Persistence of elevated deamidated gliadin peptide antibodies on a gluten-free diet indicates nonresponsive coeliac disease

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Publication data
Submitted 23 August 2013
First decision 18 September 2013
Resubmitted 10 December 2013
Accepted 11 December 2013
EV Pub Online 6 January 2014

SUMMARY

Background
Histologically nonresponsive coeliac disease (NRCD) is a potentially serious condition diagnosed during the follow-up of coeliac disease (CD) when patients have persistent villous atrophy despite following a gluten-free diet (GFD).

Aim
As current assessments of recovery are limited to invasive and costly serial duodenal biopsies, we sought to identify antibody biomarkers for CD patients that do not respond to traditional therapy.

Methods
Bacterial display peptide libraries were screened by flow cytometry to identify epitopes specifically recognised by antibodies from patients with NRCD, but not by antibodies from responsive CD patients. Deamidated gliadin was confirmed to be the antigen mimicked by library peptides using ELISA with sera from NRCD (n = 15) and responsive CD (n = 45) patients on a strict GFD for at least 1 year.

Results
The dominant consensus epitope sequence identified by unbiased library screening QPxx(A/P)FP(E/D) was highly similar to reported deamidated gliadin peptide (dGP) B-cell epitopes. Measurement of anti-dGP IgG titre by ELISA discriminated between NRCD and responsive CD patients with 87% sensitivity and 89% specificity. Importantly, dGP antibody titre correlated with the severity of mucosal damage indicating that IgG dGP titres may be useful to monitor small intestinal mucosal recovery on a GFD.

Conclusions
The finding of increased levels of anti-dGP IgG antibodies in CD patients on strict GFDS effectively identifies patients with NRCD. Finally, anti-dGP IgG assays may be useful to monitor mucosal damage and histological improvement in CD patients on a strict GFD.

Aliment Pharmacol Ther 2014; 39: 407–417
INTRODUCTION

Coeliac disease (CD) is an autoimmune disease that is activated in genetically susceptible individuals by the ingestion of gluten in wheat and similar prolamins in rye and barley. The diagnosis of CD is suggested by elevated levels of serum antibodies to tissue transglutaminase (TG2) and/or deamidated gliadin peptides (dGP) and confirmed by small bowel mucosal biopsy showing the characteristic histological features of villous atrophy and crypt hyperplasia. Diagnosis is essential to prevent morbidity and possible mortality associated with prolonged untreated CD. The only treatment currently effective for CD is a strict gluten-free diet (GFD). Even so, a majority of CD patients exhibit slow mucosal recovery rates during a GFD as measured by biopsy. An estimated 8–35% of patients recover after 2 years of GFD and 66% of patients recover after 5 years. However, 10–19% of patients do not exhibit a histological response to a GFD and are thus considered to have nonresponsive CD (NRCD). NRCD is defined as persistent small bowel mucosal villous atrophy during a GFD with or without symptoms and can only be diagnosed by follow-up intestinal biopsy. Continued exposure to gluten (36–45%) is the most common cause of NRCD. If a strict GFD is confirmed, NRCD may be due to refractory CD (RCD), which occurs in approximately 4% of CD patients. RCD is defined as the failure of a strict GFD to improve damaged intestinal architecture and relieve symptoms in patients with confirmed CD. Poor response to a GFD may also reflect the complicating coexistence of other conditions including irritable bowel syndrome (IBS), lactose intolerance, microscopic colitis and small intestine bacterial overgrowth.

Serological measures of the response of CD patients to a GFD that identify patients with NRCD would have substantial clinical value. Previous studies failed to link the disappearance of TG2 and endomysial antibodies (EMA) to CD patient recovery while on a GFD. Outside of established CD diagnostic assays, the monitoring of serum intestinal fatty acid-binding protein (I-FABP) levels, faecal fat excretion, urinary lactulose-to-mannitol excretion ratios and the maximum concentration of simvastatin in the small intestine may be useful to identify continued mucosal damage or GFD transgressions non-invasively but are not in clinical use. Thus, despite a clear need for non-invasive diagnostic methods to identify patients with NRCD, objective assessments of morphological recovery rely upon invasive and costly duodenal biopsies and are rarely made.

