Article

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Plasma Extracellular Vesicle-Associated Proteins as Promising Diagnostic Biomarkers of Age-Related Macular Degeneration

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Background: Age-related macular degeneration (AMD) is a significant factor causing blindness in adults. However, the clinical diagnosis of AMD is relatively challenging, due to the shortcomings of the existing clinical examination methods and the latent period of retinal damage before macular degeneration becomes apparent. This study aims to explore the potential of extracellular vesicles (EVs) protein chips for early diagnosis of AMD using patients' plasma samples.
Methods: To achieve early diagnosis of AMD, this study utilized a high-throughput platform for liquid biopsy based on EVs protein chips. Forty AMD patients and 41 normal individuals were recruited. Through machine learning methods, we identified that ATP-binding cassette transporter A1 (ABCA1) is an EVs protein marker for diagnosing AMD. Additionally, a validation set was constructed using the random forest method for verification.
Results: The results of the study indicated that ABCA1 is a reliable biomarker for diagnosing AMD. The validation using the random forest method confirmed the robustness and reliability of ABCA1 as a diagnostic marker. This finding suggested that ABCA1 can serve as a new promising liquid biopsy-based marker for diagnosing macular degeneration.
Conclusion: The utilization of EVs protein chips, combined with machine learning methods, can effectively identify ABCA1 as a biomarker for the early diagnosis of AMD. This approach offers a promising new method for liquid biopsy diagnostics, potentially improving the clinical diagnosis and management of macular degeneration.

Keywords: age-related macular degeneration; extracellular vesicles; liquid biopsy diagnostics; machine learning

Introduction

Age-related macular degeneration (AMD) is a prevalent degenerative eye disease primarily affecting individuals over the age of 50, and it is one of the leading causes of severe vision loss and blindness among the elderly globally [1,2]. AMD is characterized by the dysfunction of retinal pigment epithelial cells in the macular region, which leads to central vision blurring, distortion, and, in severe cases, irreversible vision loss [3,4].

AMD can be classified into two types: dry (non-exudative) and wet (exudative) [5]. Dry AMD is characterized by slow progression, involving gradual worsening of vision impairment [6,7], whereas wet AMD advances rapidly due to the formation of choroidal neovascularization, causing severe bleeding and retinal detachment. Although less common, wet AMD poses a greater threat to vision, with patients experiencing significant vision decline within a short period [8–10]. Currently, fundus examination and optical coherence tomography (OCT) are primarily relied upon for early diagnosis of AMD [11,12]. While these methods provide detailed structural information about the macular region, they typically require highly specialized technical support and equipment [13,14]. Nevertheless, their sensitivity to early-stage lesions is limited, resulting in many patients being diagnosed only when symptoms become pronounced and therefore missing the optimal treatment window [15–17]. Therefore, developing a highly sensitive, specific, and easily operable early diagnostic method is crucial for improving AMD diagnosis and treatment outcomes.

In recent years, with the advancement of liquid biopsy technology, plasma extracellular vesicle (EV)-associated proteins have emerged as promising novel biomarkers for disease diagnosis [18,19]. EVs are nanoscale vesicles secreted by cells that stably exist in the bloodstream, carrying and transmitting various bioactive molecules, including proteins, RNA, DNA, and lipids [20]. It has been shown that EVs reflect pathological changes within the body, and the specific proteins they carry can serve as potential dis-

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ease biomarkers [21]. In the context of AMD research, plasma EVs' proteins have been found to be closely related to the disease's onset and progression [22,23]. Detecting the expression levels of these proteins in plasma can provide valuable insights into early AMD lesions, thereby aiding in the diagnosis of AMD. This non-invasive and efficient diagnostic method not only enhances the early detection rate of AMD but also enables patients to undertake effective interventions before the disease exacerbates, thereby delaying or preventing vision damage [24,25].

In this study, blood samples from 40 clinically diagnosed AMD patients and 41 healthy individuals were collected. After centrifugation, the plasma samples were anonymized and divided into two independent cohorts for comprehensive analysis [26]. For this study, we designed an EVs expression array targeting a total of 57 key plasma proteins. This 57-protein array was designed based on the EV-array platform; an innovative approach specifically tailored for the detection of surface proteins on EVs in clinical settings [27]. The current armada of EV array technologies is developed for the detection of various disease biomarkers, many of which have demonstrated substantial diagnostic reliability [28–30]. In this study, we analyzed the results obtained from the EV array. Out of all 81 samples, after excluding non-qualifying samples, data from 27 samples from the AMD group and 27 samples from the normal control (NC) group were included in the statistical analysis. Subsequently, we employed various machine learning methods to conduct the analysis, with the aim of identifying several potential diagnostic biomarkers for AMD. Among them, ATP-binding cassette transporter A1 (ABCA1) was identified as the most representative marker. These biomarkers were then cross-referenced with clinical information to assess their potential for clinical application.

Overall, our work presents a novel liquid biopsy method for the diagnosis of AMD, which has the potential to facilitate early classification and diagnosis of retinal lesions.

Materials and Methods

Clinical Data

Patients participating in this study were recruited from the outpatient and inpatient departments of the Affiliated Hospital of Nanjing University of Chinese Medicine between January 2023 and March 2024. The inclusion criteria for the experimental group (AMD group) are: (1) patients with clinical diagnosis of macular degeneration; (2) patients aged between 50 and 90 years; and (3) patients who signed the informed consent, as a sign of voluntary participation. The inclusion criteria for the normal control (NC) group are (1) individuals aged between 50 and 90 years; (2) individuals without any organic eye diseases; and (3) individuals who signed informed consent, as a sign of voluntary participation. The common exclusion criteria are (1) individuals with a history of hematological disorders; (2) individuals with a personal history of malignant or borderline tumors; (3) pregnant or lactating women; (4) individuals with severe primary diseases that affect survival, such as nephrotic syndrome, primary cirrhosis, or severe infectious diseases; (5) individuals who are unable to provide full informed consent due to intellectual or behavioral disorders; (6) individuals with suspected or confirmed history of alcohol or drug abuse; and (7) patients currently participating in other clinical drug trials. To date, a total of 40 patients with AMD and 41 healthy individuals were enrolled in this study.

