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SHORT COMMUNICATION

A proteomic signature associated to atypical antipsychotic response in schizophrenia patients: a pilot study

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Abstract

A major hurdle faced by most schizophrenia patients is the poor efficacy of current antipsychotic medications. This stems from a poor understanding of the underlying pathophysiology and the lack of biomarkers for the prediction of a positive medication response. By employing state-of-the-art proteomic analysis of blood plasma from 58 patients who were either drug-naive or drug-free at the time of sample collection, we identified potential biomarkers that were predictive of a positive response after 6 weeks of treatment with antipsychotics. Complement and coagulation cascades were the most over-represented biological pathways among these proteins, consistent with the importance of these processes in schizophrenia. Although preliminary, these findings are novel and may drive future efforts in the development of predictive tests for medication efficacy and thereby have a positive influence on disease outcome.

Keywords Biomarkers · Drug response · Atypical antipsychotics · Proteins · Proteome

As an incurable disorder, schizophrenia demands expensive healthcare. A considerable part of this expense is due to the fact that patients do not respond properly to the administration of antipsychotic medications, the main method of disease management [1].

One of the reasons for failed antipsychotic response involves the fact that schizophrenia is a multivariate disorder. Treatment success varies among patients as up to 40% of schizophrenia patients do not respond properly to treatment and approximately 2/3 of patients abandon antipsychotic medication due to undesirable side effects [2]. Males are generally diagnosed with schizophrenia earlier than females at an average ages of 18 compared to 25, with severe effects on their apex intellect due to impairments of their cognitive capacities, making them less capable of study or work.

Newer and more effective medications must be developed. However, little progress has been made in this regard currently. Another option is to better characterize the response of patients to antipsychotic medications at the molecular level. While this has been attempted recently [3], we still lack suitable biomarker candidates that can be used to predict outcome and guide treatment-based decisions made by the attending physicians. This can be achieved by proteomic profiling of an easily accessible body fluid such as serum or plasma prior to treatment initiation, which can be used to link the levels of specific analytes with the outcome [4, 5].

Several proteomic studies in samples collected in vivo or postmortem from schizophrenia patients have been performed [6, 7] but none of these have investigated patient responses to medication to any significant depth [8]. In addition, only recently potential biomarker candidates have been

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proposed to predict a successful medication response using lipid-based molecules [9].

Here, we have assessed the plasma proteomes collected from living patients, employing state-of-the-art mass spectrometry-based proteomics. All patients were either drug naïve ($n=22$) or drug-free for at least 6 weeks prior to the study ($n=36$). They were sampled at baseline and then given the atypical antipsychotic drugs olanzapine ($n=18$), quetiapine ($n=14$), and risperidone ($n=26$) (Supplementary Table 1). The proteomes were then analyzed, focusing on revealing proteins that might serve as biomarker candidates to predict an effective response to the medication. Details on sample collection and preparation were as described in Martins-de-Souza et al. [3]. This collection was approved by ethics committee of the University of Magdeburg and procedures carried out in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Independently of the medication taken, patient samples were divided into two groups, which were good responders (GR) and poor responders (PR), with GR defined as $\geq 50\%$ reduction of the total Positive and Negative Syndrome Scale (PANSS) scores, as described previously [3].

Baseline plasma samples were depleted of the top 14 most abundant proteins using the MARS-14 immunodepletion system liquid chromatography (Agilent; Wokingham, UK) with a 4.6×100 mm Mars Hu14 column as previously described [10]. This procedure eliminates approximately 94% of the total protein mass as albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3 and transthyretin. The remaining protein fraction was treated consecutively with 5 mM dithiothreitol (30 min, room temperature) and 10 mM iodoacetamide (30 min, 60 °C in the dark). The samples were grouped as GR ($n=36$) and PR ($n=22$) and plasma proteins were digested using trypsin (Promega, Heidelberg) at a trypsin:protein ratio of 1:50 for 16 h at 37 °C. The peptides resulting from the trypsin digestion were analyzed using a 2D-RP/RP Acquity UPLC M-Class System (Waters Corporation) coupled to a Synapt G2-Si mass spectrometer (Waters Corporation) platform operated in data-dependant acquisition (DDA) mode.

