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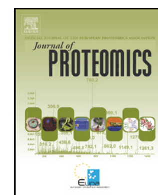

Identification of serum proteome signature of irritable bowel syndrome: Potential utility of the tool for early diagnosis and patient's stratification

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ABSTRACT

Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder with high incidence, and great heterogeneity of symptoms. Numerous factors are correlated with IBS development; however, the pathophysiology is not yet clear. In addition, there is no appropriate diagnostic tool available. The aim of this study was the identification of protein expression alterations in IBS patients compared to healthy individuals. Serum samples from 30 IBS patients (10 with IBS-Diarrhea, 10 IBS-Constipation and 10 IBS-Mixed) and 10 healthy individuals were subjected to proteomic analysis by 2-dimensional gel electrophoresis. Following evaluation of densitometrical data, protein spots exhibiting differential expression among the groups, were further characterized by matrix-assisted laser desorption tandem time-of-flight mass spectrometer and the results were confirmed by Western blot analysis. Eight significantly different expressed proteins were identified. Seven of them were overexpressed in IBS cases and only one was overexpressed in healthy individuals. These proteins were also differently expressed between the three IBS subgroups. IBS-D group overexpressed immunoglobulin light chain Lambda (LAC3) and apolipoprotein E (APOE), IBS-C group overexpressed apolipoprotein H (APOH) and collagen alpha-1 (XIV) chain (COEA1), and IBS-M group and healthy individuals overexpressed retinol-binding protein 4 (RET4). Our results show a different serum protein profile of IBS patients compared to healthy controls. Understanding the role of these eight proteins which are differently expressed in IBS patients, may contribute to a better clarification of IBS pathogenesis and to patient's stratification.

Significance: Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder with high incidence and great heterogeneity of symptoms without any appropriate diagnostic tool available. Eight significantly different expressed proteins were identified. Seven of them were overexpressed in IBS cases and only one was expressed in healthy individuals. These proteins were also differently expressed between the three IBS subgroups. Our results show that there is a different serum proteome signature in IBS compared to healthy individuals, as well as in IBS subgroups that could be used in the future for patient's stratification and as a diagnostic tool.

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1. Introduction

Irritable bowel syndrome (IBS) is a gastrointestinal disorder with high prevalence in the general population (9%–23%) and a wide range of clinical presentations [1–3]. IBS symptoms are abdominal pain or discomfort, bloating, diarrhea or constipation. The characterization of IBS subtypes is based on symptoms [4] and is categorized as: IBS with pain or discomfort and predominant constipation (IBS-C), IBS with diarrhea (IBS-D), mixed (alternating) diarrhea and constipation IBS (IBS-M) and unclassified IBS (IBS-U) [3]. The pathophysiology of IBS is not yet well understood because of the multifactorial nature of the mechanisms involved in disease development [5,6]. Visceral hypersensitivity, functional brain alterations, psychiatric factors such as anxiety, genetic alterations, diet and gender are only some of the factors which are involved in pathogenesis of IBS [7–11].

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Symptom-based diagnostic criteria hold several limitations due to the overlap with other functional disorders. Taking into consideration that no diagnostic tools are available, the development of appropriate diagnostic methods will lead to a better understanding of the pathophysiology of IBS, as well as to potential tools for early diagnosis and patient's stratification, in order to personalized medical services in the field [12]. The discovery of specific and sensitive biomarkers, which are able to distinguish between patients with different disease etiology, will lead to a better understanding of IBS, more specific treatment and evaluation of treatment effect. To achieve this, studies need to group patients according to the etiology of the disease, otherwise they will not provide valuable results in order to "identify the right patients treating them with the right medication and the right dose at the right time point" [65].

Recently, genetic analysis has revealed several potential genetic markers. A genome-wide association (GWA) study identified single nucleotide polymorphisms (SNPs) in *KDELR2* and *GRID2IP* which are associated with altered expression of these genes [13]. Moreover, the discovery of lost-of-function mutations in *SCN5A* and polymorphisms in *TRPM8* provides better understanding of the pathogenic mechanism(s) of IBS and potential therapeutic targets [14,15]. Finally, another recent study associated the increased copy numbers of *TPSAB1* with increased basal serum tryptase levels [16]. However, even if all these findings suggest a potential underlying pathogenetic mechanism, none of these genetic markers has proved to be specific and sensitive enough for clinical application.