Evaluations and Treatment

Following the clinical diagnostic criteria for AMD established by the National Collaborative Group for Fundus Diseases in 1986, all patients diagnosed with AMD underwent a complete blood count (CBC) test, fundus photography, and OCT. Concurrently, all patients in the control group had their clinical information meticulously collected to ensure they met none of the exclusion criteria. Peripheral whole blood samples (10 mL each) were collected via venipuncture in Ethylene Diamine Tetraacetic Acid (EDTA) tubes, centrifuged at 4 °C and 3000 ×g for 15 minutes to obtain plasma, which was then stored at -80 °C for long-term preservation. Clinical data for all patients, including basic information, treatment plans, laboratory test results, and fundus examination outcomes, were recorded as individual medical records. The severity of adverse events was evaluated using the Common Terminology Criteria for Adverse Events (CTCAE) 4.0. Clinical information and blood samples were usually collected within a week of the patient's initial visit, which began on 31 January 2023. Follow-up ended on 29 March 2024.

EV Array Assay

A total of 57 antibodies were used to capture EVs (listed in **Supplementary Table 1**). All antibodies were diluted in phosphate-buffered saline (PBS) supplemented with 5% glycerol and then printed onto a 3D modified slide surface (Capital Biochip Corp, Beijing, China) in duplicate at 200 µg/mL using an Arrayjet microarrayer (MERCURY 1000, Roslin, Scotland, UK).

The EV Array assay was performed as previously described by Zhang *et al*. [27]. The experiments involving the use of EV array were commissioned by EVBio_tech Company (Beijing, China). In brief, the microarray slides were incubated with 10 µL unpurified plasma sample diluted in PBS buffer (1:10) at room temperature for 2 hours followed by an overnight incubation at 4 °C. After washing, the slides were incubated in biotinylated detection antibodies (anti-human-cluster-of-differentiation antigen 9 (CD9), -CD63, and -CD81, LifeSpan BioSciences, Seattle, WA, USA) diluted in PBS buffer (1:1500). For detection, Cy3-labeled streptavidin (diluted 1:1500 in PBS buffer) was added. After incubation for 1 hour, the slides were washed

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and scanned using the GenePix 4000A microarray scanner (Molecular Devices, San Francisco, CA, USA). The fluorescent images were analyzed, and the signal intensity was extracted using the GenePix Pro image analysis software (V6.0.1.27, Molecular Devices, San Francisco, CA, USA).

Data Analysis and Statistics

The signal intensity of a given antibody was calculated by subtracting the mean of the negative triplicate (PBS spot). Differences among the subgroups were analyzed using the Mann–Whitney U test or t-test. The t-test was used for single-factor difference analysis to determine the statistical significance of differences between the AMD group and the NC group. Results with $p < 0.05$ were regarded as statistically significant. Graphs and statistics were performed using GraphPad Prism 6.0 (GraphPad Software, Inc., San Diego, CA, USA) or SPSS21.0 (IBM Corp., Chicago, IL, USA). Inter-batch data correction was performed using the online tool BatchServer (https://lifeinfo.shinyapps.io/batchserver/). The machine learning framework was implemented in the R programming language, utilizing the Boruta algorithm, Support Vector Machine Recursive Feature Elimination (SVM-RFE), and Least Absolute Shrinkage and Selection Operator (Lasso) algorithm for feature selection. The diagnostic model was constructed using the Random Forest algorithm.

Results

Patient Enrollment and Sample Testing Workflow

As depicted in Fig. 1A, a total of 41 plasma samples from clinically diagnosed AMD patients and 40 samples from healthy individuals undergoing clinical examinations were included and categorized into two groups: the AMD group and the NC group. By utilizing an EVs protein array, we detected the signals related to the expression of surface protein. Following data consistency analysis, a total of 27 samples from the AMD group and 27 samples from the NC group were included in the final statistical analysis. The mechanism diagram of the whole study is shown in Fig. 1B. We conducted a statistical analysis of the age (Fig. 1E) and gender (Fig. 1F) of the enrolled patients, all of whom were between 50 and 90 years old. There were no significant differences between the experimental and control groups in terms of age distribution. Similarly, no significant gender differences were observed between the two groups.

Design of AMD-Related EVs Protein Chip

We initially utilized the online tool Tin-X to analyze the frequency of protein-related keywords associated with AMD in published literature. The results are presented in Fig. 1C. The proteins were categorized based on importance and novelty, and divided into four sections corresponding to clinical research, drug targets, biological behavior, and others.

Additionally, we searched the GEO database and selected 77 sequencing samples diagnosed with dry AMD and wet AMD from the GSE29801 dataset as the experimental group. Correspondingly, 77 normal sequencing samples were randomly chosen from the remaining samples as controls, and a differential analysis was conducted, as illustrated in the volcano plot in Fig. 1D. In this selected data set, the overall expression of samples is shown in **Supplementary Fig. 1A**, and the data of mean difference (MD) plot is shown in **Supplementary Fig. 1B**. These analyses are used to ensure the data quality of biological information analysis.

Combining the genes identified from our analysis with those reported in the literature, we designed an array comprising 57 key proteins for this study (detailed information about all proteins is shown in **Supplementary Table 1**). The chip was subsequently designed and tested for the detection efficacy of the selected proteins.