Mass spectrometry data were processed using the MASCOT search engine for protein identification and MASCOT Distiller for label-free quantification. Cut-off criteria were: (1) identification scores above the cutoff automatically determined by MASCOT algorithm considering the current dataset; (2) at least 2 unique peptides for protein identification and quantification; and (3) at least 5 MS/MS spectra for quantification. In addition, according to previous method optimizations involving sample dilutions that we performed, the label-free method applied is not precise in the quantification of changes above fivefold. Thus, proteins above (upregulation) or below (downregulation) fivefold, were listed in Table 1 as >5 or <5, respectively, considering we observed experimentally that changes over fivefold are not accurately measured by the present quantification method (data not shown).

Mass spectrometry-based proteome analysis resulted in the detection of more than 18,000 peptides, resulting in the identification of almost 1000 proteins. The ratiometric comparison GR/PR resulted in identification of 44 proteins that were differentially expressed of which 24 were present at higher levels and 20 at lower levels in baseline samples from patients who later showed a good response to treatment (Table 1). These proteins ranged across 6 known general biological processes (Fig. 1). Of note, 30% of the differentially expressed proteins are still of unknown function, opening roads to discover new biology involved in antipsychotic treatment response.

Uploading the 44 Uniprot codes into the Reactome Pathway database (https://reactome.org/) revealed (Fig. 2a) the involvement of these proteins in several essential biological processes such as immune system (Fig. 2b) and signaling transduction (Fig. 2c). Previous studies aimed at identifying serum biomarkers found changes in biomarker candidates associated with the same pathways in first onset schizophrenia patients [4]. In addition, other processes were represented such as cell cycle, vesicle-mediated transport, protein metabolism and developmental biology (Fig. 2a). Considering statistical parameters, we found that 27 of the differentially expressed proteins between GR and PR could be mapped onto more specific pathways (Table 2). This revealed that the most over-represented pathways were the

Table 1 Proteins sampled at baseline associated with a 50% reduction in PANSS scores after 6 weeks of treatment

Entry	Entry name	Gene name	Ratio good/poor resp	Reg. in good resp	Unique peptides	Description	Biological process	Molecular class	Molecular function
O00622	CYR61_HUMAN	CYR61	> 5	Up	2	Protein CYR61	Cell communication and signaling	Extracellular matrix protein	Extracellular matrix structural constituent
Q6F5E8	LR16C_HUMAN	RLTPR	> 5	Up	2	Leucine-rich repeat-containing protein 16C	Cell communication and signaling	Unclassified	Molecular function unknown
O94887	FARP2_HUMAN	FARP2	> 5	Up	2	FERM, RhoGEF and pleckstrin domain-containing protein 2	Cell communication and signaling	Cytoskeletal protein	Structural constituent of cytoskeleton
Q5THR3	EFCB6_HUMAN	EFCAB6	4.69	Up	2	EF-hand calcium-binding domain-containing protein 6	Cell communication and signaling	Calcium binding protein	Calcium ion binding
P01019	ANGT_HUMAN	AGT	1.76	Up	19	Angiotensinogen	Cell communication and signaling	Peptide hormone	Peptide hormone
P35858	ALS_HUMAN	IGFALS	1.52	Up	12	Insulin-like growth factor-binding protein complex acid labile subunit	Cell communication and signaling	Unclassified	Molecular function unknown
Q15063	POSTN_HUMAN	POSTN	0.44	Down	4	Periostin	Cell communication and signaling	Adhesion molecule	Cell adhesion molecule activity
Q16181	SEPT7_HUMAN	SEP7_	0.38	Down	2	Septin-7	Cell communication and signaling	Cell cycle control protein	Protein binding
P30291	WEE1_HUMAN	WEE1	0.21	Down	4	Wee1-like protein kinase	Cell communication and signaling	Dual specificity kinase	Protein threonine/tyrosine kinase activity
Q9UPZ6	THS7A_HUMAN	THSD7A	< 0.2	Down	2	Thrombospondin type-1 domain-containing protein 7A	Cell communication and signaling	Unclassified	Molecular function unknown
P62736	ACTA_HUMAN	ACTA2	2.55	Up	2	Actin, aortic smooth muscle	Cell growth and maintenance	Cytoskeletal protein	Structural constituent of cytoskeleton
Q92556	ELMO1_HUMAN	ELMO1	0.25	Down	5	Engulfment and cell motility protein 1	Cell growth and maintenance	Motor protein	Motor activity
Q96AW1	ECOP_HUMAN	VOPP1	0.23	Down	2	Vesicular, overexpressed in cancer, prosurvival protein 1	Cell growth and maintenance	Transcription regulatory protein	Transcription regulator activity
P02746	C1QB_HUMAN	C1QB	3.50	Up	2	Complement C1q subcomponent subunit B	Immune response	Complement protein	Complement activity
P02747	C1QC_HUMAN	C1QC	2.46	Up	3	Complement C1q subcomponent subunit C	Immune response	Complement protein	Complement activity
P00746	CFAD_HUMAN	CFD	2.34	Up	2	Complement factor D	Immune response	Serine protease	Serine-type peptidase activity
P0C0L4	CO4A_HUMAN	C4A	1.80	Up	162	Complement C4-A	Immune response	Complement protein	Complement activity

