

RESEARCH ARTICLE

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# Potential blood-based markers of celiac disease

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## Abstract

**Background:** Blood-based diagnostics has the potential to simplify the process of diagnosing celiac disease (CD). Although high levels of autoantibodies against tissue transglutaminase (anti-TG2) are strongly indicative of active CD, several other scenarios involve a need for additional blood-based CD markers.

**Methods:** We investigated the levels of messenger RNA (mRNA) in whole blood (n = 49) and protein in plasma (n = 22) from cases with active CD (n = 20), with confirmed CD and normalized histology (n = 15), and without a CD diagnosis (n = 14). Group differences were analyzed using Kruskal-Wallis one-way analysis of variance by ranks. We also investigated correlations between levels of potential markers, histopathology according to the modified Marsh scale, and CD risk gradient based on HLA type, using Spearman rank correlation. The relation between HLA-DQ2 gene dose effect and the expression levels of selected blood-based markers was investigated using the Mann-Whitney U test. Finally, the diagnostic performance of anti-TG2, potential blood-based CD markers, and logistic regression models of combined markers was evaluated using receiver operating characteristic (ROC) curve analysis.

**Results:** *CXCL11* protein levels and *TNFRSF9* and *TNFSF13B* mRNA levels were identified as potential CD markers. These are all affected by or involved in the regulation of the NF-κB complex. *CXCL11* protein levels and *IL21* and *IL15* mRNA levels were correlated with histopathology according to the modified Marsh scale, as were the established CD markers. HLA genotype risk and HLA-DQ2 gene dose effect did not show any significant relations with either the potential CD markers or the established CD markers. ROC curve analysis revealed a slight, non-significant increase in the area under the curve for the combined use of anti-TG2 and different constellations of potential blood-based CD markers compared to anti-TG2 alone.

**Conclusions:** The CD markers identified in this study further emphasize the significance of components related to NF-κB regulation in relation to CD. However, the relevance of *CXCL11*, *TNFSF13B*, *TNFRSF9*, and other NF-κB interacting proteins recognized by pathway analysis, needs to be further investigated in relation to diagnosis and monitoring of CD.

**Keywords:** Celiac disease, Molecular diagnostics, Blood-based biological markers

## Background

Celiac disease (CD) is defined as a “chronic small intestinal immune-mediated enteropathy precipitated by exposure to dietary gluten in genetically predisposed individuals” [1]. Autoantibodies against tissue transglutaminase (TG2) can be found in the blood, as can antibodies against endomysium, deamidated gliadin (DGP), and native gliadin (GL); and infiltration of intraepithelial lymphocytes (IELs) in the epithelium, elongation of the crypts, and destruction of villi is seen in the small intestine [2]. A strong genetic component is

evident in CD, with the strongest association found in the HLA region [3,4], primarily with DQ2 (*DQA1\*05/DQB1\*02*) and in a minority of CD patients with DQ8 (*DQA1\*0301/DQB1\*0302*) [5,6]. There is a gene dose effect of HLA-DQ2 [7], and risk gradients based on HLA type have been calculated [8,9]. A number of other risk loci containing multiple candidate genes have been associated with CD [3,4,10-12].

Current CD diagnostics primarily include antibody detection, mainly of Immunoglobulin A (IgA) autoantibodies against TG2 (anti-TG2), and confirmation of the diagnosis by histopathologic assessment of small intestinal biopsies [13]. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) Working

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Group on Coeliac Disease Diagnosis suggested in 2012 that children and adolescents with clear symptoms and anti-TG2 levels over 10 times the upper limit of normal (ULN), with a remission of symptoms on a gluten-free diet (GFD), could be diagnosed with CD without histopathologic assessment of an intestinal biopsy [14]. Additionally, the presence of HLA-DQ2 or DQ8 should be verified.

We have previously developed a discriminant analysis model based on gene expression data in duodenal biopsies. This model can discriminate between biopsies with and without histopathologic alterations indicative of CD, and also indicate the level of histologic damage as well as mucosal recovery on a GFD [15]. The identification of blood-based markers which could reinforce the diagnostic value of anti-TG2, and perhaps indicate the level of histologic damage and mucosal recovery on GFD, would further simplify CD diagnostics.

In the present study, we investigated levels of candidate CD markers (messenger RNA [mRNA] and protein) in blood from celiac and non-celiac cases, and considered the possible added value of these candidate markers. We also explored the relations between candidate markers and enteropathy graded according to the modified Marsh scale [16], CD risk gradient based on HLA type [8], and HLA-DQ2 gene dose effect [7].