Differential Expression Analysis of EVs Surface Proteins

Statistical analysis of all 54 samples using the EV array identified several relevant differentially expressed proteins. The most significant candidate proteins, calculated based on p-values, were ABCA1, Human placental growth factor-2 (PIGF-2), and complement decay-accelerating factor (CD55) (Fig. 2D). Concurrently, as a control for fundamental EVs protein expression, CD81 and CD63 exhibited similar expression levels in both groups (**Supplementary Fig. 2**). This consistency in the expression of CD81 and CD63 indicated that both plasma sample groups contained comparable quantities of EVs. However, the AMD group exhibited lower expression levels of ABCA1, PIGF-2, and CD55 within these EVs. Consequently, these proteins can be considered characteristic proteins and included in the screening for diagnostic biomarkers. Additionally, we summarized the differentially expressed proteins with p-values less than 0.1 in the differential analysis. The results indicated that there were three differentially expressed proteins with p-values in the range of 0.05 to 0.1, namely endothelin-1 (ET-1), fibroblast growth factor 2 (FGF2), and glutamine synthetase (Fig. 2C). Specifically, in the AMD group, the expression of ET-1 and FGF2 was downregulated, whereas glutamine synthetase expression was upregulated **(Supplementary Fig. 3)**. The heat map of differential protein expression is shown in Fig. 2A, and the volcano map of differentially expressed proteins with p-values less than 0.05 and in the range of 0 to 0.1 are shown in Fig. 2B,C, respectively.

Diagnostic Model Analysis Based on Machine Learning

Based on the analysis of differentially expressed proteins using univariate methods, we identified six EV-associated proteins with p-values less than 0.1 that may serve as potential diagnostic biomarkers for AMD. To fur-

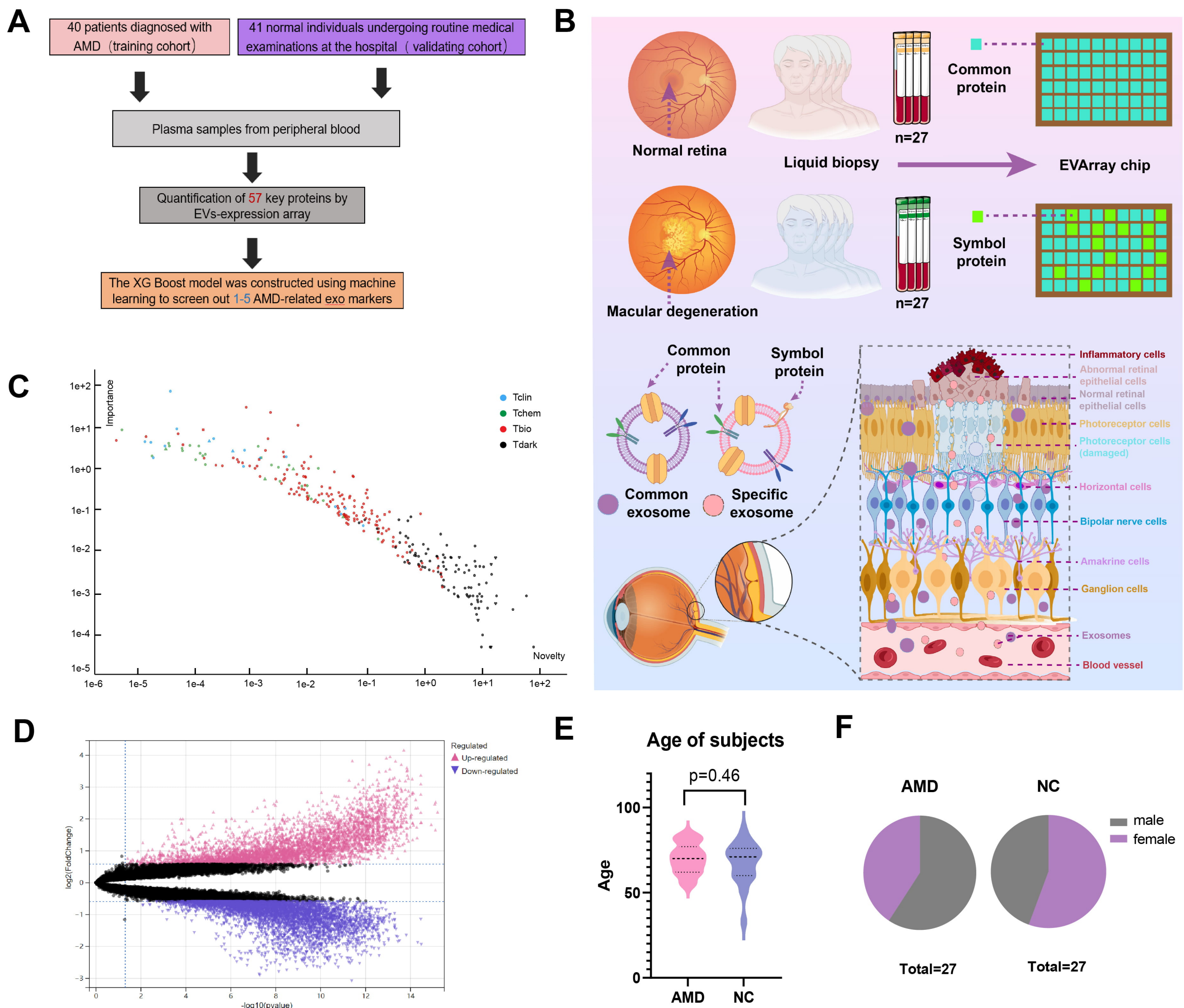

Fig. 1. Design of study protocols and collection of plasma samples. (A) The flow of patient enrollment. (B) Schematic illustration of the study's mechanism and detailed retinal structure. (C) Age-related macular degeneration (AMD)-related proteins were identified using the online tool Tin-X, annotated by clinical classification (blue), drug targets (green), biological functions (red), and others (black). (D) Differential protein analysis (volcano plot) of AMD and normal control patients from the Gene Expression Omnibus (GEO) dataset GSE29801. (E,F) Age and gender distribution of all patients whose samples were tested and analyzed. NC, normal control.

ther refine these candidate biomarkers, we employed the Boruta algorithm for feature selection (Fig. 2F), which identified three significant variables: ET-1, ABCA1, and Recombinant Protein (CRB1) (with ABCA1 and CRB1 being tentative variables).

