JOURNAL OF PROTEOMICS 98 (2014) 65–78

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Comparative proteomic study in serum of patients with primary open-angle glaucoma and pseudoexfoliation glaucoma☆

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ARTICLE INFO

Article history:
Received 5 November 2013
Accepted 9 December 2013
Available online 16 December 2013

Keywords:
Glaucoma
Serum
Equalization
Biomarkers
2D-DIGE
Networks analysis

ABSTRACT

Alterations in the sera proteins between patients with Primary Open-Angle Glaucoma (POAG), Pseudoexfoliation Glaucoma (PEXG), and healthy controls were identified through a proven approach utilizing equalization of high-abundance serum proteins with ProteoMiner™, two-dimensional fluorescent difference gel electrophoresis (2D-DIGE), MALDI-TOF/TOF, and nanoLC-MS-MS. Quantitative immunoassays of the 17 most-differentially-altered proteins identified in this analysis confirmed that they were also over expressed in the intact serum of newly recruited glaucoma patients. Overall, this report identifies a panel of candidates for glaucoma biomarkers and supports their further validation in large population studies. Additionally, functional pathway analysis of these candidate proteins suggested that they are part of a network linked to regulating immune and inflammatory-related processes. The data have been deposited to the ProteomeXchange with identifier PXD000198.

Biological significance
POAG and PEXG are major causes of age-related blindness in the world; however, treatment can be very effective if they are identified early on in the progression. Genetic linkage studies can only explain a limited number of cases, suggesting that these forms of glaucoma are multigenic in nature. Other important factors, such as modifier genes, epigenetic influences, environ-

Abbreviations: CA, correct assignment; CyDye, cyanine dyes; IPA, ingenuity pathway analysis; IOFV, Institute of Ophthalmology Fernandez-Vega; IOP, intraocular pressure; PEXG, pseudoexfoliation glaucoma; POAG, primary open-angle glaucoma; ROC, receiver operating characteristic; SNPs, single nucleotide polymorphisms.

☆ Financial disclosure: This study has been supported in part by a CENIT-CeyeC research grant CEN-20091021 from the Spanish Ministry of Innovation and Development, Fundación de Investigación Oftalmológica Fernández-Vega (http://fio.fernandez-vega.com), Fundación Mª Cristina Masaveu Peterson (http://www.fundacioncristinamasaveu.com), Fundación Rafael del Pino (http://www.frdelpino.es), and Cooperative Research Network on prevention, diagnosis and treatment of prevalent, degenerative and chronic eye diseases, Instituto de Salud Carlos III (RD07/0062/0014 and RD12/0034; http://www.retics.net). Miguel Coca-Prados is "Catedrático Rafael del Pino en Oftalmología" in the "Fundación de Investigación Oftalmológica, Instituto Oftalmológico Fernández-Vega" Oviedo, Spain. The authors declare that the results reported in this manuscript may be of commercial interest to Instituto Oftalmológico Fernández-Vega, Oviedo, Spain.

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1874-3919/$ – see front matter
http://dx.doi.org/10.1016/j.jprot.2013.12.006

mental and dietary agents, and inflammatory and oxidative effects are also believed to affect the development of these diseases. The characterization of metabolic and/or proteins changes, for example in bodily fluids, before the clinical manifestation of glaucoma is of considerable relevance for its early diagnosis. In the present work, identification of over-expressed proteins in serum of glaucoma patients (POAG and PEXG) linked to immune and inflammatory processes supports the finding that changes in these pathways also manifest systemically in patients with these pathologies. This study provides a new basis to validate the identified proteins as biomarkers of glaucoma in a large-scale-multiplexed screening in sera.

1. Introduction

A leading cause of blindness worldwide, glaucoma encompasses a complex group of neurodegenerative disorders that are multigenic and multifactorial in origin, but are all characterized by progressive degeneration of the optic nerve, retinal ganglion cell death and the loss of the visual field. Primary Open-Angle Glaucoma (POAG) and Pseudoexfoliation Glaucoma (PEXG) are among the most prevalent types of glaucoma in developed countries. Whereas an abnormal elevation in the intraocular pressure (IOP) is the best known risk factor associated with the pathogenesis and progression of POAG, the presence of pseudoexfoliation syndrome is the most common identifiable cause of PEXG, a secondary form of glaucoma. The number of people worldwide with glaucoma has been estimated to reach 80 million in 2020 [1]. In certain regions of the world, including the northwest of Spain (i.e., Asturias and Galicia) and some provinces of Saudi Arabia, the prevalence of PEXG among patients with pseudoexfoliation syndrome can reach up to 30% [2].

