Clinical Proteomics

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Cord blood platelet-rich plasma: proteomics analysis for ophthalmic applications

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Abstract

Our objective is to determine the protein and complements constituents of Cord blood Platelet-rich plasma (CB-PRP), based on the hypothesis that it contains beneficial components capable of arresting or potentially decelerating the advancement of atrophic age-related macular degeneration (dry-AMD), with the support of radiomics. Two distinct pools of CB-PRP were assessed, each pool obtained from a total of 15 umbilical cord-blood donors. One aliquot of each pool respectively was subjected to proteomic analysis in order to enhance the significance of our findings, by identifying proteins that are shared between the two sample pools and gaining insights into the pathways they are associated with. The bioinformatics analysis was developed using Reactome software. Three-hundred-seven (307) distinct proteins were found. Two hundred fifteen (215) of the elements mentioned above are shared by both pools. Seventy (70) elements are exclusive to pool S1, while pool S2 contains 22. We detected 109 representative and statistically significant pathways out of 549. We found proteins related to the immune system, signal transduction, vesicle-mediated transport, cell–cell communication, hemostasis, cellular responses to stimuli, cell cycle, and developmental biology. The analysis showed the presence of P15692-12, representing VEGF factor A, long form. With over 200 proteins, the CB-PRP can increase the immune response, including BCR, CD-22, FCGR, phospholipids, IL-10, FCGR-3A, and others. Discovering crucial trophic and complement-regulating variables is highly significant for potential applications in dry AMD. Our future research will examine the effects of intravitreal CB-PRP on dry-AMD eyes.

Keywords Proteomics, Cord blood, Platelet-rich plasma, Dry-AMD, Retinal degeneration

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Introduction

Platelet-rich plasma (PRP) is an autologous blood product characterized by an elevated platelet concentration compared to normal levels, achieved via the centrifugation of whole blood. [1]

Ehrenfest and colleagues classify PRP products into four main types based on leukocyte and fibrin content: leukocyte-rich PRP (L-PRP), pure PRP (P-PRP, which has reduced leukocyte content), leukocyte platelet-rich fibrin, and pure platelet-rich fibrin [2].

Platelet-rich plasma (PRP) is derived from a blood sample taken from the patient, with the objective of isolating and concentrating platelets from the whole blood. The production volume is contingent upon the individual's baseline platelet count, the device utilized, and the technique applied. Two primary methods exist for the preparation of PRP: the PRP method and the buffy-coat method. [3] The PRP method consists of an initial centrifugation to isolate red blood cells (RBC) followed by a second centrifugation to concentrate platelets, which are subsequently suspended in the minimal volume of plasma. The initial spin phase is performed with uniform acceleration to separate red blood cells (RBCs) from the total volume of whole blood (WB). After the initial centrifugation, whole blood (WB) separates into three distinct layers: an upper layer mainly consisting of platelets and white blood cells (WBCs), an intermediate thin layer known as the buffy coat rich in WBCs, and a lower layer primarily made up of red blood cells (RBCs). The top layer and superficial buffy coat are transferred to a sterile tube to produce pure PRP (P-PRP). To generate leucocyte-rich PRP (L-PRP), the entire buffy coat layer and a limited quantity of red blood cells are extracted. The subsequent step of the second spin is executed. The centrifugal force produced by the second spin should adequately promote the formation of soft pellets composed of erythrocytes and platelets at the tube's bottom. The upper section of the volume, primarily composed of platelet-poor plasma (PPP), is extracted. The buffy coat method involves centrifuging whole blood at high speeds to isolate and collect the buffy coat layer. A buffy coat consists of a significant concentration of leukocytes. Three distinct layers arise from differences in density: the lowermost layer consists of red blood cells (RBCs), the intermediate layer contains platelets and white blood cells (WBCs), and the uppermost layer is referred to as the platelet-poor plasma (PPP) layer. The plasma at the upper section of the container is extracted. The buffy-coat layer is subsequently transferred to a distinct sterile tube.A low-speed centrifuge is used to separate white blood cells (WBCs), or alternatively, a leucocyte filtration filter may be utilized. The pellets are homogenized in the lower one-third portion (5 ml) of plasma to generate PRP [3]. To prevent platelet activation prior to use, an anticoagulant, such as citrate dextrose A, is typically added to the blood sample. Platelets release a variety of growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta1 (TGFbeta1), vascular endothelial growth factor (VEGF-A), basic fibroblast growth factor (bFGF or FGF-2), hepatocyte growth factor (HGF), epidermal growth factor (EGF), and insulin-like growth factor-1 (IGF-1). Growth factors are essential in regulating multiple processes associated with wound healing. [4]

Umbilical cord blood (CB) comprises diverse cell types, including unrestricted somatic stem cells, embryonic-like stem cells, multipotent progenitor cells, and endothelial progenitor cells. [5].