Subsequently, we utilized the SVM-RFE algorithm, which revealed that the highest accuracy was achieved when five variables remained in the matrix (Fig. 2G). These five variables were ET-1, ABCA1, PIGF-2, complement factor I (CFI), and CD9.

Lastly, we applied the Lasso regression model to extract protein features, identifying four with non-zero coefficients out of the 57 protein features: Tissue Inhibitor of Metalloprotein (TIMP-1), ABCA1, PIGF-2, and CD55 (Fig. 2H). Consequently, we constructed three machine learning models to analyze the expression levels of the 57 relevant proteins in the samples.

We intersected these biomarker results obtained from the three machine learning models with the previous set of analysis results based on the differentially expressed protein, as illustrated in the Venn diagram (Fig. 2E). Through this screening process, we concluded that ABCA1 is the most promising EV-associated biomarker with diagnostic potential for AMD.

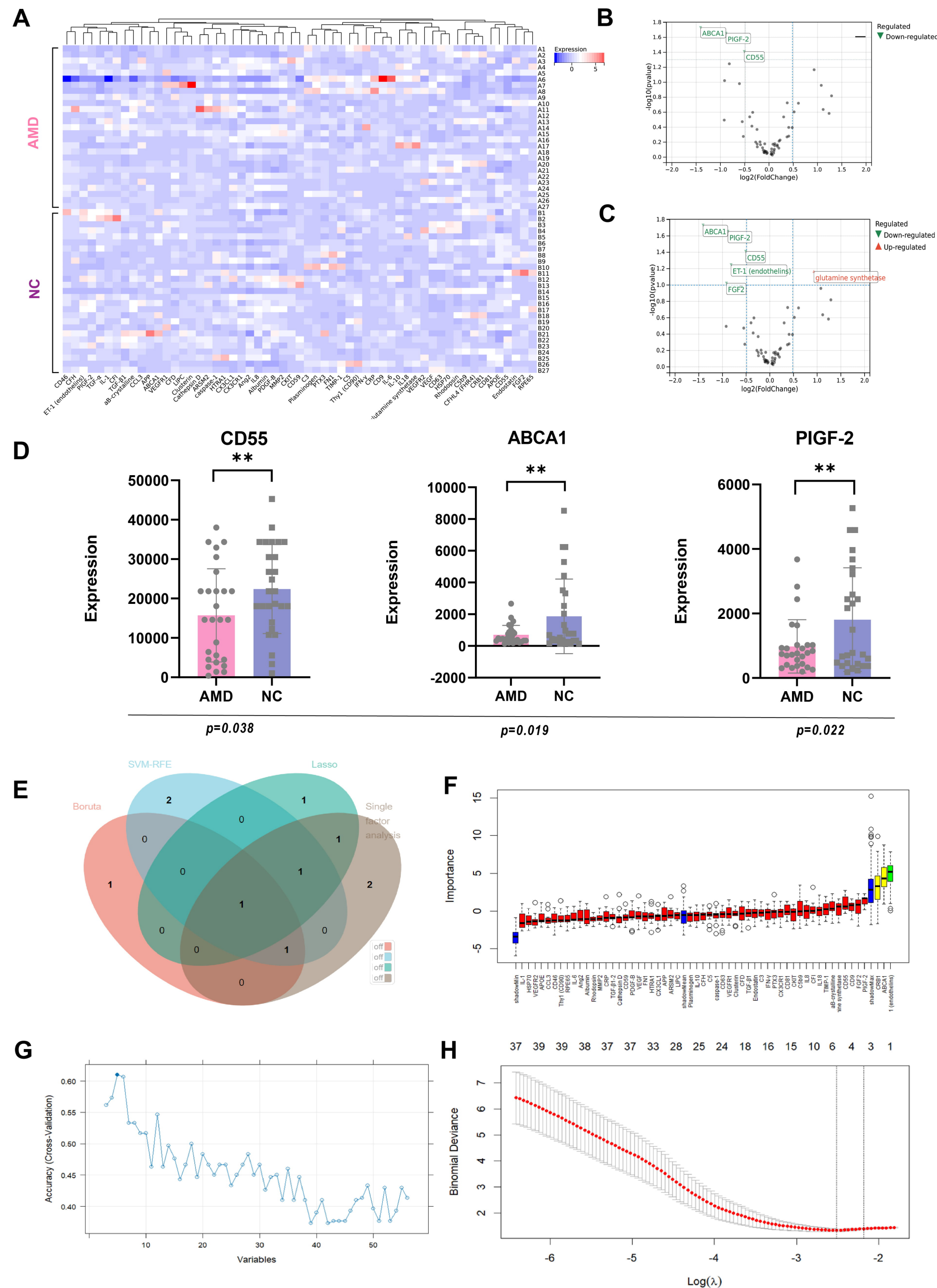

Fig. 2. Analysis of extracellular vesicle (EV) array detection results and screening of diagnostic markers. (A) Heatmap analysis of protein expression in all 54 included blood samples. The frequency of expression is indicated by color distribution. A1–A27 are samples in the AMD group, while B1–B27 are those in the NC group. (B) Volcano plot of differentially expressed proteins with $p < 0.05$ identified from EV array results (green indicates proteins with lower expression in the AMD group). (C) Volcano plot of differentially expressed proteins with $p < 0.1$ from EV array results (green indicates proteins with lower expression in the AMD group, while red denotes proteins with higher expression in the AMD group). (D) Statistical distribution of CD55/ABCA1/PIGF-2 expression in samples. **$p < 0.05$. (E) Venn diagram of differentially expressed proteins identified through univariate analysis, Boruta algorithm, SVM-RFE algorithm, and Lasso algorithm. (F) Effective diagnostic biomarkers identified by the Boruta algorithm using machine learning (green represents significant variables, and yellow represents tentative variables). (G) Effective biomarkers were identified by the SVM-RFE algorithm using machine learning (the number of top features is indicated by the highest point on the plot, with five features shown). (H) Effective biomarkers were identified by the Lasso algorithm using machine learning (there is a total of four feature proteins with non-zero coefficients). SVM-RFE, Support Vector Machine Recursive Feature Elimination; Lasso, Least Absolute Shrinkage and Selection Operator; CD55, complement decay-accelerating factor; ABCA1, ATP-binding cassette transporter A1; PIGF-2, placental growth factor-2.

Application of ABCA1 in Diagnosis of Age-Related Macular Degeneration

Following the analysis of the EV array chip detection results, we preliminarily identified ABCA1 as the most diagnostically significant biomarker among all 57 selected EV surface proteins. In this study, we developed a diagnostic model based on ABCA1 for all 57 samples, by utilizing the random forest method. The samples were randomly divided into a training set (70%) and a validation set (30%). The final results are shown in Fig. 3A. ABCA1 achieved a prediction accuracy of 0.737 and 0.625 in the training and validation sets, respectively.

To further investigate the validity of this diagnostic biomarker, we characterized ABCA1 in the samples using colloidal gold electron microscopy, as depicted in Fig. 3B. The AMD group exhibited higher expression of ABCA1 in colloidal gold immunoelectron microscopy compared to the NC group.

Additionally, during the study, we randomly collected fundus photographs and corresponding OCT images of patients with high and low ABCA1 expression (Fig. 3C). The results demonstrated that ABCA1 effectively distinguishes between different conditions of fundus changes. These findings suggested that ABCA1 can serve as an EV protein-based biomarker for clinically diagnosing AMD.