The objective of this study was to screen for serum antibody biomarkers that could serve as an economical and non-invasive diagnostic to identify NRCD and monitor morphological recovery in CD patients on a strict GFD. Application of a novel unbiased screening method to screen for serum antibodies present in patients with active CD, but not in healthy volunteers, recently enabled the identification of immunodominant B-cell epitopes in CD patients, which in turn provided exceptional diagnostic efficiency. Because of this result and the clinical need for non-invasive diagnostics of NRCD and intestinal recovery, we applied a similar unbiased method to screen for candidate biomarkers of NRCD and convalescence while on a GFD. Our results indicate that an existing assay for dGP IgG antibodies, used clinically to diagnose active CD, can also serve as a diagnostic to identify patients with NRCD and to monitor CD patient recovery while on a GFD.

MATERIALS AND METHODS

SUBJECTS

Patient sera were provided by the University of Tampere and Tampere University Hospital (Finland). The patient cohort consisted of 15 NRCD patients with persistent small bowel mucosal villous atrophy and crypt hyperplasia and 45 responsive CD patients with normal villous structure (Table 1). All patients had HLA typing and small bowel mucosal biopsies graded by the Marsh classification. To confirm adherence to a GFD, a dietitian undertook a detailed dietary analysis and a history of occasional or regular consumption of gluten-containing products by means of an interview and a 4-day record of food intake. All CD patients were confirmed to have strict GFD adherence. An estimated 70–80% of the responsive CD patients consumed oats as part of their GFD, but NRCD patients did not consume oats.

Four NRCD patients had RCD, and five other NRCD patients, who were initially asymptomatic, had reoccurring symptoms during subsequent follow-up visits. Because symptoms were required for a true RCD diagnosis, these five patients were considered to have latent RCD. The remaining six NRCD patients were asymptomatic at the time of blood draw and during follow-up. The immunophenotype of intra-epithelial lymphocytes (IELs) and the presence of abnormal clonal T-cell receptor rearrangement indicating RCD type II were detected as previously described. One patient was positive for these RCD type II markers. Eight NRCD patients had
osteoporosis or osteopaenia; two had haemolytic anaemia; two had hyposplenism; one had autoimmune hypothyroidism; one had IgA deficiency; and two had collagenous colitis. Two NRCD patients were later diagnosed with T- and B-cell lymphoma, respectively, and subsequently died from complications. Five responsive CD patients had treated autoimmune hypothyroidism; four had dermatitis herpetiformis; three had depression; and one had polyneuropathy. Serum taken at the time of initial CD diagnosis was available for 24 of the responsive CD patients.

Non-CD controls were spouses of coeliac patients who volunteered to participate in the study. They were healthy, did not have diagnosed CD, and had negative endomysial and TG2 antibodies. Disease control samples were TG2 negative and underwent a biopsy to rule out CD. Samples were shipped frozen at −20°C and aliquots were made upon initial thawing. The study protocol was approved by the Ethics Committees of Tampere University Hospital (Tampere, Finland). All individuals involved gave their written informed consent.

Coeliac disease serology

Serum samples were collected at the time of small bowel mucosal biopsy and stored at −20°C. Serum IgA anti-endomysial antibody (EMA) was determined by indirect immunofluorescence with human umbilical cord as substrate. Detectable signals with serum dilutions of 1: ≥5 were considered positive. Sera were assayed for IgA TG2 antibodies by ELISA (Celiky #18196, Phadia, Germany). The manufacturer has suggested a cut-off of 7.0 U, but cut-offs between 3.0 and 5.0 units are used in practice. We considered a unit value ≥5.0 U to be positive. Anti-dGP IgG antibody titres were determined using a QUANTA Lite Gliadin IgG II kit (INOVA Diagnostics #704520, San Diego, CA, USA) according to the manufacturer’s protocol. A cut-off of 20 units was suggested, but the manufacturer instructed each group to establish their own cut-offs, which was done by receiver-operating curve analysis. The exact sequence of the deamidated gliadin peptide used for the ELISA kit is proprietary information and therefore unknown. ELISA kits from multiple manufacturers have been shown to correlate reasonably well to each other.