## Methods

### Study subjects and samples

Following written informed consent from parents/legal guardians, blood and duodenal biopsy specimens were collected from pediatric patients (Table 1) investigated for suspected CD or at follow-up on a gluten-free or gluten-containing diet, both for diagnostic purposes and for research purposes.

For diagnostic purposes, blood was collected in a blood tube containing polymer gel and clot activator (Becton, Dickinson and Company, Franklin Lakes, NJ) and centrifuged at 2400 × g for 5 min for serum isolation. The sera were stored at room temperature for a maximum of 10 hours after centrifugation, and then at 4°C until the presence of CD-specific antibodies (anti-TG2 and antibodies against DGP [anti-DGP]) and antibodies against GL (anti-GL) was investigated, which occurred within 5 days of centrifugation. In six cases, the antibody tests were performed on plasma collected for research purposes. Multiple biopsy specimens were collected using an endoscope in all but one case, where a pediatric Watson capsule was used to extract a single biopsy specimen. The tissue was formalin-fixed and paraffin-embedded, and histopathologically assessed.

For research purposes, blood was collected both in EDTA blood tubes (Becton, Dickinson and Company) for DNA and plasma isolation, and in Tempus Blood RNA tubes (Life Technologies, Carlsbad, CA) for RNA isolation. An aliquot of EDTA blood for DNA isolation was removed, and the remaining blood centrifuged at 1500 × g for 10 min for plasma isolation. For two cases, one in the group with no indications of CD and one in the group with active CD (Table 1), blood for RNA purification was collected in EDTA blood tubes instead of Tempus tubes. Additionally, a biopsy specimen immersed in pre-chilled RNAlater RNA Stabilization Reagent (Qiagen, Hilden, Germany) was collected from all cases in the study. Biopsies and stabilized blood for RNA purification were kept at 4°C for about 18 hours, and then at -20°C. RNA from EDTA blood was, however, purified without prior storage. Plasma was stored at -80°C. A maximum of two freeze-thaw cycles was accepted for all protein analyses.

**Table 1 Descriptive data on study subjects including clinical antibody levels**

Group	No of cases <sup>a</sup> (Females)	Age (years) median; 80% CR <sup>b</sup>	Marsh grade span	Anti-TG2 (U/mL) <sup>c</sup> median; 80% CR	Anti-DGP (U/mL) <sup>d</sup> median; 80% CR	Anti-GL (U/mL) <sup>e</sup> median; 80% CR
Not CD	14 (8)	8.8; 2.2-16	Marsh 0-1	0.25; 0.05-11	0.95; 0.20-20	0.60; 0.10-2.4
Normalized CD <sup>f</sup>	15 (13)	12; 7.3-17	Marsh 0-1	3.4; 0.40-28	2.6; 1.2-9.3	0.80; 0.20-4.1
Active CD <sup>g</sup>	20 (12)	5.9; 1.5-14	Marsh 2-3C	201; 4.4-1997	64; 5.6-392	16; 2.4-167
Under investigation <sup>h</sup>	3 (3)	15; 12-16	Marsh 0-1	15; 10-16	7.1; 2.1-22	1.9; 0.50-6.5

Study subjects are divided into groups depending on the diagnosis and the histopathologic assessment.

<sup>a</sup>Initially, a total of 56 cases were included in the study, but three cases were later excluded due to low sample amount and one case was excluded due to histopathologically non-assessable biopsies, leaving 52 cases included in the analyses.

<sup>b</sup>CR = Central range.

<sup>c</sup>Tissue transglutaminase autoantibodies (anti-TG2), positive result ≥ 7 U/mL.

<sup>d</sup>Deamidated gliadin antibodies (anti-DGP), positive result ≥ 10 U/mL.

<sup>e</sup>Gliadin antibodies (anti-GL), positive result ≥ 7 U/mL.

<sup>f</sup>Cases with confirmed celiac disease and duodenal biopsies indicating a Marsh grade 0-1, on a gluten-containing diet (n = 1) or a gluten-free diet (n = 14). Two cases had suboptimal (but assessable) biopsies.

<sup>g</sup>Cases with duodenal biopsies indicating a Marsh grade 2-3C, on a gluten-containing diet (n = 18) or a gluten-free diet (n = 2). One case had patchy lesions of the small intestine.

<sup>h</sup>Cases under continued gluten challenge and observation due to elevated antibody titers but normal mucosa. Ranges for this group are represented by minimum and maximum values.

The study was conducted under the approval of the Regional Ethical Review Board in Linköping.

#### DNA purification

DNA was isolated from EDTA blood using the EZ1 DNA Blood 350 µL Kit and BioRobot EZ1 (Qiagen) according to the manufacturer's instructions.