Discussion

The current body of research on EVs in AMD has focused primarily on their biogenesis, the cargo they carry, and their functional roles in retinal homeostasis and pathology [31]. Studies have demonstrated that EVs derived from retinal pigment epithelial cells, which play a critical role in AMD, can influence inflammation, angiogenesis, and cell survival, all of which are pertinent to AMD pathogenesis [31,32]. For instance, EVs have been shown to carry pro-inflammatory cytokines, angiogenic factors, and oxidative stress-related molecules, contributing to the complex microenvironment that drives AMD progression [33,34].

Within this burgeoning field, our study identifies the EV protein ABCA1 as a novel and promising biomarker for AMD. ABCA1 is essential for regulating cholesterol and phospholipid efflux, and maintaining cellular lipid balance [35,36]. In AMD, ABCA1 plays a significant role due to its involvement in lipid metabolism, inflammation, and oxidative stress. It helps prevent lipid accumulation in retinal pigment epithelial cells, crucial for maintaining retinal function and preventing damage that can worsen AMD [37,38]. Additionally, ABCA1 inhibits inflammatory responses through reverse cholesterol transport, potentially slowing AMD progression since chronic inflammation is a key factor in the disease [39]. By modulating cholesterol transport, ABCA1 reduces oxidative stress-induced retinal damage, thereby protecting vision. Therefore, the absence of ABCA1 may be an important related factor leading to the aggravation of macular degeneration. In the present study, we analyzed 57 surface proteins on EVs by utilizing the EV array chip technology and pinpointed ABCA1 as a candidate marker with potential for AMD diagnosis. The subsequent construction of a diagnostic model using a random forest approach yielded a tool, whose high accuracy was validated across training and validation sets, underscoring ABCA1's diagnostic potential in clinical settings.

The elevated expression of ABCA1 in AMD samples, confirmed through colloidal gold electron microscopy, provides compelling evidence of its involvement in AMD pathology. Our findings suggested that ABCA1-positive EVs could be reflective of the altered lipid metabolism and transport processes that are central to AMD development. The distinct fundus changes observed in patients with varying ABCA1 expression levels further support the hypothesis that ABCA1 is not merely a marker but may also play an active role in disease progression. These insights open avenues for the potential use of ABCA1 as a biomarker for early AMD diagnosis, enabling timely intervention and management.

Additionally, through this study, we also observed that placental growth factor-2 (PIGF-2), CD55, FGF2, ET-1, and glutamine synthetase exhibited notably differential expression compared to other proteins. Although these results have not been clinically validated, they still hold promise as potential biomarkers. Among these proteins, PIGF-2, CD55, FGF2, and ET-1 are more involved in angiogenesis and inflammatory responses [40,41], playing critical roles in influencing the pathophysiology of AMD [42–44]. On the other hand, glutamine synthetase has a primary role in nutritional metabolism [45]. This is probably due to secondary changes resulting from the increased metabolic demands of the diseased retinal pigment epithelial cells [46]. Further in-depth studies are needed to understand the detailed diagnostic value of these proteins. Nevertheless, through a comprehensive analysis of these proteins, this study successfully expanded the range of potential diagnostic markers for AMD.

