluten epitopes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glia-α20</td>
</tr>
<tr>
<td>Patients</td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>tTG</td>
</tr>
<tr>
<td>JB</td>
<td></td>
</tr>
<tr>
<td>JP</td>
<td></td>
</tr>
<tr>
<td>MB</td>
<td></td>
</tr>
<tr>
<td>SV</td>
<td></td>
</tr>
<tr>
<td>NP</td>
<td></td>
</tr>
<tr>
<td>NV</td>
<td></td>
</tr>
<tr>
<td>MS</td>
<td></td>
</tr>
<tr>
<td>RR</td>
<td></td>
</tr>
<tr>
<td>KL</td>
<td></td>
</tr>
<tr>
<td>DB</td>
<td></td>
</tr>
<tr>
<td>SB</td>
<td></td>
</tr>
<tr>
<td>NB</td>
<td></td>
</tr>
<tr>
<td>BD</td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td></td>
</tr>
<tr>
<td>MaB</td>
<td></td>
</tr>
<tr>
<td>Adult1</td>
<td></td>
</tr>
<tr>
<td>Adult2</td>
<td></td>
</tr>
<tr>
<td>Adult3</td>
<td></td>
</tr>
<tr>
<td>Adult4</td>
<td></td>
</tr>
</tbody>
</table>

[Diagram of genetic data and analysis]
GCED and Grandma’s kitchen
pure unprocessed food in addition to the gluten-free diet

<table>
<thead>
<tr>
<th>Allowed</th>
<th>Not allowed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grains</td>
<td>Millet, sorghum, buckwheat or other inherently gluten-free grains, seeds, or flours</td>
</tr>
<tr>
<td>Fruits/Vegetables</td>
<td>Frozen, canned or dried</td>
</tr>
<tr>
<td>Proteins</td>
<td>Lunch meats</td>
</tr>
<tr>
<td></td>
<td>Ham, bacon</td>
</tr>
<tr>
<td></td>
<td>Other processed, self-basted or cured meat products</td>
</tr>
<tr>
<td>Eggs</td>
<td></td>
</tr>
<tr>
<td>Dried beans</td>
<td></td>
</tr>
<tr>
<td>Unseasoned nuts in the shell</td>
<td></td>
</tr>
<tr>
<td>Dairy</td>
<td>Seasoned or flavored dairy products</td>
</tr>
<tr>
<td></td>
<td>Processed cheeses</td>
</tr>
<tr>
<td></td>
<td>Flavored and malt vinegars</td>
</tr>
<tr>
<td>Condiments</td>
<td></td>
</tr>
<tr>
<td>Beverages</td>
<td></td>
</tr>
<tr>
<td>Oils, vinegar, honey, salt</td>
<td></td>
</tr>
<tr>
<td>100% fruit/vegetable</td>
<td></td>
</tr>
<tr>
<td>Gluten-free supplemental formulas</td>
<td></td>
</tr>
<tr>
<td>Gatorade, milk water</td>
<td></td>
</tr>
</tbody>
</table>

GCED is an effective therapeutic option for 80% of GFD-adherent non-responsive CD (diagnosed as ‘RCD’) patients (Hollon et al 2013)
Is wheat the only cause of CD and ncWS?

- Tolerance-breaking factors may be found in:
  - Overall feeding pattern
  - Smoking during pregnancy
  - Hygiene and drinking water quality
  - Urban versus rural lifestyle
  - Composition (quantity and quality) of the gut microflora

Unbalanced interaction of **human genotype, diet/environment** and **intestinal microbiota** may largely determine the individual’s intolerance/sensitivity

(G. Enders 2014)
Alternative grains: Traditional wheat species

- **T. monococcum** (Einkorn): only AA genome
  - Variety ‘Monlis’ was safe in food challenge (Zanini et al 2013)

- **T. turgidum** (Emmer): AABB genome
  - Some varieties no T cell proliferation (Vincentini et al 2009)

- **T. spelta** (Spelt wheat): AABBD, but low in FODMaPS
  - No/less complaints in IBD cases
Oats as Alternative

As healthy food

- Increasing fiber intake (gut microflora, immune system)
- Lowering cholesterol (HVD) (official FDA/EFSA health claims)
- Increasing satiety (obesity)
- Low-glycemic starch (obesity)
- Retarding stomach emptying (diabetes)
- Polyphenols/anti-oxidants (cancer)
- High quality proteins (meat replacer)
- High in unsaturated fatty acids
Oats and Coeliac Disease

- Contamination with wheat, rye, barley appeared to be the problem
- Safe cereal to vast majority of CD patients (Pulido et al., 2009)
- Epitopes of wheat, barley, rye are absent in oats (Londono et al., 2014)
- Allowed to be sold as Gluten-free (EC Regulation 41/2009)
- Rapidly increasing demand by CD population (consumption supported by Dutch CD patient society)
- Establishment of The Dutch Oat Chain (2006)

 Российской Академии Наук

T-cell stimulation (Koning et al., 2005)
Gluten-free oat products on the market

- Since 2011:
Dough-based oat bread (Londono et al., 2014)

Whey protein as gluten alternative (Londono et al., 2014)

Figure 1. Scheme of the formation process of whey protein particles (WPP) (after Riemndijk et al., 2011). In the first step, whey proteins form protein aggregates induced by heat (T). The second step consist of a self-aggregation process of the protein aggregates induced by a pH reduction in presence of a Locust bean gum solution. The final size of WPP is $20 \pm 4 \mu m$. 

Figure 1. Dough system (left) and baked loaf (right) made of pure oat flour.
And:

- Oat beer (gluten free)

Proost
Conclusions

- Prevention of food-based diseases through food-based and life-style solutions
- Tolerance-breaking factors
- Diagnosed vs not-diagnosed
- Challenges and Responsibilities with breeders, farmers, food industries, retail, research organisations, governments and consumers (agro-food chain)
- Multidisciplinary and interdisciplinary cooperation
- Several strategies are under development or are already active
- Quantification of CD immunogenic fragments in grain species and derived foods
- Oats as alternative
- Strict production chain separation and management

Considering these will result in reduced incidences of cereal (wheat) related disorders
Thanks

- Celiac Disease Consortium
  - Frits Koning
- PRI – Wageningen UR
  - Hetty van den Broeck
  - Jan Cordewener
  - Twan America
  - Ingrid van der Meer
  - Jan Schaart
  - Elma Salentijn
  - Diana Londono
  - Aurelie Jouanin
  - René Smulders
  - Ed Hendrix
- Partners in
  ‘De Nederlandse Haverketen’
- Brouwerij Witte Klavervier
  - Freek Ruis