The increased IOP observed among POAG subjects is usually associated with a dysfunction/obstruction of the normal exit of the aqueous humor fluid through the outflow system (i.e., trabecular meshwork). In PEXG there is an excessive production and progressive accumulation of fibrillar aggregates from all tissues of the anterior segment of the eye (i.e., corneal endothelium, iris, lens capsule, ciliary epithelium) and deposition of it on the anterior chamber structures (i.e., trabecular meshwork) leads to reduced outflow and thus to elevated IOP [3,4]. Additional clinical differences between POAG and PEXG include: (i) a more elevated IOP in PEXG patients than those with POAG; (ii) a lack of an IOP response to steroids among PEXG patients when compared to those with POAG; and (iii) quantitative histological differences of cross-sections of the optic nerve [5–7].

The frequency of onset and rate of optic degeneration in both POAG and PEXG increase with age; however, the prognosis of blindness is greater among PEXG. Among POAG patients, usually both eyes are affected, but two-thirds of PEXG patients present unilateral disease, and the chance of developing glaucoma in the fellow eye is 50% in 15 years [8]. In most cases of glaucoma, degeneration of the optic nerve head precedes detectable field loss, and it has been estimated that by the time early visual field defects are found, 25% to 35% of retinal ganglion cells may already be irrevocably lost [9]. Therefore, successfully treating glaucoma will require methods for earlier detection, thereby preventing disease progression.

In spite of the large number of linkage studies, candidate gene reports, and genome-wide association investigations conducted on POAG and PEXG populations worldwide, only genes with limited population effects have been discovered. These studies have shown, for instance, the limited involvement in POAG of *MYOC* (myocilin) [10–12] and rare variants of *CYP1B1* (Cytochrome P450 1B1) [13,14], and that the discovery of two non-synonymous single-nucleotide polymorphisms (SNPs) in the *LOXL1* (lysyl oxidase-like 1) gene are not always associated with PEXG [15]. On the other hand, investigations conducted in the field of differential proteomics in search for molecular biomarkers of glaucoma have been limited. The search for protein biomarkers in readily available biological fluids (those that do not require invasive surgery to obtain) may be beneficial in the diagnosis and management of glaucoma [16].

In the present work, we carried out a comparative differential proteomic analysis of blood serum from patients with POAG, PEXG, and healthy controls. The aim was to identify, in "equalized" serum, proteins that are quantitatively altered in glaucoma patients, and determine whether said proteins are significantly discriminatory between glaucoma patients and healthy subjects with reproducible results in "non-equalized" serum samples from newly recruited glaucoma patients.

We employed many techniques in a comprehensive proteomic workflow including equalization of high-abundance serum proteins with ProteoMiner™, two-dimensional difference gel electrophoresis (2D-DIGE) analysis, and protein identification by matrix assisted laser desorption/ionization time-of-flight/time of flight (MALDI-TOF/TOF) and nano-liquid-chromatography tandem mass spectrometry (nLC-MS/MS). This workflow yielded the identification of a panel of 35 proteins that were detected at different levels in POAG, PEXG and healthy subjects. Alterations in the top-17-ranked proteins of the 35-protein panel were verified by quantitative immunoassays such as enzyme-linked immunosorbent assay (ELISA). Lastly, bioinformatic analysis of the 35-protein panel revealed that they are linked to a network significantly enriched in immune- and inflammatory-related pathways. The present findings create an approach to validate the 35-protein panel as potential serum biomarkers for the clinical prediction, prognosis, diagnosis and monitoring of POAG and PEXG cases at large scale.

2. Materials and methods

2.1. Study design

The procedures used in the present study are grouped into two steps, outlined here, described in detail below, and summarized in Fig. 1.