One of the main strengths of our research lies in the comprehensive approach to protein analysis on EVs, leveraging the advanced EV array chip and robust statistical methods. Despite the promising results, our study is not without limitations. The foremost shortcoming of this study is the relatively small sample size employed. This calls for larger, multicentric studies to validate our findings [47]. Additionally, while this study provides preliminary insights into the role of ABCA1 in AMD, the mechanistic pathways through which ABCA1-positive EVs contribute to AMD pathogenesis are not fully elucidated in this work. Future studies should aim to dissect these pathways and explore whether modulating ABCA1 levels could have therapeutic implications.

From this study, we recognize the need of tracking AMD progression over time, which is lacking in this cross-

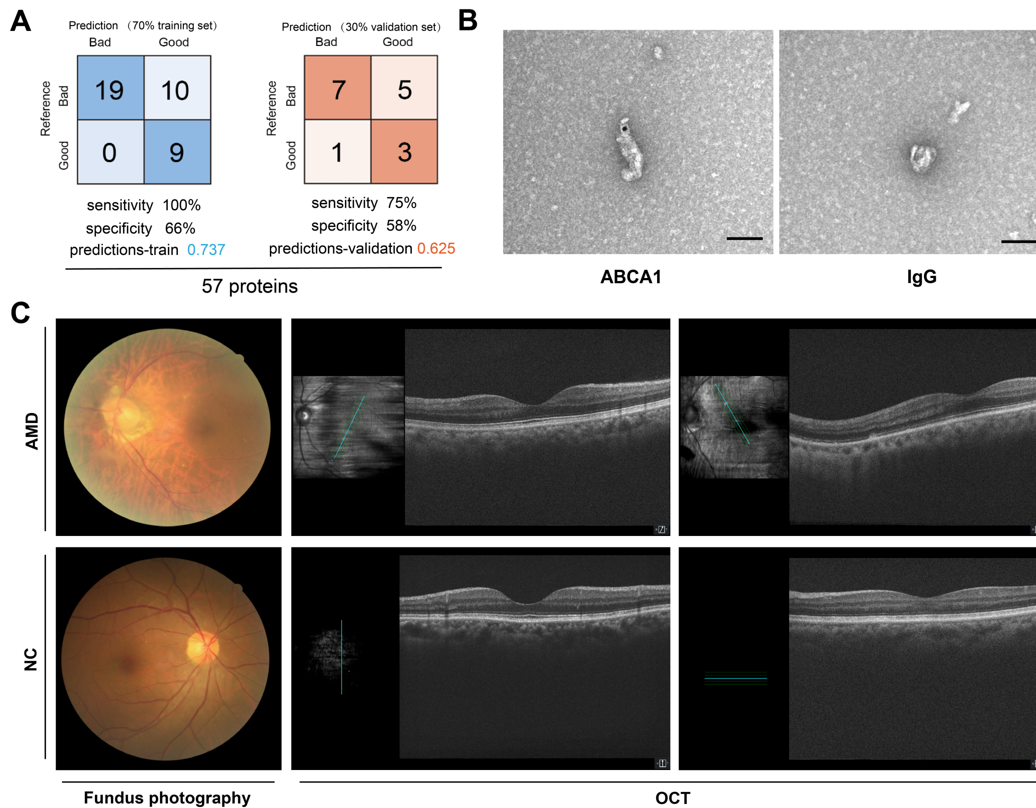

Fig. 3. Clinical validation of extracellular vesicle-associated ATP-binding cassette transporter A1 (ABCA1) protein as a diagnostic biomarker. (A) Assessment of the diagnostic efficacy of ABCA1 using a random forest algorithm with a training set (70%) and a validation set (30%) (the sensitivity and specificity were analyzed, and the predictive index was calculated). (B) Colloidal gold electron microscopy images of ABCA1 on extracellular vesicles (the black region denotes ABCA1-positive area; IgG was used as negative control; scale bar = 100 nM). (C) Fundus photography and optical coherence tomography (OCT) examination results for two representative patients with relatively high ABCA1 expression and two representative patients with low ABCA1 expression.

sectional study, to further investigate the clinical significance of the ABCA1 protein. Additionally, it is essential to explore the relationship between biomarkers and the disease mechanisms of AMD more thoroughly. Given the distinct physiological bases of dry and wet AMD, in future research, it is also crucial to identify more discriminating biomarkers with the diagnostic potential to clarify these differences.

The present study highlights the potential of the EV protein ABCA1 as a diagnostic marker for AMD. Our analysis using the EV array chip identified that ABCA1 stands out as the most promising candidate among the 57 surface proteins analyzed. The diagnostic model, constructed with a random forest approach (**Supplementary material Part 3**), demonstrates significant accuracy, as validated in the training and validation sets. Furthermore, the elevated expression of ABCA1 in AMD samples, confirmed through colloidal gold electron microscopy, underscores its relevance to the pathological context of AMD. The distinction in fundus changes between patients with different ABCA1 expression levels further supports the biomarker's diagnostic utility. Collectively, these findings position ABCA1 as a viable biomarker for AMD, with potential implications for early diagnosis and tailored therapeutic strategies. Further research is warranted to explore the mechanistic role of ABCA1 in AMD and to validate its clinical application in larger cohorts.

Conclusion

In summary, as effectively pinpointed through EV protein chip analysis assisted with machine learning techniques, ABCA1 stands out as a biomarker for the detection of AMD. The innovative approach employed represents a promising advancement in liquid biopsy diagnostics, providing an impetus to improving the clinical diagnosis and management of AMD.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

WS and TL conceived the idea and supervised the research; RC and YW designed the experiments; RC, YW, and YF collected clinical specimens; RC and YW finished the data analysis; RC, YW, TL, and WS co-wrote the paper; All authors discussed the results and commented on the manuscript. All authors were involved in the drafting and critical revision of the manuscript. All authors have read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The procedures involved in clinical sample collection had been reviewed and approved by the Ethics Committee of the Affiliated Hospital of Nanjing University of Chinese Medicine (2024NL-086-02). Prior to the collection of blood samples, all enrolled patients gave their informed consent, and all sample collection procedures for this study were performed in accordance with the "Declaration of Helsinki of the World Medical Congress".