#### RNA purification and reverse transcription

RNA from stabilized blood was purified using the Tempus Spin RNA Isolation Reagent kit (Life Technologies), and RNA from EDTA blood was purified using the QIAamp RNA Blood Mini kit (Qiagen), in both cases according to the manufacturer's instructions. The quality of the RNA from stabilized blood and EDTA blood was verified, and the RNA was reverse transcribed using a previously documented procedure [15]. The resulting cDNA and the remaining RNA were stored at  $-80^{\circ}\text{C}$ .

#### Histopathologic assessment

Biopsies were assessed by a single experienced pathologist, blinded to all case data, in accordance with instructions for quality assurance and standardization assembled by the Swedish Society of Pathology. The status of the villi and crypts and the number of IELs were assessed for each biopsy. In cases where hematoxylin-eosin staining revealed an IEL number close to the ULN (25 IELs per 100 epithelial cells), an additional staining for CD3 was performed to better assess the number of IELs; when using CD3 staining, there should be  $>30$  IELs per 100 epithelial cells to be indicative of CD. Hematoxylin-eosin staining was performed using the Tissue-Tek DRS 2000 Slide Stainer (Sakura, Alphen aan den Rijn, The Netherlands), and CD3 staining was performed using antibodies against CD3 (Dako, Glostrup, Denmark) and IntelliPATH FLX (Biocare Medical, Concord, CA). The histological changes were reported according to the modified Marsh scale (0, 1, 2, 3A, 3B, or 3C) [16].

#### Clinical antibody tests

Detection of IgA anti-TG2, IgA anti-GL, and Immunoglobulin G (IgG) anti-DGP in serum or plasma was performed using EliA Celikey IgA (positive result  $\geq 7$  U/mL), EliA Gliadin IgA (positive result  $\geq 7$  U/mL), and EliA GliadinDP IgG (positive result  $\geq 10$  U/mL), respectively, on Phadia250 (Thermo Fisher Scientific, Waltham, MA) as described by the manufacturer. In cases with total IgA levels below 0.07 g/L, detection of IgG anti-TG2 replaced IgA anti-TG2 (EliA Celikey IgG, Thermo Fisher Scientific). In order to distinguish results below the detection limit of an assay from missing data, the former were replaced with the detection limit divided by two.

#### HLA typing and risk assessment

DNA from each case was HLA-typed for *DRB1*, *DQA1* and *DQB1* using a sequence-specific primer PCR method and capillary gel electrophoresis [17,18]. The risk gradient for CD based on HLA type was calculated for each case using relative genotype risks extracted from a Scandinavian population [8].

#### Selection of genes for analysis

Potential reference genes for the mRNA analysis were investigated using a Human Endogenous Control Plate (Life Technologies) containing assays for 32 potential reference genes, and cDNA from a total of nine blood RNA samples including three samples from cases with no mucosal injury (Marsh 0) and six with varying degrees of mucosal injury (Marsh 2-3C). Three potential reference genes (Additional file 1) were selected based on low sample-to-sample variation in mRNA levels, as investigated using the NormFinder algorithm [19] in version 5.4.2 of the Genex software package (MultiD Analyses, Göteborg, Sweden). The three selected reference genes were analyzed in the complete dataset, and the final selection of reference gene/s was established using the complete dataset by means of both low sample-to-sample variation and an absence of group differences in expression.

Genes for analysis of mRNA and protein levels were selected by reviewing published studies on blood mRNA/protein expression in CD, and by functional context (Additional file 1).

#### Protein analysis

Multiplex detection of proteins in plasma was performed using Milliplex kits (Millipore, Billerica, MA) based on the Luminex xMAP technology, according to the manufacturer's instructions (Additional file 1). The analyses were performed on the Bio-Plex 200 system (Bio-Rad, Hercules, CA). The *CDI63* soluble protein was detected using an enzyme-linked immunosorbent assay (ELISA) according to instructions from the manufacturer (Additional file 1), and the results were obtained and analyzed using a Sunrise microplate absorbance reader combined with version 7.0 of the Magellan software package (Tecan Group Ltd, Männedorf, Switzerland). Standard curves were included in all protein analyses, and optimized using Bio-Plex Manager 6.1 (Bio-Rad) for all Milliplex assays, and the Akima method for curve fitting in Magellan v.7.0 for the ELISA. In order to distinguish results below the detection limit of an assay from missing data, the former were replaced with the lowest detected value of the assay divided by two.