PRP, accessible from both adult peripheral blood and cord blood, demonstrates variations in the type and quantity of factors derived from these sources. [6] Previous studies have shown that CB-PRP possesses a greater concentration of growth factors and anti-inflammatory molecules than PRP sourced from peripheral blood. [7]

PRP is commonly utilized in clinical settings to address various pathological conditions, including erectile dysfunction, dry eye syndrome, peripheral nerve damage, alopecia, and tendinopathies. [8] [9]CB-PRP is intended for various diseases that necessitate multiple metabolic supports without adverse effects. Validated applications in ophthalmology primarily focus on ocular surface diseases. [10, 11]

Additionally, evidence suggests that PRP is effective in vitreoretinal surgery, especially for the closure of large macular holes in patients with high myopia. [12, 13]

Recent studies have indicated that subretinal administration of CB-PRP may serve as a potential treatment for photoreceptor degeneration associated with dry age-related macular degeneration (dry-AMD). [14] The authors presented the safety profile of CB-PRP injection administered under the retina, noting a significantly low rate of atrophy progression blockage in comparison to the untreated eye one month post-administration. However, a single injection did not result in efficacy to halt the progression of retinal atrophy one year after administration.

This study aimed to analyze the protein and complement factor composition in CB-PRP, based on the hypothesis that it contains beneficial components that may halt or slow the progression of atrophic age-related macular degeneration.

Methods

Donor selection

Umbilical cord blood units were collected at the public UNICATT cord blood bank (CBB) of Fondazione Policlinico A. Gemelli, IRCCS from full term neonates whose

parents decided to solidary donate cord blood. Only after counseling with CBB medical staff can couples donate cord blood. The Italian regulation "Provisions relating to the quality and safety requirements of blood and blood components" (https://www.gazzettaufficiale.it/eli/id/2015/12/28/15A09709/sg) prohibits donors with bloodborne infectious, neoplasm, hematological, hereditary, or autoimmune diseases. Couples give written informed agreement to donate cord blood for transplant or clinical/research usage if units are unsuitable during counseling. After couple eligibility, midwives transmit the cord blood unit and maternal blood samples to the CBB if pregnancy goes uneventful to term (>37 weeks). During the initial examination, the CBB discards units older than 48 h, those with a volume of less than 40 ml, clots, or nonconformities such improperly sealed bags. Then, a cord unit CBC and DAT are performed, and maternal samples are screened for syphilis (serology) and HIV, HBV, and HCV (serology and genome testing). Cord blood units with negative DAT, normal CBC, negative infection test results, and at least 1400 million total nucleated cells (TNC) are preserved for transplant. Researchers use units with negative DAT, normal CBC, negative viral tests, and less than 1200 millions of TNC. We perform blood cultures for fungal, aerobic, and anaerobic bacteria in both situations. When a newborn donating cord blood is discharged from the hospital, a neonatologist sends the CBB a certification certifying the infant's health, allowing the CBB to discharge units from neonates with undiagnosed health issues. Finally, this study used only cord blood units from full-term healthy neonates with negative DAT, normal CBC, and negative viral tests and blood cultures.

CB-PRP preparation

CB-PRP refers to a blood product that is manufactured in accordance with the guidelines outlined by the Italian legislation on blood components, specifically for purposes other than transfusion. The fundamental components utilized in the manufacturing of platelet-rich plasma (PRP) consist of cord blood units obtained from the UNICATT Cord Blood Bank, which operates under the Fondazione Policlinico A. Gemelli, IRCCS.

The CB-PRP will be comprised of a collection of 15 units. Following the initial collection, each unit will be subjected to a process of soft-spin centrifugation in order to obtain Platelet Concentrate (PC), which will be referred to as PRP throughout the manuscript. The Platelet Concentrate (PC), which will be standardized to a value of 1×10^9/L, will be obtained using hard-spin centrifugation and the subsequent removal of any excess of platelet-poor plasma. The CB-PRP that has been retrieved will undergo microbiological analysis and will be preserved at a temperature of − 80 °C until results will be proved negative. After acquiring 15 units of cryopreserved platelet-rich plasma (CB-PRP) with negative test results, a procedure will be implemented to prevent any discrepancies in growth factor concentration among the units. This involves thawing the units and combining them into a single bag specifically suited to produce blood products intended for non-transfusion purposes. Subsequently, the pool will be divided into aliquots of 1 ml each, which will be placed in sterile vials that are securely sealed. An additional microbiological analysis for fungi and bacteria is performed and the vials will then be maintained at a temperature of − 80 °C until microbial test will be proved negative and CB-PRP vials are ready to be utilized. This procedure will result in a standardized intervention for all participants involved in the study. The entirety of the procedures required to prepare the CB-PRP pool will be executed within aseptic cryogenic blood component bags. These bags possess sterile connectors that facilitate the transfer of CB-PRP from one bag to another, as well as the fractionation of CB-PRP aliquots.