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Conflict of Interest

The authors declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.24976/Discov.Med.202436191.217.

References

[1] Guymer RH, Campbell TG. Age-related macular degeneration. Lancet (London, England). 2023; 401: 1459–1472.
[2] Flores R, Carneiro Â, Vieira M, Tenreiro S, Seabra MC. Age-Related Macular Degeneration: Pathophysiology, Management, and Future Perspectives. Ophthalmologica. Journal International D'ophtalmologie. International Journal of Ophthalmology. Zeitschrift Fur Augenheilkunde. 2021; 244: 495–511.
[3] Nashine S. Potential Therapeutic Candidates for Age-Related Macular Degeneration (AMD). Cells. 2021; 10: 2483.
[4] Al-Zamil WM, Yassin SA. Recent developments in age-related macular degeneration: a review. Clinical Interventions in Aging. 2017; 12: 1313–1330.
[5] Thomas CJ, Mirza RG, Gill MK. Age-Related Macular Degeneration. The Medical Clinics of North America. 2021; 105: 473–491.
[6] Fabre M, Mateo L, Lamaa D, Baillif S, Pagès G, Demange L, *et al*. Recent Advances in Age-Related Macular Degeneration Therapies. Molecules (Basel, Switzerland). 2022; 27: 5089.
[7] Wheeler S, Mahmoudzadeh R, Randolph J. Treatment for dry age-related macular degeneration: where we stand in 2024. Current Opinion in Ophthalmology. 2024; 35: 359–364.
[8] Fleckenstein M, Schmitz-Valckenberg S, Chakravarthy U. Age-Related Macular Degeneration: A Review. JAMA. 2024; 331: 147–157.
[9] Guymer RH. Age-Related Macular Degeneration: Not So Wet and Dry. Ophthalmology. Retina. 2020; 4: 249.
[10] Kulkarni AD, Kuppermann BD. Wet age-related macular degeneration. Advanced Drug Delivery Reviews. 2005; 57: 1994–2009.
[11] Stahl A. The Diagnosis and Treatment of Age-Related Macular Degeneration. Deutsches Arzteblatt International. 2020; 117: 513–520.
[12] Cho YK, Park DH, Jeon IC. Medication Trends for Age-Related Macular Degeneration. International Journal of Molecular Sciences. 2021; 22: 11837.
[13] Podoleanu AG. Optical coherence tomography. Journal of Microscopy. 2012; 247: 209–219.
[14] Dauerman HL. Optical Coherence Tomography - Light and Truth. The New England Journal of Medicine. 2023; 389: 1523–1525.
[15] Keenan TDL, Cukras CA, Chew EY. Age-Related Macular Degeneration: Epidemiology and Clinical Aspects. Advances in Experimental Medicine and Biology. 2021; 1256: 1–31.
[16] Cicinelli MV, Rabiolo A, Sacconi R, Carnevali A, Querques L, Bandello F, *et al*. Optical coherence tomography angiography in dry age-related macular degeneration. Survey of Ophthalmology. 2018; 63: 236–244.
[17] Vidal-Oliver L, Montolío-Marzo E, Gallego-Pinazo R, Dolz-Marco R. Optical coherence tomography biomarkers in early and intermediate age-related macular degeneration: A clinical guide. Clinical & Experimental Ophthalmology. 2024; 52: 207–219.
[18] Lin AA, Nimgaonkar V, Issadore D, Carpenter EL. Extracellular Vesicle-Based Multianalyte Liquid Biopsy as a Diagnostic for Cancer. Annual Review of Biomedical Data Science. 2022; 5: 269–292.
[19] Min L, Wang B, Bao H, Li X, Zhao L, Meng J, *et al*. Advanced Nanotechnologies for Extracellular Vesicle-Based Liquid Biopsy. Advanced Science (Weinheim, Baden-Wurttemberg, Germany). 2021; 8: e2102789.
[20] Zheng X, Li X, Wang X. Extracellular vesicle-based liquid biopsy holds great promise for the management of ovarian cancer. Biochimica et Biophysica Acta. Reviews on Cancer. 2020; 1874: 188395.
[21] Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, *et al*. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Molecular Cancer. 2022; 21: 79.
[22] Wang AL, Lukas TJ, Yuan M, Du N, Tso MO, Neufeld AH. Autophagy and exosomes in the aged retinal pigment epithelium: possible relevance to drusen formation and age-related macular degeneration. PloS One. 2009; 4: e4160.
[23] Liu J, Jiang F, Jiang Y, Wang Y, Li Z, Shi X, *et al*. Roles of Exosomes in Ocular Diseases. International Journal of Nanomedicine. 2020; 15: 10519–10538.
[24] Klingeborn M, Dismuke WM, Bowes Rickman C, Stamer WD. Roles of exosomes in the normal and diseased eye. Progress in Retinal and Eye Research. 2017; 59: 158–177.
[25] Hernandez BJ, Skiba NP, Plössl K, Strain M, Liu Y, Grigsby D, *et al*. Polarized Desmosome and Hemidesmosome Shedding via Small Extracellular Vesicles is an Early Indicator of Outer