#### mRNA analysis

Levels of mRNA were investigated using custom made TaqMan Array Cards (Life Technologies) containing 47

gene expression assays including assays for the reference genes, or by using single assays and a previously documented procedure [15] (Additional file 1). Gene expression analysis on the TaqMan Array Cards was performed using the TaqMan Universal Master mix II without UNG and the recommended thermal profile (Life Technologies) on 300 ng cDNA in duplicates. Cards were prepared as recommended by the manufacturer, including analysis on the 7900HT Fast Real-time PCR system (Life Technologies).

For both TaqMan Array Cards and single assay results, quantification cycle ( $C_q$ ) values were established using version 1.0.2 of the ExpressionSuite software package (Life Technologies). The auto-baseline algorithm in the software was used to compensate for background noise for each amplification curve, and the thresholds were adjusted to the log-linear range and set to the same level for all samples within one assay. Missing  $C_q$  values due to low copy numbers were replaced by the highest  $C_q$  value available for the gene in question, increased by one cycle. The resulting  $C_q$  values were normalized against selected reference gene/s (Genex).

### Statistical analysis

Version 10 of the STATISTICA software package (StatSoft, Tulsa, OK) was used in all statistical analyses. Differential expression was investigated using Kruskal–Wallis one-way analysis of variance by ranks, except for the analysis of differential expression in cases stratified based on having one or two *DQB1\*02* alleles, where the Mann–Whitney U test was used. Post-hoc comparisons of mean ranks of all pairs of groups were performed (significance level  $p < 0.05$ , two-sided significance levels with a Bonferroni adjustment) [20]. Spearman rank correlation was used to investigate relations between mRNA/protein levels and histopathology and CD risk gradient based on HLA type. For all statistical analyses, except the post-hoc comparisons, a false-discovery rate was used and set to 5% [21].

The diagnostic performance of individual assays and logistic regression models of assay combinations was evaluated using receiver operating characteristic (ROC) curve analysis (MedCalc Statistical Software version 13.1.2, MedCalc Software, Ostend, Belgium).

## Results

### Differential blood marker expression

Detectable levels were found for all protein markers ( $n = 22$ , Additional file 1), and all mRNA markers except for *IL25* ( $n = 48$ , Additional file 1). *CDKN1B* was selected as the most stable reference gene, and used for normalization of all target mRNA levels.

In cases with active CD (Table 1, Active CD), significantly increased levels were observed for *CXCL11* protein ( $p = 0.003$ , Figure 1, Additional file 2) and *TNFSF13B*

mRNA ( $p = 0.001$ , Figure 1, Additional file 2), in comparison to cases without a CD diagnosis (Table 1; Not CD). Additionally, *TNFSF13B* mRNA levels were significantly elevated in cases with confirmed CD and normalized histology (Table 1; Normalized CD), in comparison to cases without a CD diagnosis ( $p = 0.001$ , Figure 1, Additional file 2). Levels of *TNFRSF9* mRNA were significantly decreased in cases with active CD and in cases without a CD diagnosis, in comparison to CD cases with a normalized histology ( $p = 0.005$  and  $p = 0.02$ , respectively, Figure 1, Additional file 2).

Previously established CD markers (anti-TG2, anti-DGP, and anti-GL) were differentially expressed in active CD cases in comparison to cases with normalized CD ( $p < 0.005$ ) as well as cases without a CD diagnosis ( $p < 0.00005$ ).

Stratification based on having one or two *DQB1\*02* alleles revealed no significant differences in levels of potential CD markers or in levels of previously established CD markers (anti-TG2, anti-DGP, and anti-GL), either when examining active CD cases only, or when looking at all CD cases together. Differences in expression in normalized CD cases alone could not be investigated, because all but two cases had one *DQB1\*02* allele.

### Correlation analysis

Significant correlations were observed between Marsh grade (all cases) and levels of *CXCL11* protein (Figure 2; Spearman rank correlation coefficient [ $r_s$ ] = 0.50), and *IL21* and *IL15* mRNA ( $r_s = -0.46$  for both, data not shown).

Clinical antibody levels (anti-TG2, anti-DGP, and anti-GL) displayed significant correlations with Marsh grade ( $r_s = 0.73$ , 0.75, and 0.75, respectively; Figure 3).

