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Non Celiac Gluten Sensitivity: Lack of Response to In Vitro Gliadin Challenge and Basophils Activation Assay

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In recent years it has become more frequent for physicians to come across types of gluten sensitivity other than celiac disease (CD). Regardless of the absence of any serological CD markers or intestinal damage, gluten sensitive (GS) patients often report intestinal and extra-intestinal symptoms shortly after the ingestion of gluten and the disappearance of such symptoms on a gluten free diet (GFD). Regrettably, at present, there is no evidence of any mucosal or serological modifications in GS patients, or that gluten is really the trigger factor in gluten sensitivity. We aimed at evaluating the impact of gliadin challenge on two experimental settings, i.e. in vitro gliadin challenge of duodenal mucosa and peripheral blood basophils in GS patients compared to CD patients and healthy controls. We consecutively enrolled patients referred to tertiary centers for food intolerance and celiac disease. Participants underwent a complete clinical screening in order to rule out CD (serum a-tTG, EMA, total IgA and HLA status, where appropriate), an interview for evaluation of diet and symptoms, a skin prick test to exclude wheat allergy, upper endoscopy for duodenal biopsies used for both histological assessment and for the in vitro evaluation of the gliadin-induced mucosal expression of early and late inflammatory markers: PY99, epithelial HLA-DR, ICAM-1, crypt HLA-DR, CD3, CD25 and CD69. In addition, a basophils activation assay, in which patients' basophils were challenged with a gliadin extract, was performed. Basophils were analyzed for the expression of hematopoietic membrane antigens CD203c, CD63 and CD45 by flow cytometry looking at differences in antigen expression. 119 subjects were screened for CD and GS and, according to the results, four groups were obtained: GS patients (no.16), CD on gluten free diet (no.34), CD on gluten containing diet (no.35) and healthy controls (no.34). As for the in vitro gliadin challenge, all CD patients, regardless of their dietetic status, showed a clear mucosal activation (increased immunofluorescence intensity both for early and delayed inflammation markers) when stimulated with gliadin, while only 3 controls and 3 GS patients showed an weak response for some, not all, inflammation markers. Also, there were no significant differences in the skin prick test results or in the level of CD63 and CD203c basophils expression, used as a marker of cell activation, among the groups. The present study shows that in GS patients, gliadin challenge test did not show any significant modifications in the expression of those mucosal inflammation markers found increased in CD patients. Therefore, this tool should not be used as a diagnostic instrument in case of GS. Moreover, the lack of differences in basophils activation and skin prick test seems to exclude this form of cellular sensitivity in our GS series also.

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Utilizing High Throughput Discovery Approach to Identify Optimal Candidate Deamidated Gliadin Peptides for the Identification of Celiac Disease.

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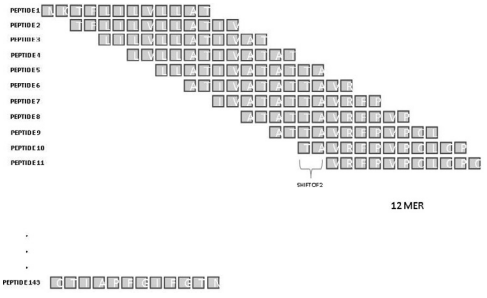
Celiac disease (CeD) is an immune response to gluten especially to deamidated gliadin. It is detected by antibodies to tissue transglutaminase (tTg) or deamidated gliadin(DG). The performance of DGP antibodies is quite variable and limits their use compared to tTg antibodies. The current DG tests have been based on epitopes recognized by t cells or by use of random libraries. The sequences developed commercially are trade secrets. Goals: Identify sequences of the deamidated gliadin family (alpha, beta, gamma, omega gliadin) that are most predictive of celiac disease using a high-density in situ synthesized peptide microarrays in combinatorial analysis. Methods: Sera from untreated CeD (n=50) were compared to controls (n=50), both on a gluten-containing diet. A 2-stage process was utilized. In the 1st stage high-density microarrays with all native and systemically deamidated gliadin 12 mer overlapping sequences. Each microarray consisting of over 55,000 unique peptides with duplicates were in-situ synthesized onto an area of 2.35mm2. The most informative 3-mer subsequences were paired and in-situ synthesized into the 2nd phase of arrays into 6mers in forward and reverse combinations. Using high-density analysis, specific 3-mer subsequences paired into novel 6-mers, which provided the highest percentage of positives, were identified. A matrix of subsequences and the highest percentage of sequences showing IgG and IgA antibody response among all positive samples were then filtered out using a novel algorithm. A matrix of diagnostic utility was constructed by combining the sequences with the highest occurrence. Novel sequences could then be synthesized with learning from the first library, and this set is continuously evolving as more samples are run. Results: With this, it is possible to demonstrate that combining subsequences, each with 3 amino acids, in a specific order shows a high accuracy for CeD. (Table) Interestingly, in reversing the subsequences substantially impairs the recognition. This suggests that antibody recognition of epitopes of DG in CeD is highly sequence specific and only a few sequences are recognized across the entire spectrum of patients with CeD. Similar sequences are recognized by IgA and IgG but thus far the IgA isotype is much more sensitive than IgG (90% vs 68%) Conclusion: A systematic approach to the determination of antibody recognition of deamidated gliadin peptides in celiac disease allows for greater precision of testing. It also identifies potentially immunodominant epitopes in a way independent of t cells epitopes. This mass scale analysis of targeted DG recognition in CeD, is expected to be further refined as more samples are run. This method is entirely scalable and allows for the precise interrogation of humoral immunity in subjects with celiac disease and other autoimmune diseases.