Reagents and strains

Bacterial display experiments were performed using E. coli strain MC1061. A pool of three bacterial display random peptide libraries with the format X_{15}, X_{12}CX_3 or X_4CX_7CX_4 (where X is any amino acid and C represents a site restricted to cysteine) displayed at the N-terminus of the enhanced circularly permuted OmpX (eCPX) display scaffold were used for peptide discovery. Bacterial cultures were grown at 37°C with vigorous shaking in Luria-Bertani (LB) media supplemented with chloramphenicol (CM) (34 μg/mL) for expansion. Medium was supplemented with arabinose (final concentration of 0.02–0.04% w/v) to induce the peptide display. Reagents were obtained as follows: streptavidin-R-phycoerythrin (SA-PE) (Invitrogen, Carlsbad, CA, USA), Protein A/G magnetic beads (Thermo Fisher Scientific, Waltham, MA, USA), biotin-SP-conjugated AffiniPure goat anti-human IgA and biotin-SP-conjugated AffiniPure goat anti-human IgG (Jackson ImmunoResearch, West Grove, PA, USA).

Library Screening

E. coli-binding serum antibodies were removed, after which bacterial display random peptide libraries were
screened as described\textsuperscript{21, 31} with the following modification: after the library size was sufficiently reduced by pre-enrichment, fluorescence-activated cell sorting (FACS) was used for subtractions rather than magnetic-activated cell sorting (MACS). Cycles of FACS enrichment were performed with diluted pooled NRCD patient sera (1:250 dilution), and cycles of FACS subtraction were performed with diluted pooled healthy control, disease control and/or Marsh 0 responsive CD patient sera (1:150 dilution). Typical patient pool sizes were three and five patients for NRCD and controls, respectively, and the patients in a pool were switched during each round of sorting. Biotin-conjugated anti-human serum IgA and IgG were used simultaneously as secondary antibodies. Incubation with SA-PE allowed for fluorescent detection at 576 nm using a FACSARia (Becton Dickinson). To reduce the library diversity to one suitable for single clone analysis and achieve the desired NRCD patient cross-reactivity and specificity, five rounds of enrichment and four rounds of subtraction were completed. Peptide sequences were identified by DNA sequencing from plated colonies of cells from sorted library populations. All antibody-binding assays by flow cytometry were performed with a 1:250 dilution of serum into PBS.

Statistical analysis
To measure serum antibody reactivity using flow cytometry, the fluorescence intensity obtained from binding to \textit{E. coli} that do not display peptides was subtracted from that obtained with peptide-displaying bacteria. The Wilcoxon rank-sum test was used for all nonparametric comparisons with a \( P \) value < 0.05 considered significant. The Spearman’s rank correlation coefficient was used for correlations. Specificity and sensitivity were calculated as previously described.\textsuperscript{31} All statistics including the generation of receiver-operating characteristic (ROC) curves were generated using GraphPad Prism. Zero or negative values after background subtraction for FACS assays were normalised to 0.1\% for the purposes of visual display on the logarithmic scale.