Table 1 (continued)

Entry	Entry name	Gene name	Ratio good/poor resp	Reg. in good resp	Unique peptides	Description	Biological process	Molecular class	Molecular function
Q04756	HGFA_HUMAN	HGFAC	2.75	Up	3	Hepatocyte growth factor activator	Protein metabolism	Serine protease	Serine-type peptidase activity
P00740	FA9_HUMAN	F9	2.37	Up	2	Coagulation factor IX	Protein metabolism	Coagulation factor	Extracellular matrix binding
P02743	SAMP_HUMAN	APCS	1.60	Up	9	Serum amyloid P-component	Protein metabolism	Secreted polypeptide	Binding
P01011	AACT_HUMAN	SERPINA3	1.56	Up	34	Alpha-1-antichymotrypsin	Protein metabolism	Protease inhibitor	Protease inhibitor activity
P03952	KLKB1_HUMAN	KLKB1	1.48	Up	14	Plasma kallikrein	Protein metabolism	Serine protease	Serine-type peptidase activity
P01023	A2MG_HUMAN	A2M	0.34	Down	3	Alpha-2-macroglobulin	Protein metabolism	Protease inhibitor	Protease inhibitor activity
O75592	MYCB2_HUMAN	MYCBP2	> 5	Up	2	Probable E3 ubiquitin-protein ligase MYCBP2	Reg. nucleic acid metab	Transcription regulatory protein	Transcription regulator activity
Q9UHC1	MLH3_HUMAN	MLH3	0.30	Down	2	DNA mismatch repair protein Mlh3	Reg. nucleic acid metab	DNA repair protein	Protein binding
Q9UL58	ZN215_HUMAN	ZNF215	0.22	Down	2	Zinc finger protein 215	Reg. nucleic acid metab	DNA binding protein	DNA binding
Q9UGN5	PARP2_HUMAN	PARP2	< 0.2	Down	2	Poly [ADP-ribose] polymerase 2	Reg. nucleic acid metab	DNA binding protein	Catalytic activity
Q7L014	DDX46_HUMAN	DDX46	< 0.2	Down	11	Probable ATP-dependent RNA helicase DDX46	Reg. nucleic acid metab	RNA helicase	Helicase activity
Q8IZQ8	MYCD_HUMAN	MYOCD	< 0.2	Down	2	Myocardin	Reg. nucleic acid metab	Transcription factor	Transcription factor activity
P21796	VDAC1_HUMAN	VDAC1	> 5	Up	2	Voltage-dependent anion-selective channel protein 1	Transport	Voltage gated channel	Voltage-gated ion channel activity
O14994	SYN3_HUMAN	SYN3	< 0.2	Down	2	Synapsin-3	Transport	Transport/cargo protein	Transporter activity
A6NNU9	SSX11_HUMAN	SSX11	> 5	Up	2	Protein SSX11	Unknown	Unclassified	Molecular function unknown
Q7Z6W1	TMCO2_HUMAN	TMCO2	> 5	Up	2	Transmembrane and coiled-coil domain-containing protein 2	Unknown	Integral membrane protein	Molecular function unknown
Q6IPU0	CENPP_HUMAN	CENPP	> 5	Up	2	Centromere protein P	Unknown	Unclassified	Molecular function unknown
A1L167	YE019_HUMAN	UBE2QL1	> 5	Up	2	Ubiquitin-conjugating enzyme E2Q-like protein 1	Unknown	Unclassified	Molecular function unknown