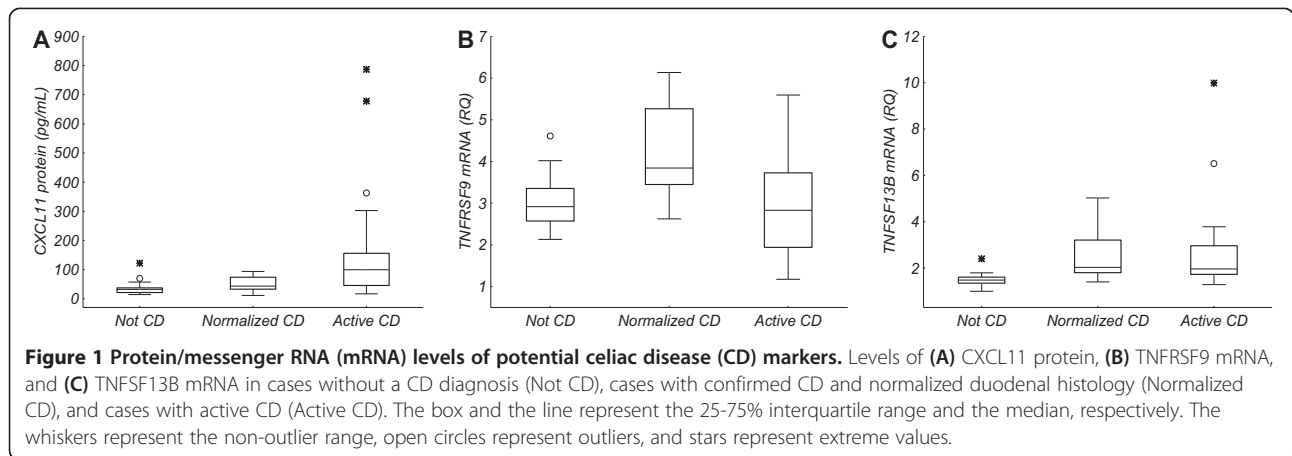
Levels of potential and previously established CD markers were not significantly correlated with HLA genotype risk for CD.

### Clinical antibody tests

All cases on a gluten-containing diet with anti-TG2 levels 10 times ULN or more ( $n = 13$ ) showed a Marsh grade of 3A-3C and received a CD diagnosis. Correspondingly high levels of anti-DGP ( $n = 7$ ) and anti-GL ( $n = 4$ ) were less common, but all occurred in cases with an active CD diagnosis and a Marsh grade 3A-3C.

Of all cases with active CD on a gluten-containing diet ( $n = 18$ ) and all cases without CD diagnosis ( $n = 14$ ), anti-TG2 failed to identify one case with active CD (Marsh 3C, anti-TG2 3.4 U/mL) and gave a false positive result in two cases without CD diagnosis (Marsh 0–1, anti-TG2 11–23 U/mL).

Anti-DGP yielded the same number of misclassifications as anti-TG2, but affected partially different cases, whereas anti-GL yielded substantially more misclassifications with



four false negative results, and one false positive result (data not shown).

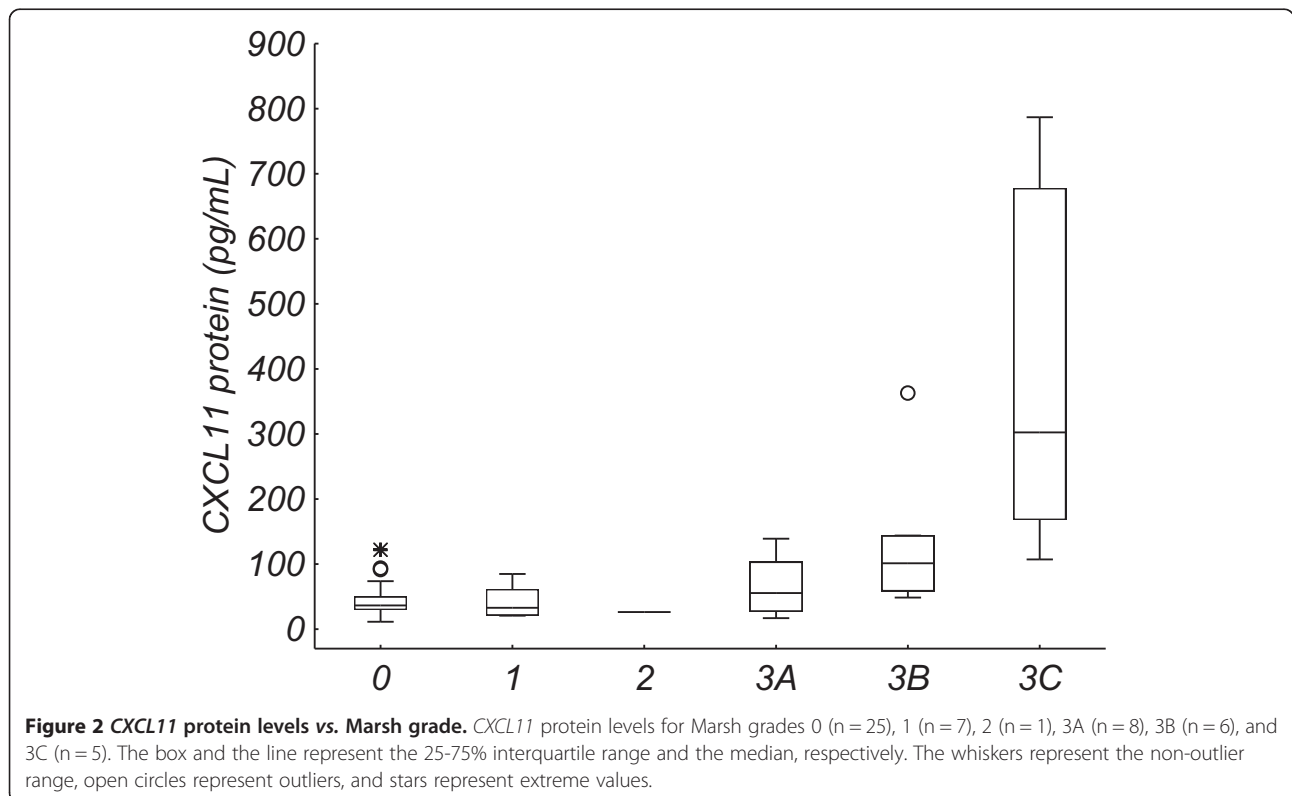
Two cases with remaining enteropathy on a GFD (Marsh 2 - 3A, anti-TG2 2.0 - 5.4 U/mL) had normalized levels of all antibodies.