In the last decades, proteomic techniques, mainly mass spectrometry (MS), are often used in order to elucidate disease pathophysiology and protein expression alterations. Proteomics have greatly contributed to the discovery of new biomarkers in several diseases, such as inflammatory bowel disease and cancer, by screening blood, urine, saliva and tissues [17–21]. A disease specific biomarker panel needs to have diagnostic value in reflecting the progress of a disease or the response to therapy and has to be non-invasive. However, despite the extensive use of proteomics in biomarker identification in other diseases, only a few studies have been performed in the field of IBS [22–24]. Most of these studies used tissue or fecal samples [24–27]. Goo et al. [23] used urine proteomic analysis to identify possible proteins implicated in IBS. Their results revealed an elevated expression of gelsolin (GSN) in female IBS patients with abdominal pain and higher trefoil factor 3 (TFF3) levels compared to healthy controls. However, up to now no specific serum or urine proteomic signature of IBS has been reported and applied in clinical practice. In the current study, we aimed to characterize serum proteomic features, as a minimally invasive platform for proteomic biomarkers, in patients with IBS.

2. Material and methods

2.1. Patients

Thirty adult IBS patients fulfilling the Rome III diagnostic criteria for IBS [3] and 10 healthy volunteers, who were attending the Outpatient Clinic of "Evangelismos" General Hospital and the "Aretaieio" University Hospital, were enrolled in this study. Patients and controls gave written informed consent. The study was approved by the ethical committee in the participating hospitals. Specifically this study enrolled 10 native Greek patients with IBS-D, 10 patients with IBS-C, and 10 patients with IBS-M [3]. The control group (n = 10) consisted of healthy individuals matched for age and gender to the patient population. The healthy status of the controls was assessed by a detailed medical history, thorough physical examination, including regular laboratory tests prior to study enrollment, which confirmed the absence of any chronic gastrointestinal or psychological disease. Patient's clinical characteristics are presented in Table 1.

Table 1
Characteristics of patients with irritable bowel syndrome and healthy controls.

	IBS-D (n = 10)	IBS-C (n = 10)	IBS-M (n = 10)	Controls (n = 10)
Age (years)				
18–30	1 (10%)	1 (10%)	1 (10%)	1 (10%)
31–40	1 (10%)	1 (10%)	3 (30%)	3 (30%)
41–50	1 (10%)	2 (20%)	1 (10%)	2 (20%)
>51	7 (70%)	6 (60%)	5 (50%)	5 (50%)
Gender				
Male	2 (20%)	1 (10%)	3 (30%)	2 (20%)
Female	8 (80%)	9 (90%)	7 (70%)	8 (80%)
Smoking				
Yes	4 (40%)	4 (40%)	5 (50%)	4 (40%)
No	6 (60%)	6 (60%)	5 (50%)	6 (60%)
Family history of IBS				
Yes	2 (20%)	2 (20%)	1 (10%)	0
No	8 (80%)	8 (80%)	9 (90%)	10 (100%)

2.2. Proteomic analysis

One blood sample was taken from each IBS patient and healthy individual in tubes contained EDTA. Following centrifugation, sera were collected and stored at −80 °C until use. The sera protein content determination, and the experimental procedure of two-dimensional gel electrophoresis (2-DE), was performed as previously described [28,29].

Gel images were scanned in a GS-800 Calibrated Densitometer (Bio-Rad), and stored on a PC for further analysis.

2.3. Image analysis

Protein spots from all gel analyses were detected, aligned, matched, and quantified using the PD-Quest v8.0 image processing software (Bio-Rad), according to the manufacturer's instructions, as previously described [28].

2.4. Protein identification by mass MALDI-TOF-MS

For Matrix-Assisted Laser Desorption Tandem TOF Mass Spectrometer (MALDI-TOF-MS) analysis, protein spots of interest were manually annotated using the Melanie 4.02 software and excised from 2-DE gels using a Proteiner SPII instrument (Bruker Daltonics, Bremen, Germany) [28,30].

2.5. Western blot analysis

Equal amounts of proteins extracted from plasma samples were separated by 12% SDS-PAGE. Following electrophoresis, proteins were electrotransferred to Hybond-ECL nitrocellulose membranes (Amersham Biosciences, Uppsala, Sweden) and blots were blocked for 1 h at room temperature with 3% nonfat milk in PBS. The monoclonal antibodies against apolipoprotein E (APOE, clone 2B5), plasma retinol-binding protein (RETBP, clone F-12), and clusterin (CLUS, clone L-20) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), diluted 1:500 in blocking buffer were used. Blots were developed using the ECL Western blotting detection system (Amersham Biosciences, Uppsala, Sweden). Mean protein quantification after Western blot analysis was performed by three independent experiments, designed to contain per blot 4 plasma samples from healthy individuals and IBS patients, randomly selected.

2.6. Statistical analysis

To ensure confidence in our experimental approach, we employed a design which involved duplicate 2-DE gels per sample (i.e., to determine

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