Table 1 (continued)

Entry	Entry name	Gene name	Ratio good/poor resp	Reg. in good resp	Unique peptides	Description	Biological process	Molecular class	Molecular function
Q6P4F2	ADXL_HUMAN	FDX1L	> 5	Up	2	Adrenodoxin-like protein, mitochondrial	Unknown	Unclassified	Molecular function unknown
Q8N485	LIX1_HUMAN	LIX1	1.57	Up	5	Protein limb expression 1 homolog	Unknown	Unclassified	Molecular function unknown
Q9H6S0	YTDC2_HUMAN	YTHDC2	0.39	Down	2	Probable ATP-dependent RNA helicase YTHDC2	Unknown	Unclassified	Molecular function unknown
Q6ZVN6	YO003_HUMAN	HSD47	0.37	Down	4	Uncharacterized protein HSD47	Unknown	Unclassified	Molecular function unknown
Q86TV6	TTC7B_HUMAN	TTC7B	0.31	Down	2	Tetratricopeptide repeat protein 7B	Unknown	Unclassified	Molecular function unknown
Q008S8	LFDH_HUMAN	ECT2L	< 0.2	Down	3	Epithelial cell-transforming sequence 2 oncogene-like	Unknown	Unclassified	Molecular function unknown
Q969T7	5NT3L_HUMAN	NT5C3B	< 0.2	Down	3	7-methylguanosine phosphate-specific 5′-nucleotidase	Unknown	Unclassified	Molecular function unknown
Q8WTR4	GDPD5_HUMAN	GDPD5	< 0.2	Down	2	Glycerophosphodiester phosphodiesterase domain-containing protein 5	Unknown	Unclassified	Molecular function unknown
Q5JU67	CI117_HUMAN	C9orf117	< 0.2	Down	2	Uncharacterized protein C9orf117	Unknown	Unclassified	Molecular function unknown

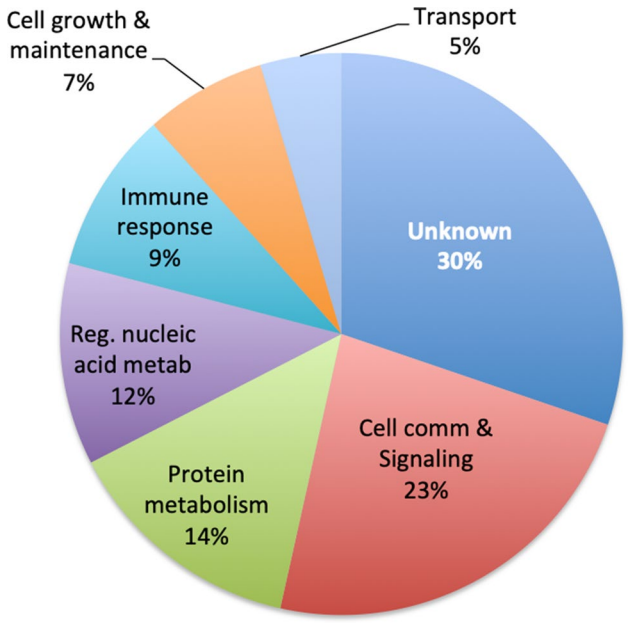

Fig. 1 Biological processes associated to the differentially expressed proteins between GR and PR

intrinsic pathway of fibrin clot formation, initial triggering of complement, formation of fibrin clot, and the complement cascade. The finding that proteins involved in the complement and coagulation cascades could be used to predict response is interesting considering that proteins associated with these pathways are known to be altered in first onset schizophrenia patients [11]. Genetic variations in complement 4 (*C4*) genes lead to alterations in the levels of *C4A* and *C4B* expression in the human brain in schizophrenia patients and pre-clinical models [12]. This may, in turn, lead to alterations in synaptic pruning [13] in a biological mechanism involving microglia [14]. In addition, a recent study showed that changes in complement proteins are associated with symptom severity [13]. Furthermore, complement and coagulation cascades were found to be altered in pre-symptomatic psychotic patients during childhood, in a study that revealed changes in blood proteins from children with increased risk for psychotic disorder at later stages [15].