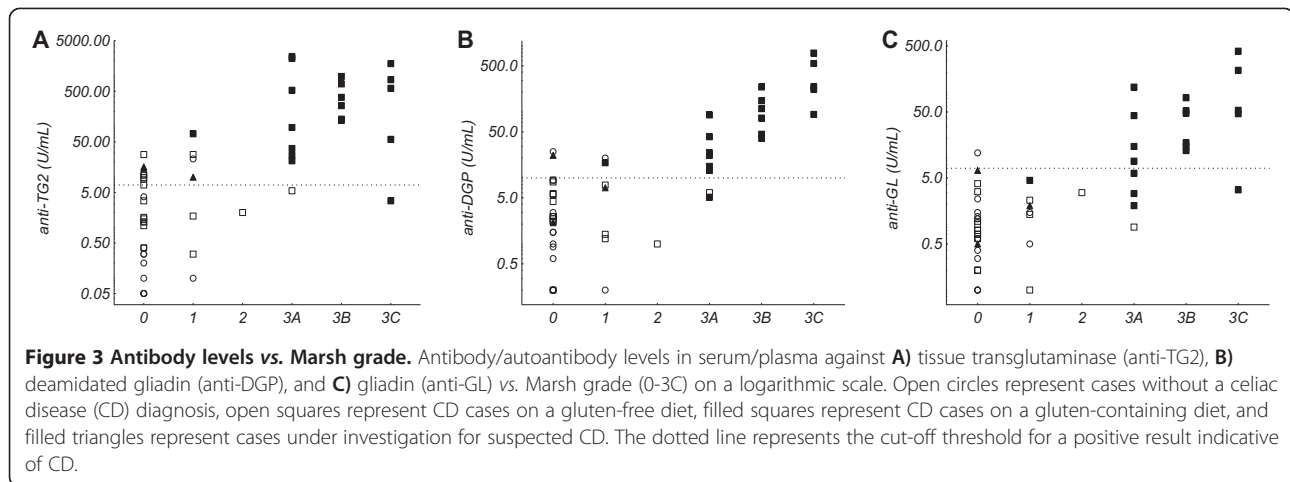
**Misclassified cases and cases under clinical investigation**

The case with active CD on a gluten-containing diet that was misclassified based on levels of anti-TG2 (see Clinical antibody tests) showed levels of the two CD up-regulated markers *CXCL11* protein and *TNFSF13B* mRNA (Figure 1) that were above the 80% central range (CR) of the group

without CD and within the 80% CR for the group with active CD (Additional file 2). For one of the two cases without CD that were misclassified based on levels of anti-TG2 (see Clinical antibody tests), levels of *CXCL11* protein were below the 80% CR of the group with active CD and thus corresponded to the “Not CD” group (Additional file 2).

One of the two CD cases with remaining enteropathy on a GFD that were misclassified based on anti-TG2 (see Clinical antibody tests) showed a level of the *TNFRSF9* mRNA marker for normalized CD (Figure 1) that was below the 80% CR of the group with normalized CD





and within the 80% CR for the group with active CD (Additional file 2).

The remaining results fell within the 80% CR for more than one group.