RESULTS

Screening for antibody biomarkers of NRCD using bacterial display peptide libraries
To identify candidate antibody biomarkers of NRCD, we applied a quantitative, specificity-based screening method to identify peptide mimotopes from random peptide libraries that capture disease-specific antibodies.\textsuperscript{21, 31} Pools of two to four NRCD patients’ sera, a pool of three recovered (Marsh 0) CD patients’ sera and pools of four to five non-CD control patients’ sera were used for library screening. Non-CD controls included healthy individuals without symptoms and negative TG2 serology as well as symptomatic disease controls with normal small bowel mucosal biopsies. To disfavour nonspecific peptide binding to antibodies in non-CD individuals, four rounds of subtractive FACS were performed by sorting library peptides that did not bind to control patients’ sera \((n = 33)\). A bacterial display peptide library comprised of three distinct pooled libraries of the form \(X_{15}, X_{12}CX_3\) and \(X_4CX_2CX_4\) (where \(X\) is any amino acid and \(C\) represents a site restricted to cysteine) with \(1.2 \times 10^{10}\) members was used. Random peptide libraries of 15 amino acids are capable of mimicking diverse linear and discontinuous epitopes. Typical linear B-cell epitopes contain six to nine amino acids. Although discontinuous epitopes can span 9–22 amino acids, the majority of conformational epitopes include at least one linear stretch of four to seven residues.\textsuperscript{32} A library of \(10^{10}\) random 15-mer peptides contains, in theory, each of the \(1.3 \times 10^9\) possible unique 7-mers with greater than 95\% confidence.\textsuperscript{33} Cells from the final rounds of screening were plated and peptide sequences from individual colonies were determined (Table S1). Peptides obtained by screening exhibited a consensus of Q\textsubscript{xxx}(A/S/P)FP(E/D), and the frequency of peptides with this motif increased substantially during subsequent cycles of FACS (Figure 1a). To reveal other potentially important flanking residues, the amino acid sequences of peptides from the final enriched library population were aligned (Figure 1b). The position of the motif sequence within the peptide was highly conserved, which indicated the importance of the Gln at the N-terminus. This Gln was part of the N-terminal linker sequence (GQSGG) upstream of the randomised 15-mer of each peptide library. The consensus motif was \(Q(P/V/A)(V/E)(A/D/Q)(A/P)FP(E/D)(A/R/Q)\), which shared eight identical residues with an immunodominant B-cell epitope (DGP3) in patients with active CD\textsuperscript{21} (Figure 1c). These mimotopes were similar to previously described B-cell epitopes of \(\gamma\)-gliadin\textsuperscript{34, 35} (Figure 1c) and T-cell epitopes from \(\alpha\)-gliadin, secalin and hordein (not shown).\textsuperscript{36, 37}

Down selection and characterisation of library-isolated peptides
To identify clones exhibiting cross-reactivity and specificity for antibodies in NRCD sera, individual unique library
clones were assayed for binding to serum IgA and IgG from six NRCD patients and four responsive CD patients (Figure S1). The clones that reacted above background levels with at least four of the six NRCD patients were then assayed with the original cohort of 11 NRCD and 25 responsive CD patients. Two of the best performing clones (C83 and C139) from the final library population, along with previously optimised clone DGP3, were further assayed using only IgG secondary antibodies with all 15 NRCD and 45 responsive CD patients (Figure 2a). Sera from NRCD and responsive CD exhibited a significant difference in reactivity with each clone (Wilcoxon rank-sum test $P < 0.0001$, 0.0003, 0.0001 for DGP3, C83 and C139 respectively). Among responsive patients with serology available at diagnosis, 23/24 showed a reduction in response to DGP3 after 1 year of GFD as expected (Figure 2b). Assays for IgA reactivity revealed a similar trend, but with more overlap between NRCD and responsive CD patient titres, because the mean signal intensities were decreased overall (Figure S2). Thus, random peptide library screening revealed an immunodominant dGP epitope recognised by serum IgG from patients with NRCD, but not by those with responsive CD.

