Gene expression profiling discriminates biopsies

– in paediatric patients investigated for suspected celiac disease

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Background and aim

Celiac disease (CD) is an inflammatory disease, triggered by an immunologic response to gluten in wheat, which manifests itself by symptoms such as diarrhoea, abdominal pain and malabsorption. CD results in an inflamed small intestine with varying degree of villous atrophy, crypt hyperplasia, increased intraepithelial lymphocytes and increased paracellular permeability of the intestinal epithelium. According to current guidelines, the diagnosis of CD requires intestinal biopsy and is set by histopathological findings. The aim of this study was to investigate the possibility of establishing molecular classifiers using gene expression data.

Results

Sixteen of the investigated genes were differentially expressed (healthy vs. Marsh type 2–3):
all 5 villi markers
3 crypt markers
8 apical junctional complex (AJC) genes

All of the differentially expressed genes except two of the crypt markers exhibited higher expression (1.4–3.6-fold) in healthy biopsies. The two crypt markers that departed from this expression pattern exhibited a 0.61- and 0.72-fold, respectively, lower expression in healthy biopsies compared to those of Marsh type 2–3. Based on the degree of intestinal mucosal lesion classified according to Marsh criteria, principal component analysis (PCA) (Fig. 1) and cluster analysis (Fig. 2) resulted in the following three relevant groups: healthy /Marsh type 0/Marsh type 1, Marsh type 2, and Marsh type 3.

Materials and methods

Unselected paediatric patients investigated for suspected CD were consecutively included in the study. Small intestinal biopsies were obtained from four healthy individuals, ten with CD (histopathologically Marsh 1–3C) and one biopsy after introduction of gluten-free diet. Total RNA was isolated using the RNeasy Protect Mini kit (Qiagen), and RNA integrity examined using the Agilent 2100 Bioanalyzer (Agilent Technologies). For each sample RNA was reverse transcribed (RT) as duplicates using the High Capacity cDNA reverse transcription kit with RNase inhibitor (Applied Biosystems). Gene expression was investigated by means of Taqman Gene Expression assays (Applied Biosystems) and the TaqMan® Fast Universal PCR Master Mix (Applied Biosystems) using a 7500 Fast real-time PCR instrument (Applied Biosystems).

Conclusions

Preliminary data indicate that gene expression profiling of intestinal biopsies is a feasible approach to classification and that this may prove useful in establishing the diagnosis.

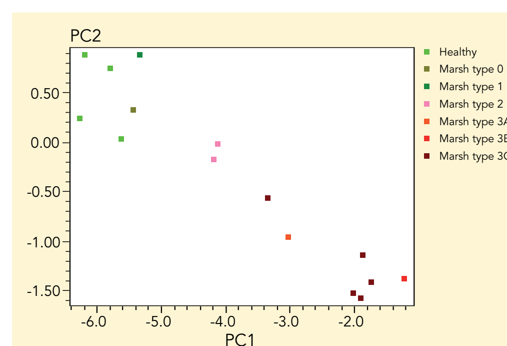

Figure 1. PCA plot.

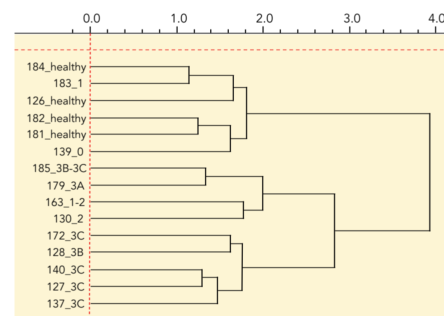

Figure 2. Hierarchical clustering.

A total of 35 genes were analysed: 2 reference genes, 5 villi markers, 11 crypt markers and 17 AJC genes. Relative quantities were determined after averaging of RT repeats and normalization using two reference genes that exhibited low sample-to-sample variation (M-value of 0.19 according to the geNorm algorithm), and differential expression was statistically investigated using Mann-Whitney ($p<0.05$, uncorrected for multiple testing) (Genex, MultiD Analyses). Sample classification based on gene expression profiling was explored by means of PCA and hierarchical clustering (Genex, MultiD Analyses). The study was conducted under approval by the regional ethics committee.

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