Matrix Combination of Gliadin Subsequences

	QPE	PEQ	EQP	QPF	FPE	PFP	FPQ	PQP	PQQ
QPE	11%	26%	22%	92%	22%	84%	37%	10%	3%
PEQ	12.%	42%	38%	19%	23%	90%	42%	10%	4%
EQP	15%	30%	48%	30%	25%	25%	42%	10%	4%
QPF	26%	40%	37%	19%	0	14%	4	16%	4%
FPE	12%	8%	8%	7%	1%	4%	3%	1%	0%
PFP	27	25	37%	19%	1%	14%	7%	3%	1%
FPQ	3%	18%	16%	15%	0%	12%	7%	3%	1%
PQP	5%	5%	23%	22%	0%	19%	10%	1%	0%
PQQ	0%	7%	7%	7%	0%	7%	4%	0%	0%

% accuracy of 6mer combination of 3 mer subsequences



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Screening for Celiac Disease in Women With Preterm Childbirth

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Celiac disease (CD) is a chronic, immuno-mediated entheropathy caused by ingestion of gluten in genetically predisposed individuals. It may present with a variety of symptoms (such as diarrhea, weight loss, iron deficient anemia, etc…) or completely silent. The association between CD and pregnancy outcome has been investigated, with contrasting results. Our aim was to screen for CD women with preterm childbirth or low birth weight. In our prospective study, we performed a CD screening in a group of women with preterm childbirth (defined as <37 weeks of gestational age) or with low birth weight (< 2500 gr), using RIA IgA anti-transglutaminase antibodies (tTGAb). Positive patients were referred to our Operative Unit on Celiac disease, where they were submitted to a further serological evaluation. Confirmed positive patients were submitted to upper gastrointestinal endoscopy with multiple duodenal biopsies, and if diagnosed to be celiac, started the gluten-free diet (GFD). A total of 75 women (age range: 19-49 years) participated in the study. Four (5.3%) women resulted tTGAb positive (age range: 20-31 years, gestational age range: 31weeks+6 days- 35weeks+ 6 days, birth weight range: 1650-2720 gr). The four women were all primiparae but one, who already had a preterm child. All the patients were confirmed at the second serum sample. One of these women performed upper endoscopy with duodenal biopsies, showing subtotal villous atrophy (3b sec. Marsh modified by Oberhuber) and started the GFD. The other three women are still under evaluation, waiting to perform the upper endoscopy. In conclusion, our study demonstrates that CD prevalence is higher in women with preterm childbirth or low birth weight rather than in the Italian population. Screening for CD could be advisable as part of the diagnostic flow-chart in these patients. In fact, a timely diagnosis and the prompt GFD, in otherwise asymptomatic patients, could improve the pregnancy outcome.

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Lamina Propria Lymphocytes Are Positive for IL-15 in (Refractory) Celiac Disease

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A small fraction of celiac disease (CD) patients does not recover even after strict adherence to the gluten-free diet (GFD). The diagnosis of refractory celiac disease (RCD) in these patients is only possible by exclusion of other enteropathies. In RCD type I (RCDI) patients the IEL have a normal phenotype (surface CD3 and CD8 positive), while in RCDII patients a large proportion of the IEL consists of aberrant cells (surface CD3, CD4 and CD8 negative but positive for cytoplasmic CD3). Inappropriate activation of intraepithelial lymphocytes (IEL) by enterocyte derived IL-15 is thought to contribute to villous atrophy. Furthermore, IL-15 is thought to be an important growth and survival factor for aberrant IEL found in RCDII patients, and anti-IL-15 is therefore currently being explored as a therapy for RCD. We hypothesised that IL-15 levels may be a useful marker for the diagnosis of RCD. Here, we investigated IL-15 levels in enterocytes of small bowel biopsies of active CD (ACD), GFD, RCD-I and RCD-II patients. The biopsies were separated into epithelial layer containing enterocytes and IEL and lamina propria containing other immune cells including T-lymphocytes and dendritic cells. As IL-15 is mostly receptor-bound and hardly secreted in a soluble form, we measured IL-15 in cell lysates from freshly isolated cell fractions or by FACS on intact specific cell types. When IL-15 was measured in lysates of the complete epithelial layer, including IEL, levels were the lowest in RCD-I. Interestingly, after removal of lymphocytes from the epithelial fraction by CD45-MACS, IL-15 levels were found to be considerably lower. In the lamina propria lysates, the levels of IL-15 were comparable to those of the