We evaluated two different pools each derived from 15 umbilical cord-blood donators. One aliquot underwent to proteomic analysis to increase the significance of our results, looking for proteins common to the two pools and understanding the pathways in which they were involved (S1 and S2). The study was approved by the Catholic University/Fondazione Policlinico Universitario A. Gemelli IRCCS Institutional Ethics Committee (protocol ID number: 4995, NCT05706896).

Proteomic analysis

We used as working samples two pools obtained from 15 different donors each. After proteins total content determination detected by Bradford Assay (BioRad), samples were processed by the FASP protocol using Microcon 10 k centrifugal ultrafiltration units (Merck, Darmstadt) operated at 10 000 g. [15] Aliquots containing 50 µg of total protein were mixed with 100 µL of 8 M urea in 0.1 M Tris/HCl, pH 8.5 (UA), in the ultrafiltration unit and then centrifuged at 20 °C, for 15 min10 000 g. The eluates were discarded; 200 µL of UA was pipetted into the filtration unit, and the units were centrifuged again (for 3 times). 100 ul of DTT 8 mM were added and incubated for 15 min at 56 °C. Then were centrifuged for 10 min at 10 000 g, 100 ul of UA were added and were centrifuged for 30 min at 13 000 g. The eluates were discarded for each step. Then, 100 µL of 50 mM IAA in UA was added to the filters, and samples were incubated in darkness for 20 min at room temperature. Then the samples were centrifuged for 10 min at 13 000 g. Filters were washed with 100 µL of UA and centrifuged for 30 min at 11.000 g. Quenched excess of IAA adding

100ul of DTT and incubated 15 min 56 °C. Centrifuged for 10 min at 13.000 g. 100 ul of UA were added to the filters to remove DTT; centrifuge for 30 min at 13 000 g. 100 ul of NH_4HCO_3 50 mM were added and centrifuged for 10 min at 11 000 g (for 2 times). New collection tubes were used and 50 ul of trypsin (MS grade), at an enzyme to protein ratio of 1:50 w/w was added and incubated at 37 °C for 18 h in a wet chamber. The released peptides were collected by centrifugation at 11 000 g for 30 min followed by two washes with 50 ul of NH_4HCO_3 50 mM. The reaction was blocked with 20ul TFA 1%.

For bottom-up analyses, the chromatographic column used was EASY-Spray column 15 cm × 50 µm ID, PepMap C18 (2 µm particles, 100 Å pore size) in coupling with Acclaim PepMap100 cartridge (C18, 5 µm, 100 Å, 300 µm i.d. × 5 mm) (Thermo Fisher Scientific) in gradient elution using eluent A (FA 0.1%, v/v) and solvent B (ACN:FA 99.9:0.1, v/v) and the following steps:

5% B (2 min), (ii) from 5 to 55% B (130 min), (iii) from 55 to 99% B (15 min), (iv) 99% B (10 min), (v) from 99 to 5% B (2 min), (v) 5% B (13 min) at a flow rate of 0.3 µL/min. The injection volume was 5 µL, corresponding to 0.25 µg of total protein concentration after opportune sample dilution with 0.1% (v/v) FA aqueous solution. Chromatographic separations were performed in triplicate at a thermostated temperature of 40 °C. The Orbitrap Fusion Lumos instrument was operating in positive ionization mode at a resolution of 1200,000 in 350–1500 m/z scan filter range in Data-Dependent Scan (DDS) mode and performing MS/MS fragmentation by High Collision Dissociation (HCD) of the most intense signals of each full scan MS spectrum. The minimum signal was set to 500.0 and the isolation width to 2.00 m/z. Normalized collision energy was set at 35.0. Capillary temperature was 250 °C, and the source voltage was + 1.5 kV. MS/MS spectra acquisition was performed in the linear ion trap at normal scan rate. Acquisition started at 4 min in order to avoid salt source contamination in the first minute of elution. All samples were analyzed in triplicate. Figure 1 shows the procedure from preparing the CB-PRP sample (1) to identifying and quantifying the protein material it contains (5). Point 2 represents the phase of cell lysis, point 3 represents the phase of the FASP protocol (filter-assisted sample preparation: digestion and cleanup), and point 4 represents the phase of liquid chromatography mass spectrometry.

Data analysis

The analysis was based on a label free proteomic approach, performed using an Orbitrap Fusion Lumos Tribrid mass spectrometer (Thermo Scientific). Raw data were analyzed using the Proteome Discoverer 2.4 software, based on SEQUEST HT algorithm and UniprotKB/Swiss-prot *Homo Sapiens* database.