Cases included in the group under investigation (Table 1, Under investigation) were on a gluten-containing diet and under continuous monitoring for suspected CD. Considering markers *CXCL11* protein and *TNFSF13B* mRNA, the median of the group fell within the 80% CR for both the group without a CD diagnosis and the group with active CD (Additional file 2).

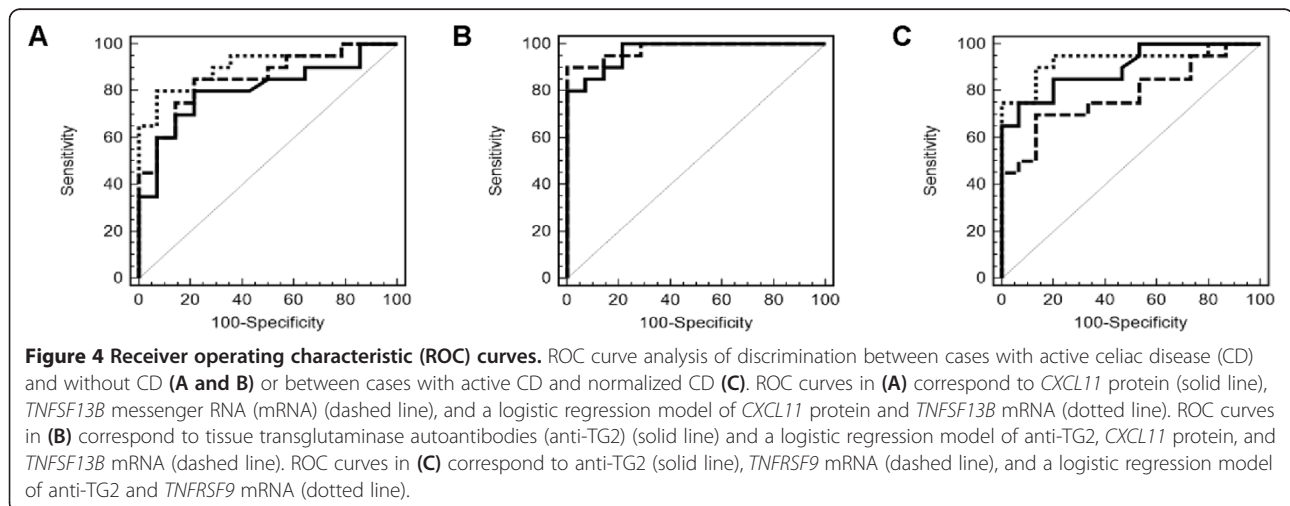
#### ROC curve analysis

ROC curve analysis of discrimination between cases with active CD and without CD (Figure 4A and B) revealed a larger area under the curve (AUC) for anti-TG2 (AUC = 0.97) in comparison to *CXCL11* protein (AUC = 0.81), *TNFSF13B* mRNA (AUC = 0.85), and a logistic regression model based on *CXCL11* protein and *TNFSF13B* mRNA (AUC = 0.91). A logistic regression model based on anti-TG2, *CXCL11* protein, and *TNFSF13B* mRNA

resulted in the highest AUC (0.98). However, compared to anti-TG2 alone, this improvement was not significant ( $p = 0.54$ ). ROC curve analysis of discrimination between cases with active CD and normalized CD (Figure 4C) revealed an AUC of 0.90 for anti-TG2 and 0.78 for *TNFRSF9* mRNA. Compared to anti-TG2 alone, a logistic regression model based on anti-TG2 and *TNFRSF9* mRNA resulted in an increased AUC of 0.93 ( $p = 0.54$ ).

#### Discussion

The prospect of a blood-based diagnostic procedure for CD is appealing. This is reflected by the new recommendations from ESPGHAN, which offer the option to omit biopsies in patients with anti-TG2 titers of 10 times ULN or more [14]. In patients with high pretest probability for CD in combination with anti-TG2 levels of 10 times ULN or more, the probability of having CD is high, but it decreases somewhat in patients with lower pretest probabilities [22]. All patients with anti-TG2 levels of 10 times ULN or more in the current study were diagnosed with CD. However, in patients with lower antibody levels



and/or of young age (<18 months), blood-based diagnostics seem to be less clear-cut [23,24], and additional blood-based CD markers could be useful. Three potential blood-based CD markers (*CXCL11* protein, *TNFRSF9* mRNA, and *TNFSF13B* mRNA) were identified in the current study.