The pathways listed in Table 2 were also represented in the analysis performed using the STRING database, highlighting the major role player involved in these processes (Fig. 3). This analysis can be accessed and modified in: https://string-db.org/cgi/network.pl?taskId=OtAC4S3s0ZTZ. We employed *kmeans clustering* available in STRING, which clustered proteins in four major groups involved in complement pathways (turquoise blue), structural proteins (yellow) and two clusters (green and red), which together play roles in signaling pathways, eventually involving hormones such as the renin–angiotensin system, bradykinin and non-cannonical complement pathways (Fig. 3). Regarding cellular localization in the STRING analysis, differentially expressed proteins were present in blood microparticle, extracellular space, membrane-bounded vesicle, extracellular exosome and extracellular region part, as expected from a blood proteome.

Our data propose for the first time protein biomarker candidates associated predominantly with the complement and coagulation cascades, which may be used to predict a successful antipsychotic response. The potential markers identified here were not able to distinguish the different medication used, as found by unsupervised PCA (data not shown), but may be useful to stimulate further research in this area. For example, certain protein patterns may be more predictive of successful outcomes using specific classes of antipsychotics. We are taking forward other tasks such as validation in larger and distinct cohorts and the development of a method to evaluate the potential markers in a targeted manner. Thus, the results of this manuscript should be taken as preliminary. Nevertheless, it is important to divulge this dataset to the community given the potential interest in clinical implementation and validation experiments being conducted elsewhere. These might be beneficial to patients in the future.

This is just the first step towards antipsychotic effectiveness prediction, which may avoid treatment drop out and inefficacy, helping to minimize the progression of disease severity and improve disease outcomes.