The following parameters were set: minimum precursor mass 350 Da; maximum precursor mass 10,000 Da; total intensity threshold 0.0; minimum peak count 1; Signal to Noise (S/N) threshold 1.5; mass tolerance 10 ppm; fragment mass tolerance 0.5 Da; use average precursor mass False; use average fragment mass False. Trypsin enzyme was set with a maximum of 2 missed cleavage sites. For data elaborations, the minimum and maximum peptide length was 6 and 144 residues, respectively. Dynamic methionine oxidation (+ 15.99 Da) and static carbamidomethylation of cysteine (+ 57.02 Da) were also set. Protein and peptide spectra matches were validated by the calculation of false discovery rate (FDR) using the Percolator node. The strict target FDR value was set at 0.01, while the relaxed value was set at 0.05. Protein identification results were further filtered for high peptide confidence identification; peptide rank 1; 2 peptides per protein; peptide length ≥ 9 amino acid residues, according to the Human Proteome Project Mass Spectrometry Data Interpretation Guidelines [16]. The pathway analysis was conducted using the Reactome software (www.reactome.org) with the following settings. The proteins

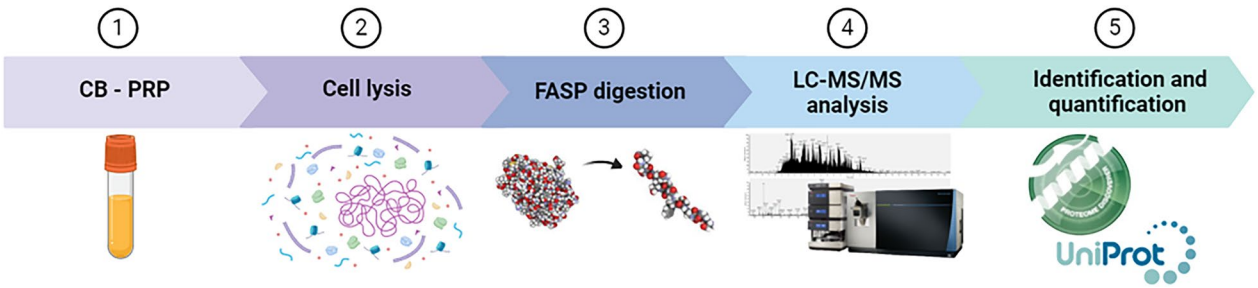

Fig. 1 Workflow for proteomic analysis of CB-PRP samples. Starting from CB-PRP pool (1) the sample were prepared and quantified (2); then were submitted to a FASP protocol (filter-assisted sample preparation: digestion and cleanup) (3). After digestion, the samples were analyzed using a liquid chromatography mass spectrometry shot gun proteomic approach (4) and the last step was the identification and quantification with specific bioinformatics tools (5)

common to the two samples were listed using the Uniprot accession, corresponding genes were identified and associated with representative pathways, sorted by p-value and corrected for FDR using the Benjamini–Hochberg method. The pathways were then filtered for significance, retaining those with a p-value lower than 1×10^3.

Results

A comprehensive examination of the two different pools each of 15 umbilical cord-blood donators, S1 and S2, yielded a total of 307 distinct proteins. Among the aforementioned elements, a total of 215 are shared by both pools, while 70 elements exclusively belong to pool S1 and 22 elements exclusively belong to pool S2. The Venn diagram illustrates the proteins that are shared across the two samples (215) as well as those that are uniquely detected in either one of the samples, showed in Fig. 2.

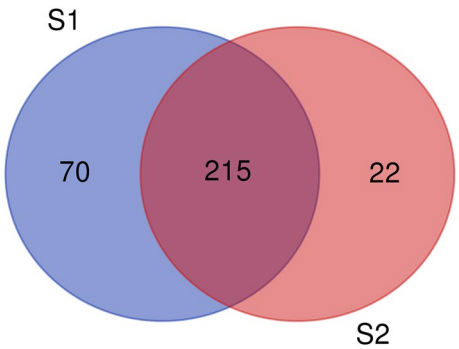

Fig. 2 Venn diagrams show the distribution of the two different pools (S1–S2) each of 15 umbilical cord-blood donators. A total of 215 elements are shared by both pools, while 70 elements exclusively belong to pool S1 and 22 elements exclusively belong to pool S2

We have listed the 215 common proteins in the two pools, 70 elements included exclusively in S1 and 22 elements included exclusively in S2 (See supplementary Tables).

Additionally, we conducted investigations to determine the specific pathways in which these proteins were implicated. Each pathway can be assigned a false discovery rate (FDR), which is determined by the quantity of the protein present in the analyzed material.

The pathways in which CB-PRP is hypothesized to be implicated are illustrated in Fig. 3 according to the proteins it contains and analyzed by reactome pathway database (https://reactome.org/). Reactome is pathway database which provides intuitive bioinformatics tools for the visualization, interpretation and analysis of pathway knowledge.

