Proteomics of Vitreous Humor in Age-related Macular Degeneration

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PURPOSE
Age-related Macular Degeneration (AMD) is the major cause of central vision loss among the elderly in Western countries [1]. It is a multifactorial disease involving a complex and poorly perceived interplay of genetic, environmental, metabolic and functional factors. Therefore, the pathogenesis remains partially understood and further research is needed [2]. We performed a clinical experimental study to identify potential future biomarkers and new targets of therapy. The study procedure is depicted in Figure 1.

1. Vitreous humor
2. Mass spectrometry
3. Statistical analysis

SPSS Version 21.0 * WEB-based GEnEx SetAnalysis Toolkit

Figure 1. Study procedure
CE-MS = capillary electrophoresis coupled to time-of-flight mass spectrometry. LC-MS/MS = liquid chromatography coupled to tandem mass spectrometry.

After tryptic digestion analysis was performed using capillary electrophoresis coupled online to a time-of-flight mass spectrometer (CE-MS), as well as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). The two mass spectrometry techniques employed in this study are used in a complementary fashion. Due to its high reproducibility, CE-MS is used for detection and semi-quantification of peptides while LC-MS/MS is used for the determination of peptide amino acid sequences.

RESULTS
We were able to analyze vitreous samples of 108 nAMD patients and 26 patients with idiopathic floaters. Epidemiological data of both groups are listed in Table 1. In our analysis 878 successfully sequenced peptides were detected, relating to 136 proteins. 50 proteins, composed of 116 peptides, were expressed significantly different between sample and control group (P<3.48E-4 to 4.91E-2), 42 proteins could be analyzed via WebGestalt; most frequent proteins of the three formed main categories are listed. Biological Process: response to stimulus (n=33), multidimensional organisal process (33), biological regulation (28). Cellular component: extracellular space (23), membrane-enclosed lumen (17), extracellular matrix (13). Molecular Function: protein binding (26), structural molecular activity (14), enzyme regulator activity (13). See Figure 2 / Table 2 for further information.

Table 1. Epidemiology

<table>
<thead>
<tr>
<th></th>
<th>N=</th>
<th>Age in years (mean±SD)</th>
<th>Male</th>
<th>Female</th>
<th>Right</th>
<th>Left</th>
<th>Phakic</th>
<th>Phakoduphagic</th>
</tr>
</thead>
<tbody>
<tr>
<td>nAMD</td>
<td>108</td>
<td>77±13.5</td>
<td>40</td>
<td>68</td>
<td>53</td>
<td>55</td>
<td>48</td>
<td>60</td>
</tr>
<tr>
<td>Controls</td>
<td>26</td>
<td>61.5±12.0</td>
<td>14</td>
<td>12</td>
<td>18</td>
<td>8</td>
<td>13</td>
<td>13</td>
</tr>
</tbody>
</table>

* N: Number of patients in each group, nAMD: Neovascular Age-related Macular Degeneration

Figure 2. Gene Ontology-Terms (GO-Terms)
GO-Term analysis of significant vitreous humor proteins (n=50) when comparing neovascular age-related macular degeneration (nAMD) and control samples. Distribution of GO-Terms is shown for the main categories "Biological Process", "Cellular Component" and "Molecular Function" created via WebGestalt (WEB-based Gene SetAnalysis Toolkit) and GO Slim Classification.

PROSPECT
GO-Term analysis is the first step of our research, that enables us to provide a general overview of changes in the proteome of vitreous humor in nAMD. We plan to perform a thorough statistical analysis considering clinical diagnosis groups of nAMD with the aim of identifying potential future biomarkers or even new targets of therapy. We will focus on the analysis of subgroups to filter the influence of bleeding on determination of potential biomarker candidates. Coupled with one of the highest sample numbers in literature we thus strive to generate new pathophysiological data.

Table 2. Gene Ontology-Terms (GO-Terms) of five sample proteins
The three most abundant GO-Terms in each main category are highlighted in red.