**IgG antibodies to dGP are an effective marker of CD recovery**

The sequence similarity of the best performing mimotopes to known deamidated gliadin B-cell epitopes (Figure 1c) coupled with the responsiveness of antibody titres to DGP3 to a GFD (Figure 2b) indicated that the discovered peptides mimicked dGP. We confirmed this hypothesis with a commercially available anti-dGP IgG antibody ELISA (Figure 3a). The mean antibody titre of NRCD patients was seven-fold greater than that of responsive CD patients, and the differences in ELISA response between the two groups were significant ($P < 0.0001$). A positive threshold value of 12 units yielded 87% sensitivity and 89% specificity for NRCD based solely on dGP serology. There was no statistical difference between titres for RCD patients (current and latent RCD, $n = 9$) and the remaining NRCD patients ($n = 6$) ($P = 0.95$). Furthermore, anti-dGP IgG titres correlated with the severity of mucosal damage represented by Marsh classifications (Figure 3b). The ELISA signal differences between Marsh 0 and Marsh 1 patients or Marsh 2 and Marsh 3a/3b patients were nonsignificant, but each of the other Marsh binary comparisons was significant (Table S2). To further verify that dGP was the antigen mimicked by bacterial display peptide mimotopes, the antibody reactivity from the individual patient assays with DGP3 and C139 was compared to dGP ELISA values. Serum IgG-class dGP ELISA values correlated with cytometry measurements (Spearman $\rho = 0.52$ and $P < 0.0001$ for C139; $\rho = 0.46$ and $P = 0.0002$ for DGP3) (Figure 3c).

The true-positive rate (sensitivity) was plotted against the false-positive rate (1-specificity) in an ROC curve as
a function of varying ELISA unit cut-offs (Figure 3d). The positive threshold of 12 units yielded 87% sensitivity and 89% specificity, and a cut-off of 10 units yielded 93% sensitivity and 84% specificity. Because only one NRCD patient was positive for TG2 and four others were weakly positive (Table 1), the serum IgG dGP ELISA substantially outperforms the IgA anti-TG2 ELISA with an ROC area under the curve (AUC) of 0.94 vs. 0.61 respectively (Figure S3). An optimal serum IgA-class TG2 ELISA positive threshold of 5 U/mL yielded 33% sensitivity and 100% specificity, misclassifying 10 of 15 NRCD patients as GFD responders. Assays with DGP3 and C139 (AUC = 0.88 and 0.87 respectively) displayed on bacteria using flow cytometry also outperformed TG2 (Figure S3), but did not exceed the diagnostic accuracy of the dGP ELISA (Figure 3d). On the basis of our novel finding of the persistence of elevated IgG dGP antibodies, we have suggested a revised diagnostic algorithm using non-invasive serological tests for the monitoring of CD patient recovery during a GFD (Figure 4).

**DISCUSSION**

There exists an unmet diagnostic need for serological assays to monitor the recovery of CD patients on a GFD and identify those nonresponsive individuals that may require more aggressive therapy. Here, we have identified and validated dGP IgG assays as an effective serological assay to meet this need. Similarity between the best performing library-isolated peptides and known deamidated gliadin B- and T-cell epitopes led to the confirmation of dGP as the targeted antigen. In a cohort of 15 NRCD cases and 45 responsive controls, we observed 87% sensitivity and 89% specificity, which support the utility of IgG dGP testing in monitoring response to a GFD. Nevertheless, a more rigorous assessment of diagnostic performance will require a large prospective cohort of CD cases on a GFD to determine positive and negative predictive values. Our results demonstrate that bacterial display technology can provide both diagnostically useful reagents and an effective route to antigen discovery without prior knowledge of the cause or mechanisms of disease.