Protein pathways with the highest statistical significance are most strongly represented in yellow in Fig. 3 and were more distinguishable belong to: immune system, signal transduction, vesicle-mediated transport;

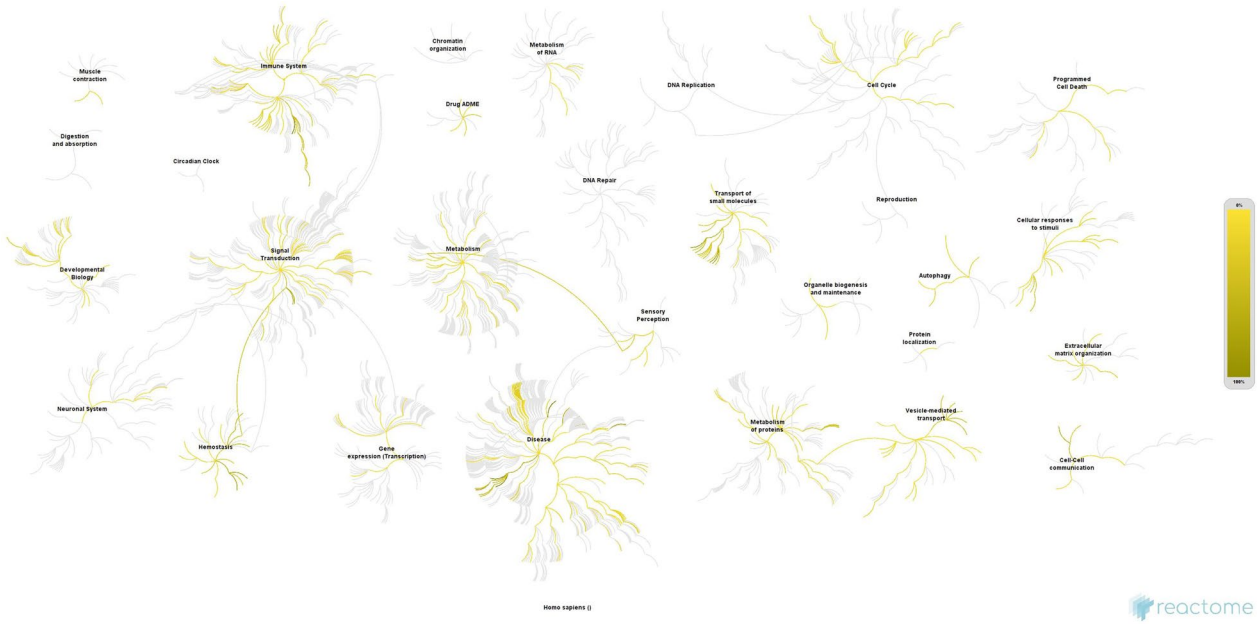

Fig. 3 All reactome pathways are shown, in blocks of 20 pathways, ranked by the p-value obtained from over-representation analysis. Among the most prevalent proteins, we found those belonging to: immune system, signal transduction, vesicle-mediated transport; cell–cell communication, hemostasis, cellular responses to stimuli, cell cycle, and developmental biology

cell–cell communication, hemostasis, cellular responses to stimuli, cell cycle, developmental biology.

Based on the analysis conducted, we identified 109/549 pathways that were statistically significant representative and reported in Table 1.

Further analysis for vascular endothelial growth factor (VEGF) signal were performed. The analysis showed the presence of P15692-12, representing VEGF factor A, long form.

Discussion

The application of advanced methodologies in proteomics facilitated the identification of numerous proteins in CB-PRP. Our study demonstrated that proteomics identified several pathways that were statistically significant in a 15-pool of CB-PRP samples. We examined the pathway demonstrating the highest statistical significance, which may be linked to favorable outcomes in age-related macular degeneration.

Following the findings of Longo et al., we identified distinct proteins that demonstrated pro-inflammatory and anti-inflammatory properties, as well as effects on angiogenesis, cell proliferation, and tissue remodeling. The proteins comprised extracellular matrix molecules, proteases and peptidases along with their inhibitors, peroxidase activity, and calcium ion binding. [17]

The haptoglobin pathway serves as a prominent example, functioning as a scavenger of heme groups. Free heme intercalates into biological membranes, disrupting lipid bilayers and promoting the conversion of low-density lipoprotein to cytotoxic oxidized products, resulting in tissue damage. Moreover, it serves as a source of redox-active iron, generating oxygen radicals through its involvement in the Fenton reaction. This protein may provide advantages in the context of hemorrhagic conditions. [18]

Additional scavenger and binding proteins were identified in CB-PRP, playing significant roles in innate immunity, the prevention of thrombotic events, and the regulation of dyslipidaemia [19, 20]. The presence of scavengers from various classes may enhance the proper turnover and defense mechanisms of the organism.

Other important pathway is linked with hemostasis and platelet activation. For example, we found proteins that support the activation of phospholipase C enzymes. This enzyme raise the intracellular Ca++, fundamental element for several protein. [21]These pathways could help to block hemorrhagic conditions [21, 22].

Another fundamental pathway pertains to immunity, encompassing both innate and specific responses. CB-PRP can enhance the immune response due to over 200 types of proteins, including B cell receptor (BCR), CD-22, Fcgamma receptor (FCGR), phospholipids, IL-10, and FCGR-3A, among others [23–27].