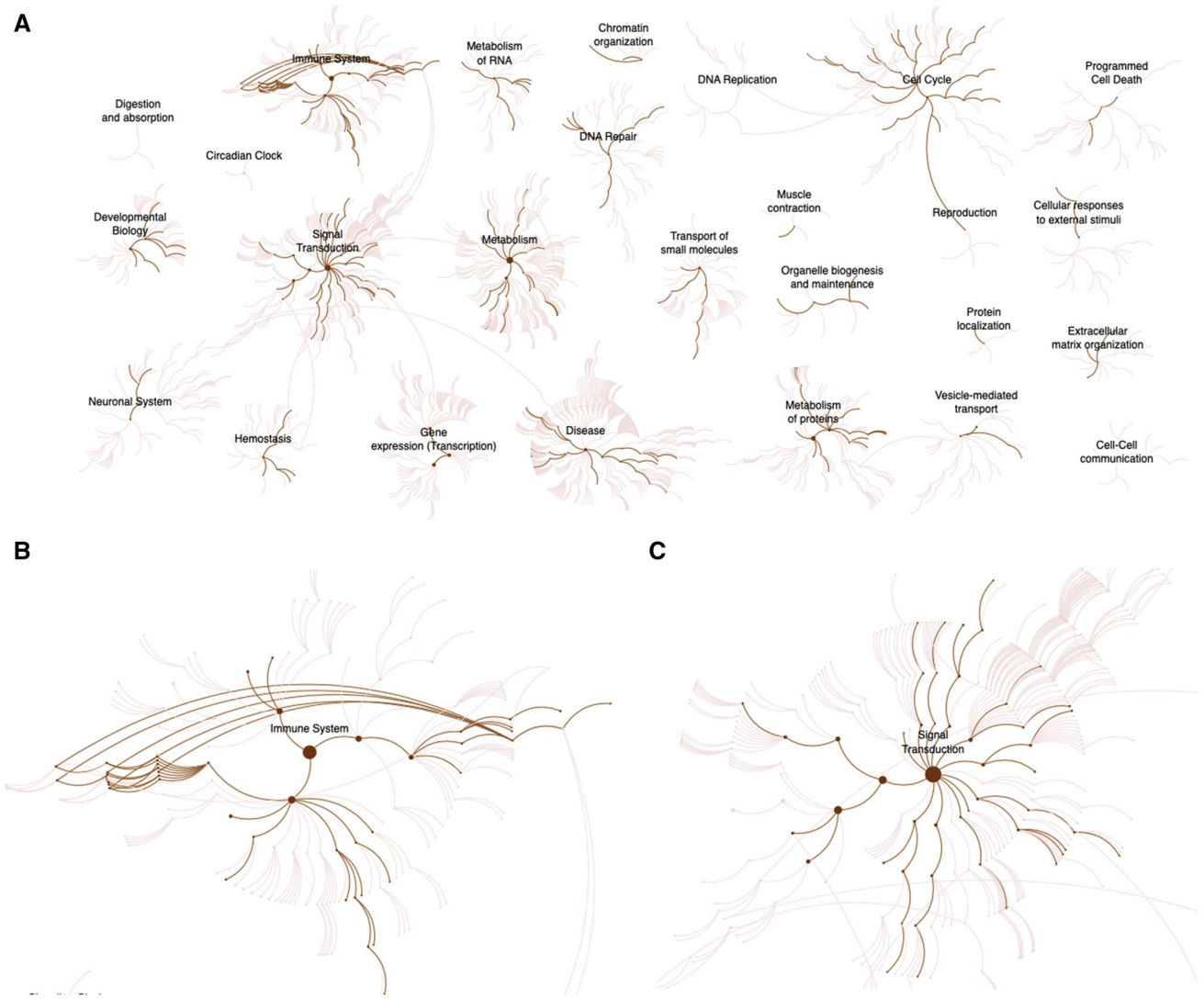

Fig. 2 **a** Biochemical pathways covered by the differentially expressed proteins between GR and PR according to Reactome; **b** detailed representation covered into Signal Transduction pathways; **c** detailed representation covered into Immune System pathways

Table 2 Most relevant pathways sorted by *p* value using the Reactome Pathway Analysis Report

Pathway name	Entities				Reactions	
	Found	Ratio	*p* value	FDR*	Found	Ratio
Intrinsic pathway of fibrin clot formation	3/41	0.002	4.7e-4	0.197	10/20	0.002
Initial triggering of complement	4/141	0.007	0.002	0.371	8/21	0.002
Formation of fibrin clot	3/95	0.005	0.005	0.633	11/57	0.005
Complement cascade	4/210	0.011	0.007	0.633	28/71	0.006

*Pathway over-representation analysis through overlay of quantitative expression data onto the Reactome annotated protein database. This uses binomial tests to calculate the probability shown for each result and *p* values are corrected for false discovery rate (FDR) from evaluating the submitted list of identifiers against every pathway. Only those pathways with a *p* value > 0.01 are shown here

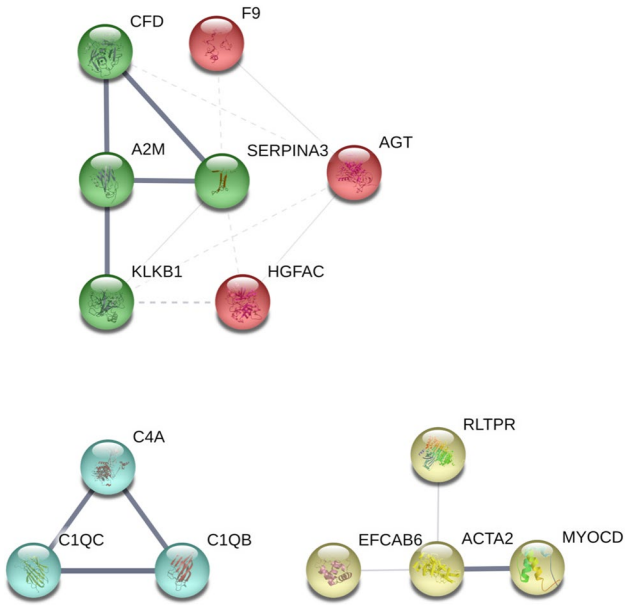

Fig. 3 Protein clusters associated to complement pathways (turquoise blue), structural proteins (yellow) and signaling pathways (green and red), according to STRING

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Compliance with ethical standards

Conflict of interest We declare no conflict of interest.

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