Refactory CD (RCD) is defined by persistent villous atrophy and CD-associated symptoms despite a year or more of strict GFD after CD diagnosis and no evidence of other small bowel disorders. Early detection of RCD is critical because of the increased risk of ulcerative jejunitis, enteropathy-associated T-cell lymphoma (EATL) and non-Hodgkin’s lymphomas, epithelial neoplasms,
oesophageal and pharyngeal squamous cell carcinomas, and concomitant autoimmune diseases. RCD diagnosis depends on the exclusion of other possible causes of villous atrophy and increased IELs. The only definitive test for RCD requires detection of an abnormal IEL phenotype specific to RCD type II. Our observations that EMA and TG2 did not detect persistent mucosal damage confirm previous results that neither test can substitute for intestinal biopsies for diagnosing NRCD or RCD. However, negative EMA and TG2 assays within our cohort supported strict GFD adherence. Overall, 96.7% and 91.7% of our patients were negative for EMA and TG2 respectively. These results are in agreement with a previous study wherein EMA and TG2 were negative in 97.5% and 97.2% of GFD-adherent patients respectively. Although occasional accidental gluten intake cannot be completely ruled out, the combination of the low anti-TG2 titre and comprehensive dietary assessment with dietitians is the gold standard for monitoring the strictness of GFDs.

IgG-class antibodies against dGP were significantly elevated in NRCD patients compared with responsive CD patients as detected by flow cytometry and ELISA. Only one misclassified responsive CD patient was on a GFD for more than 2 years. The titres of the remaining responsive patients could be expected to continue to decline (below the positive threshold) upon further follow-up. This observation is supported by the change in dGP antibody reactivity before and after 1 year of GFD, as the two patients with the highest titres at diagnosis also had the highest titres 1 year later. In this study, 14/15 NRCD patients were on a strict GFD for more than 3 years. Previous studies suggest that a period of 3 years of GFD is sufficient to allow titres to decline to baseline.

Figure 3 | Confirmation of deamidated gliadin as the primary antigen mimicked by library-isolated peptides and a marker of coeliac disease (CD) patient recovery. (a) Anti-deamidated gliadin peptide (dGP) IgG antibody ELISA discriminates nonresponsive CD (NRCD) (n = 15) and responsive CD patients (n = 45) (P < 0.0001). An adjusted ELISA cut-off of 12 units yielded 87% sensitivity and 89% specificity. (b) Anti-dGP titre increases with the severity of mucosal damage as described by Marsh staging (see Supplementary Table S2 for binary significance comparisons). (c) Correlation between flow cytometry assays with bacterial display peptides and anti-dGP IgG ELISA (P < 0.0001 for C139 and P = 0.0002 for DGP3). Each point represents an individual patient’s reactivity with C139 (▲) and DGP3 (●). (d) Receiver-operating characteristic (ROC) curve for the anti-dGP IgG antibody ELISA test. The area under the curve (AUC) was 0.94 (95% confidence interval, 0.88–0.99).
levels in responsive patients. Comparing the difference in dGP titre between Marsh 3c NRCD and fully recovered Marsh 0 patients, classification accuracy improves further to >90%. Given that there were no differences in serological or histological findings between RCD and NRCD patients, the definition of RCD could potentially be revised to include asymptomatic NRCD patients in cases where a strict diet is confirmed and other associated causes of refractory disease are ruled out.

Previous studies have proposed that the detection of antibodies specific for deamidated gliadin may be helpful in CD follow-up. One study reported that six of nine NRCD patients and 6/33 responsive CD patients maintained IgA dGP antibodies after 1 year of a strict GFD. Our assays using IgA antibodies had comparable NRCD sensitivity and specificity, but we observed that IgG-class dGP antibodies were significantly more sensitive and specific for NRCD when compared with IgA-class antibodies. Although IgA outperforms IgG in anti-TG2 assays, IgG anti-dGP ELISA has a greater diagnostic performance with untreated CD patients than its IgA counterpart. Another study reported that 10/15 NRCD patients on a GFD for 1 year had elevated anti-dGP IgA and IgG titres, but 7/10 positive cases were patients that had ‘low compliance’ with their GFD. Consequently, it was not possible to link dGP titre to NRCD. We did not include any patients with low or moderate adherence to GFD in our study. Thus, the present finding that anti-dGP IgG antibodies accurately discriminate NRCD from responsive CD provides compelling evidence that dGP testing may be useful to identify individuals with NRCD. Our study thus provides support for a prospective study in a large cohort of newly diagnosed CD patients on a GFD for identifying NRCD cases.
One possible explanation of the persistence of antibodies against dGP despite complete removal of gluten from the diet is the presence of T-cell clones that have evolved antigen independence and continue to stimulate dGP antibody-secreting plasma cells. This phenomenon has been previously described, and pools of memory T- and B-cells can be maintained at constant levels for years even in the absence of the eliciting antigen. In addition, plasma cells can continuously secrete antibody even after the disappearance of memory cells. Further studies will be necessary to confirm the presence of dGP-specific memory B-cells or plasma cells in NRCD patients. Therefore, the mechanism responsible for the persistence of anti-dGP IgG antibodies remains to be elucidated.