*CXCL11* protein, which is an IFN- $\gamma$  and IFN- $\beta$  induced chemokine [25], and mRNA from *TNFSF13B*, which encodes a cytokine with a major role in B cell growth and survival [26], showed elevated levels in cases with active CD compared to cases without a CD diagnosis, which is consistent with previous studies of *CXCL11* mRNA in small intestinal biopsies [15,27] and *TNFSF13B* in serum [28]. An increased level of *TNFSF13B* mRNA was also evident in cases with CD and normalized histology compared to cases without a CD diagnosis, which is also consistent with previous results [28]. Increased serum levels of *TNFSF13B* protein have previously been found in autoimmune diseases [26], and *CXCL11* has also been implicated in autoimmune diseases [29].

*CXCL11* protein level correlated significantly with Marsh grade, as did mRNA levels of *IL21*, which is involved in the control of the innate and adaptive immune responses [30], and *IL15*, which is involved in the innate immune response in CD [31].

A decreased level of mRNA from *TNFRSF9*, which is a receptor induced on the surface of CD4+ and CD8+ T cells during activation [32], was found in cases with active CD and in cases without a CD diagnosis, compared with CD cases with normalized histology. This result differs from that in small intestinal biopsies [15], indicating tissue-specific regulation.

Potentially, *CXCL11* protein, *TNFRSF9* mRNA, and *TNFSF13B* mRNA might assist in the clinical diagnosis of CD. The established blood-based CD marker anti-TG2 misclassified one case with active CD and two cases without enteropathy on a gluten-containing diet, and two cases with remaining enteropathy on a GFD. In cases on a gluten-containing diet, *CXCL11* protein showed the greatest potential as a marker, with results in accordance with histopathology for two out of three cases misclassified by anti-TG2. For cases on a GFD with remaining enteropathy, results for *TNFRSF9* mRNA were in accordance with histopathology for one out of two cases.

ROC curve analysis showed that, as a single test, the already established anti-TG2 assay outperformed the new potential blood-based CD markers. However, it might be possible to increase the diagnostic performance by considering several assay results jointly. Adding new markers to the anti-TG2 assay produced a slight, though non-significant, increase in diagnostic performance. At present, the data is too limited for any firm conclusions regarding added diagnostic value, and our analysis

provided no suggestions as to the potential CD status of cases in the group under investigation.

Several proteins in serum/plasma have previously been suggested as potential blood-based CD markers, for example regenerating gene I $\alpha$  protein and intestinal fatty acid binding protein [33,34]. Recently, Galatola *et al.* proposed a discriminant model based on the expression of four genes including *REL* and *TNFAIP3* [35], which along with *TNFSF13B* and *TNFRSF9* (identified as potential CD markers in the current study) are involved in regulation of the NF- $\kappa$ B complex [32,36,37]. Furthermore, the NF- $\kappa$ B complex is involved in the IFN- $\beta$  induced transcription of *CXCL11* [38], and *REL* is involved in the transcriptional activation of *TNFSF13B* [26]. This indicates that additional investigations into NF- $\kappa$ B interacting proteins could reveal new potential markers for diagnosis and monitoring of CD.

## Conclusions

The CD markers identified in this study further emphasize the significance of components related to NF- $\kappa$ B regulation in relation to CD. However, the diagnostic relevance of *CXCL11*, *TNFSF13B*, *TNFRSF9*, and other NF- $\kappa$ B interacting proteins recognized by pathway analysis needs to be further investigated.

## Human genes

The human genes discussed in this article are presented in Additional file 1.

## Additional files

**Additional file 1: Genes selected for messenger RNA (mRNA) and/or protein detection in human blood.** Protein/mRNA levels were investigated in cases with active celiac disease (CD), cases with confirmed CD and normalized histology, cases without a CD diagnosis, and cases under investigation for suspected CD. Some genes were selected based on their context; others were selected based on information from published studies.

**Additional file 2: Descriptive data on differentially expressed potential blood-based celiac disease (CD) markers.** Study subjects are divided into groups depending on the diagnosis and the histopathologic assessment.

## Abbreviations

CD: Celiac disease; TG2: Tissue transglutaminase; DGP: Deamidated gliadin; GL: Native gliadin; IELs: Intraepithelial lymphocytes; anti-TG2: Autoantibodies against tissue transglutaminase; ESPGHAN: European Society of Paediatric Gastroenterology, Hepatology and Nutrition; ULN: Upper limit of normal; mRNA: Messenger RNA; anti-DGP: Antibodies against deamidated gliadin; anti-GL: Antibodies against native gliadin; C<sub>q</sub>: Quantification cycle; ROC: Receiver operating characteristic; CR: Central range; AUC: Area under the curve.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

JS conceived of the study, and all authors participated in the study design. HB, UJ, and JS collected material for the study. HB carried out all the laboratory work. HB, JS, and MF contributed to data analysis and

interpretation. HB drafted the manuscript, with help from all other authors, especially JS. All authors read and approved the final manuscript.

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