<table>
<thead>
<tr>
<th>Protein</th>
<th>IPI-ID</th>
<th>Cellular Component</th>
<th>Biological Process</th>
<th>Molecular Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-1-antitrypsin</td>
<td>IP00553177</td>
<td>CC4, CC5, CC6, CC10</td>
<td>BP1, BP2, BP9, BP10, BP11</td>
<td>MF2, MF5</td>
</tr>
<tr>
<td>Antithrombin-III</td>
<td>IP00023179</td>
<td>CC5, CC8</td>
<td>BP1, BP2, BP10, BP11</td>
<td>MF2, MF3, MF5</td>
</tr>
<tr>
<td>Apolipoprotein E</td>
<td>IP00021842</td>
<td>CC1, CC2, CC3, CC5, CC6, CC7, CC8, BP2, BP3, BP4, BP5, BP6, BP7, BP8, BP9, BP10, BP11</td>
<td>MF1, MF2, MF3, MF4, MF5, MF6</td>
<td></td>
</tr>
<tr>
<td>Complement C3</td>
<td>IP00783987</td>
<td>CC5, CC8</td>
<td>BP1, BP2, BP4, BP6, BP9, BP10, BP11</td>
<td>MF2, MF5</td>
</tr>
<tr>
<td>Kininogen-1</td>
<td>IP00092459</td>
<td>CC5, CC8, CC10</td>
<td>BP1, BP5, BP8, BP9, BP10, BP11, MF1, MF2, MF5</td>
<td></td>
</tr>
</tbody>
</table>

CC1 = cell projection, CC2 = cytokoelel, CC3 = endosome, CC4 = extracellular matrix, CC5 = extracellular space, CC6 = Golgi apparatus, CC7 = macromolecular complex, CC8 = membrane, CC9 = membrane-enclosed lumen, CC10 = vesicle
BP1 = biological regulation, BP2 = cell communication, BP3 = cell proliferation, BP4 = cell component organization, BP5 = death, BP6 = developmental process, BP7 = growth, BP8 = localization, BP9 = metabolic process, BP10 = multidimensional organisal process, BP11 = response to stimulus
MF1 = antioxidant activity, MF2 = enzyme regulator activity, MF3 = ion binding, MF4 = lipid binding, MF5 = protein binding, MF6 = transporter activity

CONCLUSION
In this clinical-experimental study with one of the highest number of nAMD samples in the literature we could identify proteins, that were associated with different vascular retinal diseases before [4,5]. Response to stimulus, multidimensional organisal process and biological regulation are the most common subgroups when subdividing the detected significant proteins regarding their biological processes. Therefore, proteins assigned to these subgroups may have an important function in the pathogenesis of nAMD.

REFERENCES
Vitreous samples of patients with neovascular age-related macular degeneration (nAMD, sample group) or idiopathic floaters (control group) were analyzed. After tryptic digestion analysis was performed using capillary electrophoresis coupled online to a time-of-flight mass spectrometer (CE-MS), as well as liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

Significant peptides were identified by comparing signal intensity of nAMD-samples and controls and were matched to corresponding proteins. Gene Ontology-Terms (GO-Terms) were assigned to every IPI number via WebGestalt (WEB-base Gene SeT AnaLysis Toolkit).
In our analysis 878 successfully sequenced peptides were detected, relating to 136 proteins. 50 proteins, composed of 116 peptides, were expressed significantly different between sample and control group ($P=3.48E-8$ to $4.91E-2$).

42 proteins could be analyzed via WebGestalt; most frequent GO-Terms of the three formed main categories are listed. Biological Process: response to stimulus ($n=33$), multicellular organismal process (33), biological regulation (28). Cellular component: extracellular space (23), membrane-enclosed lumen (17), extracellular matrix (13). Molecular Function: protein binding (26), structural molecule activity (14), enzyme regulator activity (13).
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