We propose a revised diagnostic algorithm using non-invasive serological tests for the monitoring of CD patient recovery during a GFD (Figure 4). The first step in the follow-up of CD is to confirm compliance with a strict GFD. Although IgA anti-TG2 antibodies do not identify a majority of diet noncompliers (low positive predictive value (PPV)), our results suggest that a negative TG2 ELISA may be useful to assess strict adherence to GFD. However, consultation with an expert dietitian or nutritionist is still considered the gold standard. If trace gluten contamination is suspected, a modified diet including only fresh and unprocessed foods may be beneficial for a subset of NRCD patients. Once diet adherence is confirmed and other associated causes of villous atrophy have been excluded, an anti-dGP IgG ELISA test should be performed 1 year after the initial CD diagnosis. A negative result may reassure a physician that their patient is recovering and may help patients maintain compliance to a GFD. Such patients would not require serial follow-up biopsies unless their symptoms reoccur. A positive dGP test identifies probable nonresponders, and NRCD patients could seek alternative therapies (parenteral nutrition, corticosteroids and/or immunosuppressive drugs) and undergo a biopsy to test for RCD type II.

The antibody repertoire analysis method used here may have broad utility for the development of diagnostics. Here, we have isolated and characterised mimotope peptide sequences from bacterial display random peptide libraries that did not require a priori knowledge about refractory or nonresponsive CD. The use of convenient IgG dGP blood tests could aid the effective care of recovering CD patients on a GFD and reduce the need for costly and invasive endoscopy/biopsy procedures. Furthermore, the dGP assay can efficiently identify patients that require alternative treatment options to reduce the morbidity and mortality risks associated with NRCD. Our results strongly support the use of dGP serology in the clinical follow-up of individuals with CD.

AUTHORSHIP

Guarantor of the article: Patrick Daugherty.

Author contributions: BNS performed the experiments. BNS, MFK and PSD conceived and designed the experiments. BNS, KK, MFK and PSD analysed and interpreted the data. KK, PC and MM contributed patient and control sera. BNS and PSD wrote the manuscript. All authors approved the final version of the submitted manuscript.

ACKNOWLEDGEMENTS

Declaration of personal interests: None.

Declaration of funding interests: National Institutes of Health grant AI09224 to PSD; the coeliac disease study group has been financially supported by the Academy of Finland, the Sigrid Juselius Foundation, the Competitive State Research Financing of the Expert Responsibility Area of Tampere University Hospital (grant numbers 9H166, 9P020 and 9P033) to PC, MM, KK; National Institutes of Health grant DK35108 and a grant from the Wm. K. Warren Foundation to MFK.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Heat map representation of the reactivity of clones remaining in the peptide library during the final rounds of screening.

Figure S2. Performance of clones with highest specificity for NRCD using IgA-type serum antibodies.

Figure S3. Receiver-operating characteristic (ROC) curve analysis for TG2 ELISA and select library clones by flow cytometry.

Table S1. All sequences obtained from colonies from the final five rounds of library screening.

Table S2. Wilcoxon rank-sum P values comparing the dGP ELISA response of patients grouped by their Marsh classification index from small bowel mucosal biopsies. Raw data derived from Figure 3b in the main text. P < 0.05 is considered significant; ns, not significant.
REFERENCES

dGP antibodies indicate nonresponsive CD