Normal eye function requires several protein pathways to ensure physiological functionality. In CB-PRP, we identified proteins involved in retinoid metabolism and transport, proteins associated with the visual phototransduction pathway, as well as cofactors or proteins implicated in the metabolism of both water-soluble and fat-soluble compounds. This may signify a crucial support for photoreceptor health.

Additionally, we have identified a class of proteins in our sample consisting of the heavy chains of immunoglobulins. The latter would function as immunomodulating agents by binding to receptors for heavy chains (FcγRs) present on all immune system cells. [28] The Fc portion of IgG antibodies binds to Fc gamma receptors (FCGRs) on the surface of various immune cells. [29] There are different types of FCGRs, and they can be either activators or inhibitors. FCGRs are expressed on a wide range of immune cell types, including macrophages, neutrophils, dendritic cells, natural killer (NK) cells, and B cells. [30] The interaction, influenced by the type of FCGR and its signaling pathways, may result in either the activation or inhibition of immune responses. This is essential for sustaining a balanced and regulated immune system.

Vasodilator-Stimulated Phosphoprotein (VASP) is also present in both samples. It plays a role in regulating actin dynamics, promoting cell adhesion, and modulating cell migration. It additionally functions to inhibit platelet aggregation and adhesion to endothelial cells. [31]

VASP could play a positive role in maintaining proper communication among photoreceptors and between them and the extracellular matrix. [32] However, by reducing platelet aggregation and adhesion, it could also represent a mechanism that promotes bleeding.

Other class of important protein is represented by the antioxidant proteins. Redox enzymes and cellular processes like respiration produce reactive oxygen species like superoxide (O2.-), peroxides (ROOR), singlet oxygen, peroxynitrite (ONOO-), and hydroxyl radical (OH.). These species are necessary for signaling but can be harmful because of their high reactivity [33]. Reactive oxygen species are detoxified by aerobic cells' defensive mechanisms, which change them into less reactive forms. Thanks to these proteins the potential effect against DNA and membrane could prevented.

Complement factors and immunoglobulins primarily account for most discrepancies observed between the two samples. Platelet function variability in normal populations is significant, stemming from various genotypic and phenotypic differences among individuals. The final composition of PRP is significantly

Table 1 Prevalent Pathway of CB-PRP contents

Pathway identifier	Pathway name	#Entities found	#Entities total	Entities FDR
R-HSA-2168880	Scavenging of heme from plasma	25	99	4,33E-15
R-HSA-166786	Creation of C4 and C2 activators	25	103	4,33E-15
R-HSA-173623	Classical antibody-mediated complement activation	23	95	4,33E-15
R-HSA-166658	Complement cascade	48	146	4,33E-15
R-HSA-977606	Regulation of Complement cascade	46	135	4,33E-15
R-HSA-166663	Initial triggering of complement	28	111	4,33E-15
R-HSA-2173782	Binding and Uptake of Ligands by Scavenger Receptors	31	129	4,33E-15
R-HSA-140877	Formation of Fibrin Clot (Clotting Cascade)	20	39	4,33E-15
R-HSA-114608	Platelet degranulation	50	128	4,33E-15
R-HSA-76005	Response to elevated platelet cytosolic Ca2 +	50	133	4,33E-15
R-HSA-109582	Hemostasis	85	726	4,33E-15
R-HSA-76002	Platelet activation, signaling and aggregation	55	265	4,33E-15
R-HSA-168249	Innate Immune System	85	1197	4,33E-15
R-HSA-168256	Immune System	97	2188	4,33E-15
R-HSA-140875	Common Pathway of Fibrin Clot Formation	13	22	2,88E-14
R-HSA-140837	Intrinsic Pathway of Fibrin Clot Formation	13	23	4,91E-14
R-HSA-381426	Regulation of Insulin-like Growth Factor (IGF) transport and uptake by Insulin-like Growth Factor Binding Proteins (IGFBPs)	22	124	1,63E-13
R-HSA-5690714	CD22 mediated BCR regulation	17	70	1,61E-12
R-HSA-2029481	FCGR activation	19	101	4,16E-12
R-HSA-2029482	Regulation of actin dynamics for phagocytic cup formation	22	150	5,94E-12
R-HSA-8957275	Post-translational protein phosphorylation	19	107	1,01E-11
R-HSA-2029485	Role of phospholipids in phagocytosis	19	114	2,89E-11
R-HSA-2029480	Fcgamma receptor (FCGR) dependent phagocytosis	22	175	1,04E-10
R-HSA-166665	Terminal pathway of complement	8	8	1,17E-10
R-HSA-983695	Antigen activates B Cell Receptor (BCR) leading to generation of second messengers	17	95	1,36E-10
R-HSA-9664323	FCGR3A-mediated IL10 synthesis	19	128	1,74E-10
R-HSA-202733	Cell surface interactions at the vascular wall	25	246	2,45E-10
R-HSA-9664417	Leishmania phagocytosis	20	149	2,45E-10
R-HSA-9664407	Parasite infection	20	149	2,45E-10
R-HSA-9664422	FCGR3A-mediated phagocytosis	20	149	2,45E-10
R-HSA-9664433	Leishmania parasite growth and survival	20	168	1,86E-09
R-HSA-9662851	Anti-inflammatory response favouring Leishmania parasite infection	20	168	1,86E-09
R-HSA-8963898	Plasma lipoprotein assembly	9	19	2,65E-09
R-HSA-2730905	Role of LAT2/NTAL/LAB on calcium mobilization	16	102	2,82E-09
R-HSA-76009	Platelet Aggregation (Plug Formation)	11	40	6,74E-09
R-HSA-9679191	Potential therapeutics for SARS	19	164	7,65E-09
R-HSA-372708	p130Cas linkage to MAPK signaling for integrins	8	15	9,06E-09
R-HSA-354194	GRB2:SOS provides linkage to MAPK signaling for Integrins	8	15	9,06E-09
R-HSA-9651496	Defects of contact activation system (CAS) and kallikrein/kinin system (KKS)	8	16	1,49E-08
R-HSA-9671793	Diseases of hemostasis	8	16	1,49E-08
R-HSA-2871809	FCERI mediated Ca + 2 mobilization	16	117	1,55E-08
R-HSA-8963888	Chylomicron assembly	7	10	1,55E-08
R-HSA-8963901	Chylomicron remodeling	7	10	1,55E-08
R-HSA-2871796	FCERI mediated MAPK activation	16	119	1,88E-08
R-HSA-8963899	Plasma lipoprotein remodeling	10	35	2,16E-08
R-HSA-8964058	HDL remodeling	7	11	2,73E-08
R-HSA-5653656	Vesicle-mediated transport	40	762	4,46E-08
R-HSA-983705	Signaling by the B Cell Receptor (BCR)	18	176	1,09E-07