Blood-Retina Barrier Dysfunction. Journal of Extracellular Biology. 2023; 2: e116.
[26] Newman AM, Gallo NB, Hancox LS, Miller NJ, Radeke CM, Maloney MA, *et al*. Systems-level analysis of age-related macular degeneration reveals global biomarkers and phenotype-specific functional networks. Genome Medicine. 2012; 4: 16.
[27] Zhang C, Chong X, Jiang F, Gao J, Chen Y, Jia K, *et al*. Plasma extracellular vesicle derived protein profile predicting and monitoring immunotherapeutic outcomes of gastric cancer. Journal of Extracellular Vesicles. 2022; 11: e12209.
[28] Chen D, Li L, Wang M, Hu X, Jiang J, Li W, *et al*. Efficacy and safety of immunotherapy combined with single-agent chemotherapy as second- or later-line therapy for metastatic non-small cell lung cancer. Frontiers in Immunology. 2023; 14: 1086479.
[29] Jung AL, Møller Jørgensen M, Bæk R, Artho M, Griss K, Han M, *et al*. Surface proteome of plasma extracellular vesicles as mechanistic and clinical biomarkers for malaria. Infection. 2023; 51: 1491–1501.
[30] Jørgensen M, Bæk R, Pedersen S, Søndergaard EKL, Kristensen SR, Varming K. Extracellular Vesicle (EV) Array: microarray capturing of exosomes and other extracellular vesicles for multiplexed phenotyping. Journal of Extracellular Vesicles. 2013; 2.
[31] Manai F, Smedowski A, Kaarniranta K, Comincini S, Amadio M. Extracellular vesicles in degenerative retinal diseases: A new therapeutic paradigm. Journal of Controlled Release: Official Journal of the Controlled Release Society. 2024; 365: 448–468.
[32] Ahn JY, Datta S, Bandeira E, Cano M, Mallick E, Rai U, *et al*. Release of extracellular vesicle miR-494-3p by ARPE-19 cells with impaired mitochondria. Biochimica et Biophysica Acta. General Subjects. 2021; 1865: 129598.
[33] Wooff Y, Cioanca AV, Chu-Tan JA, Aggio-Bruce R, Schumann U, Natoli R. Small-Medium Extracellular Vesicles and Their miRNA Cargo in Retinal Health and Degeneration: Mediators of Homeostasis, and Vehicles for Targeted Gene Therapy. Frontiers in Cellular Neuroscience. 2020; 14: 160.
[34] Mukai A, Otsuki Y, Ito E, Fujita T, Ueno M, Maeda T, *et al*. Mitochondrial miRNA494-3p in extracellular vesicles participates in cellular interplay of iPS-Derived human retinal pigment epithelium with macrophages. Experimental Eye Research. 2021; 208: 108621.
[35] Wang Y, Guo M, Tang CK. History and Development of ABCA1. Current Problems in Cardiology. 2024; 49: 102036.
[36] Segrest JP, Tang C, Song HD, Jones MK, Davidson WS, Aller SG, *et al*. ABCA1 is an extracellular phospholipid translocase. Nature Communications. 2022; 13: 4812.
[37] Dai B, Lei C, Lin R, Tao L, Bin Y, Peng H, *et al*. Activation of liver X receptor α protects amyloid β_{1-40} induced inflammatory and senescent responses in human retinal pigment epithelial cells. Inflammation Research. 2017; 66: 523–534.
[38] Farhan F, Almarhoun M, Wong A, Findlay AS, Bartholomew C, Williams MTS, *et al*. Deletion of TSPO Causes Dysregulation of Cholesterol Metabolism in Mouse Retina. Cells. 2021; 10: 3066.
[39] Duncan KG, Hosseini K, Bailey KR, Yang H, Lowe RJ, Matthes MT, *et al*. Expression of reverse cholesterol transport proteins ATP-binding cassette A1 (ABCA1) and scavenger receptor BI (SR-BI) in the retina and retinal pigment epithelium. The British Journal of Ophthalmology. 2009; 93: 1116–1120.
[40] Engelbrecht C, Sardinha LR, Rizzo LV. Cytokine and Chemokine Concentration in the Tear of Patients with Age-Related Cataract. Current Eye Research. 2020; 45: 1101–1106.
[41] Liukkonen MPK, Paterno JJ, Kivinen N, Siintamo L, Koskela AKJ, Kaarniranta K. Epithelial-mesenchymal transition-related serum markers ET-1, IL-8 and TGF-β2 are elevated in a Finnish wet age-related macular degeneration cohort. Acta Ophthalmologica. 2022; 100: e1153–e1162.
[42] Barillari G, Albonici L, Franzese O, Modesti A, Liberati F, Barillari P, *et al*. The basic residues of placenta growth factor type 2 retrieve sequestered angiogenic factors into a soluble form: implications for tumor angiogenesis. The American Journal of Pathology. 1998; 152: 1161–1166.
[43] Han X, Gharahkhani P, Mitchell P, Liew G, Hewitt AW, MacGregor S. Genome-wide meta-analysis identifies novel loci associated with age-related macular degeneration. Journal of Human Genetics. 2020; 65: 657–665.
[44] Haas P, Aggermann T, Nagl M, Steindl-Kuscher K, Krugluger W, Binder S. Implication of CD21, CD35, and CD55 in the pathogenesis of age-related macular degeneration. American Journal of Ophthalmology. 2011; 152: 396–399.e1.
[45] Eelen G, Dubois C, Cantelmo AR, Goveia J, Brüning U, DeRan M, *et al*. Role of glutamine synthetase in angiogenesis beyond glutamine synthesis. Nature. 2018; 561: 63–69.
[46] Huan T, Cheng SY, Tian B, Punzo C, Lin H, Daly M, *et al*. Identifying Novel Genes and Variants in Immune and Coagulation Pathways Associated with Macular Degeneration. Ophthalmology Science. 2022; 3: 100206.
[47] Jørgensen MM, Bæk R, Varming K. Potentials and capabilities of the Extracellular Vesicle (EV) Array. Journal of Extracellular Vesicles. 2015; 4: 26048.