Table 1 (continued)

Pathway identifier	Pathway name	#Entities found	#Entities total	Entities FDR
R-HSA-975634	Retinoid metabolism and transport	10	44	1,67E-07
R-HSA-9824443	Parasitic Infection Pathways	21	254	2,17E-07
R-HSA-9658195	Leishmania infection	21	254	2,17E-07
R-HSA-174824	Plasma lipoprotein assembly, remodeling, and clearance	12	76	3,28E-07
R-HSA-6798695	Neutrophil degranulation	29	478	3,29E-07
R-HSA-6806667	Metabolism of fat-soluble vitamins	10	48	3,37E-07
R-HSA-6802948	Signaling by high-kinase activity BRAF mutants	9	37	4,48E-07
R-HSA-446353	Cell-extracellular matrix interactions	7	18	6,28E-07
R-HSA-354192	Integrin signaling	8	28	7,50E-07
R-HSA-5674135	MAP2K and MAPK activation	9	41	1,06E-06
R-HSA-9656223	Signaling by RAF1 mutants	9	42	1,15E-06
R-HSA-2871837	FCERI mediated NF-kB activation	16	167	1,29E-06
R-HSA-9649948	Signaling downstream of RAS mutants	9	47	2,92E-06
R-HSA-6802946	Signaling by moderate kinase activity BRAF mutants	9	47	2,92E-06
R-HSA-6802955	Paradoxical activation of RAF signaling by kinase inactive BRAF	9	47	2,92E-06
R-HSA-6802949	Signaling by RAS mutants	9	47	2,92E-06
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	18	230	4,04E-06
R-HSA-3000480	Scavenging by Class A Receptors	6	19	1,42E-05
R-HSA-5686938	Regulation of TLR by endogenous ligand	6	21	2,51E-05
R-HSA-430116	GP1b-IX-V activation signalling	5	12	2,74E-05
R-HSA-2454202	Fc epsilon receptor (FCERI) signaling	16	218	3,46E-05
R-HSA-6802952	Signaling by BRAF and RAF1 fusions	9	66	3,98E-05
R-HSA-174577	Activation of C3 and C5	4	7	7,74E-05
R-HSA-446388	Regulation of cytoskeletal remodeling and cell spreading by IPP complex components	4	8	1,30E-04
R-HSA-2187338	Visual phototransduction	10	100	1,71E-04
R-HSA-977225	Amyloid fiber formation	9	81	1,71E-04
R-HSA-9657689	Defective SERPING1 causes hereditary angioedema	3	3	1,71E-04
R-HSA-9657688	Defective factor XII causes hereditary angioedema	3	3	1,71E-04
R-HSA-6802957	Oncogenic MAPK signaling	9	84	2,24E-04
R-HSA-216083	Integrin cell surface interactions	9	85	2,45E-04
R-HSA-9029569	NR1H3 & NR1H2 regulate gene expression linked to cholesterol transport and efflux	6	38	5,79E-04
R-HSA-9679506	SARS-CoV Infections	23	509	6,51E-04
R-HSA-1280218	Adaptive Immune System	33	879	7,31E-04
R-HSA-445355	Smooth Muscle Contraction	6	44	1,27E-03
R-HSA-9024446	NR1H2 and NR1H3-mediated signaling	6	48	2,01E-03
R-HSA-3000484	Scavenging by Class F Receptors	3	7	2,05E-03
R-HSA-9662001	Defective factor VIII causes hemophilia A	3	7	2,05E-03
R-HSA-5602498	MyD88 deficiency (TLR2/4)	4	19	3,02E-03
R-HSA-5627117	RHO GTPases Activate ROCKs	4	19	3,02E-03
R-HSA-70263	Gluconeogenesis	5	35	3,23E-03
R-HSA-5603041	IRAK4 deficiency (TLR2/4)	4	20	3,23E-03
R-HSA-1474244	Extracellular matrix organization	15	300	3,23E-03
R-HSA-9668250	Defective factor IX causes hemophilia B	3	9	3,54E-03
R-HSA-159763	Transport of gamma-carboxylated protein precursors from the endoplasmic reticulum to the Golgi apparatus	3	9	3,54E-03
R-HSA-5627123	RHO GTPases activate PAKs	4	21	3,65E-03
R-HSA-1643685	Disease	59	2101	3,83E-03
R-HSA-70171	Glycolysis	7	80	4,49E-03

Table 1 (continued)

Pathway identifier	Pathway name	#Entities found	#Entities total	Entities FDR
R-HSA-3299685	Detoxification of Reactive Oxygen Species	5	39	4,71E-03
R-HSA-381183	ATF6 (ATF6-alpha) activates chaperone genes	3	10	4,79E-03
R-HSA-159782	Removal of aminoterminal propeptides from gamma-carboxylated proteins	3	10	4,79E-03
R-HSA-159740	Gamma-carboxylation of protein precursors	3	10	4,79E-03
R-HSA-196854	Metabolism of vitamins and cofactors	11	192	5,88E-03
R-HSA-159854	Gamma-carboxylation, transport, and amino-terminal cleavage of proteins	3	11	6,28E-03
R-HSA-373755	Semaphorin interactions	6	64	7,36E-03
R-HSA-9636667	Manipulation of host energy metabolism	2	3	7,66E-03
R-HSA-9672391	Defective F8 cleavage by thrombin	2	3	7,66E-03
R-HSA-381033	ATF6 (ATF6-alpha) activates chaperones	3	12	8,04E-03
R-HSA-3000170	Syndecan interactions	4	27	8,07E-03
R-HSA-446728	Cell junction organization	8	118	8,07E-03
R-HSA-75205	Dissolution of Fibrin Clot	3	13	8,07E-03
R-HSA-1236974	ER-Phagosome pathway	7	93	8,40E-03

influenced by variations among donors and the patient's condition at the time of blood collection. This may indicate a limitation of the study [34, 35].

The role of oxidizing agents in atrophic macular degeneration is well-documented, and the importance of antioxidants may be critical in slowing the progression of the disease [36].

The presence of VEGF and complement factors may suggest a potential transition from atrophic to neo-vascular disease; however, this is deemed highly improbable in light of our safety findings. Antioxidant and anti-inflammatory factors are considered more effective in slowing the progression of dry AMD. [14]

Conclusions

Our study identified 307 unique proteins and 109 pathways that are statistically significant in CB-PRP. At present, our comprehension of the behavior of these molecules in the eye is restricted to hypotheses, informed by their activity within the organism and their mechanisms of action. The precise influence of these molecules on the treatment of age-related macular degeneration is currently unclear. Although research on the role of CB-PRP in ophthalmology is limited, numerous ongoing investigations are anticipated to improve understanding in this area in the future. Identifying essential trophic and complement-regulating variables is important for potential applications in dry AMD. The future objective is to investigate the potential effects of intravitreal administration of CB-PRP in eyes with dry age-related macular degeneration (AMD).

Data availability

The authors declare that the data supporting the findings of this study are available within the paper and its Supplementary Information files. Should any raw data files be needed in another format they are available from the corresponding author upon reasonable request.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12014-024-09524-2.

Supplementary material 1.

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Author contributions

Authors' contributions: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by MCS, LT and FI. The first draft of the manuscript was written by FG,VC and AS and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Dr. MCS and FG equally contributed to the paper and should be considered equal first authors. Prof.FI and SR share co-senior authorship ECA.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The study was approved by the Catholic University/Fondazione Policlinico Universitario A. Gemelli IRCCS Institutional Ethics Committee (protocol ID number: 4995, NCT05706896).

Consent for publication
Informed consent was obtained from all individual participants included in the study.

Competing interests
The authors declare no competing interests.

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