Ageing- and AAA-associated differentially expressed proteins identified by proteomic analysis in mice

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ABSTRACT

Background: Abdominal aortic aneurysm (AAA) is a disease of high prevalence in old age, and its incidence gradually increases with increasing age. There were few studies about differences in the circulatory system in the incidence of AAA, mainly because younger patients with AAA are fewer and more comorbid nonatherosclerotic factors.

Method: We induced AAA in ApoE−/− male mice of different ages (10 or 24 weeks) and obtained plasma samples. After the top 14 most abundant proteins were detected, the plasma was analyzed by a proteomic study using the data-dependent acquisition (DDA) technique. The proteomic results were compared between different groups to identify age-related differentially expressed proteins (DEPs) in the circulation that contribute to AAA formation. Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and protein–protein interaction (PPI) network analyses were performed by R software. The top 10 proteins were determined with the MCC method of Cytoscape, and transcription factor (TF) prediction of the DEPs was performed with iRegulon (Cytoscape).

Results: The aortic diameter fold increase was higher in the aged group than in the youth group (p < 0.01). Overall, 92 DEPs related to age and involved in AAA formation were identified. GO analysis of the DEPs showed enrichment of the terms wounding healing, response to oxidative stress, regulation of body fluid levels, ribose phosphate metabolic process, and blood coagulation. The KEGG pathway analysis showed enrichment of the terms platelet activation, complement and coagulation cascades, glycolysis/gluconeogenesis, carbon metabolism, biosynthesis of amino acids, and ECM-receptor interaction. The top 10 proteins were Tpi1, Eno1, Prdx1, Ppia, Prdx6, Vwf, Fga, Fgg, and Fgb, and the predicted TFs of these proteins were Nfe2, Srf, Epas1, Tbp, and Hoxc8.

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Conclusion: The identified proteins related to age and involved in AAA formation were associated with the response to oxidative stress, coagulation and platelet activation, and complement and inflammation pathways, and the TFs of these proteins might be potential targets for AAA treatments. Further experimental and biological studies are needed to elucidate the role of these age-associated and AAA-related proteins in the progression of AAA.

Subjects  Cardiology, Pathology
Keywords  Abdominal aortic aneurysm, Age-associated circulation differences, Proteomic analysis, LC–MS/MS

INTRODUCTION
Abdominal aortic aneurysms (AAAs) are the most common form of aortic aneurysm, and AAA rupture and associated catastrophic physiological damage result in an overall mortality rate of over 80%; morbidity has been shown in up to 8% of men aged >65 years (George et al., 2015; Sakalihasan, Limet & Defawe, 2005). The incidence rate of AAA in males aged >65 years increases by 40% every 5 years, indicating that age is a significant risk factor for AAA (Chichester Aneurysm Screening Group et al., 2001; Nordon et al., 2011). Histopathologically, AAA is related to increased inflammatory cell infiltration, abnormal oxidative stress, medial elastin degradation, and medial collagen deposition (Daugherty & Cassis, 2002; Maegdefessel, Dalman & Tsao, 2014). With age-related alterations, several alterations in the vasculature, such as enhanced inflammatory response, vascular stiffening, and oxidative stress, predispose aged arteries to vascular disease (Bourantas et al., 2018; Dominic et al., 2020). Furthermore, the relationship between AAA and aging is also reflected at the cellular level. Cellular senescence is a hallmark of aging and might play a vital role in the development of AAA, and this link has been shown in many previous studies. In intra-aortic walls, senescence of vascular mesenchymal stromal cells impairs their remodeling ability, which can support the formation and development of AAA, linking vascular senescence and inflammation (Chen et al., 2016; Teti et al., 2021). Moreover, the accumulation of senescent cells in the perivascular adipose tissue and abdominal aortic tissue promotes the formation of aneurysms through induction of inflammation, oxidative stress, and leukocyte adhesion (Parvizi et al., 2021).

In addition to the changes in the aortic wall and surrounding microenvironment that affect the formation of AAA, changes in the composition of the circulatory system, which result in high AAA morbidity in older people, might play an essential role in the formation of AAA. Increased insulin-like growth factor 1 (IGF1) expression and an increased IGF1/IGFBP3 ratio were reported to be associated with AAA, whereas IGFBP1 was independently associated with increased aortic diameter. Components of the IGF1 system may contribute to or be markers of aortic dilatation in older adults (Yeap et al., 2012). Elevated tHcy is associated with the presence of AAA in older men (Wong et al., 2013). Levels of the biomarkers C-reactive protein (CRP), pronectensin (PNT), copeptin (CPT), lipoprotein-associated phospholipase 2 (Lp-PLA2), cystatin C (Cyst C), midregional proatrial natriuretic peptide (MR-proANP), and midregional...
proadrenomedullin (MR-proADM) were not associated with the aortic diameter at ultrasound examination after 14–19 years of follow-up (Taimour et al., 2017). Given that AAA cases can be sporadic and/or associated with genetic diseases and infections, exploration of AAA models in different age groups is warranted.

Overall, it is still unclear why elderly patients (>65 years) are most likely to develop AAA, and the mechanisms for the significantly accelerated development and progression of AAA in these patients remain to be determined. We hypothesize that aging is associated with the formation of AAA, and there may be age-related changes in the circulatory system in addition to changes in the senescence of cells in the aortic wall. Therefore, we generated AAA models by using mice of different ages, and we examined the changes in their plasma using proteomic techniques to uncover age-related changes in the circulatory system that promote AAA formation.

**METHODS AND MATERIALS**

**Construction of the AAA animal model and sample collection**

For the mice model used in this study, ApoE knockout (KO) mice on a C57BL/6 background were obtained from the Beijing Vitalriver Laboratory Animal Co. (Beijing, China). All mice were raised in specific-pathogen-free conditions under 12/12 cycle of light at room temperature (24–27 °C) and allowed free access to food and water in a specific pathogen-free environment. The AAA model was generated by administering Ang II (1,000 ng/kg per minute; Sigma–Aldrich, St. Louis, MO, USA) or saline to male mice (10 weeks of age in the youth group or 24 weeks of age in the aged group) for 28 d via implantation of ALZET mini-osmotic pumps (model 2007; DURECT, Cupertino, CA, USA), as in our previous studies (Ren et al., 2015; Zhang et al., 2020). The mice were, mice were euthanized by cervical dislocation after inhalational anesthesia with 0.1% pentobarbital, and the blood of mice was collected by cardiac puncture at 16 weeks of age for the youth group (or at 28 weeks for the aged group) for further histological and molecular analyses. The diameter of the abdominal aorta in mice was measured by vernier caliper, and the diameter increased more than fifty percent, which was identified as aneurysm formation. All animal studies were approved by the Institutional Animal Care and Use Committee of Peking Union Medical College Hospital (JS-2629), and experiments conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication no. 85–23, 1996). The plasma was separated from the blood by centrifugation at 2,000 rpm/min for 10 min at 4 °C, and all samples were stored at −80 °C.

**Separation of the top 14 most abundant proteins**

The top 14 most abundant proteins in plasma were detected with Thermo Scientific High-Selected Top 14 Abundant Protein Detection Resin (A36370; Thermo Fisher Scientific, Waltham, MA, USA). The depletion spin column was equilibrated to room temperature, the column screw cap was removed, and up to 10 µL of plasma was added directly to the resin slurry in the column. The column was capped and inverted several times until the resin solution was completely homogenous. The mixture was incubated in the column with gentle end-over-end mixing for 10 min at room temperature. The sample
was ensured to mix with the resin during incubation period. Alternatively, the sample was gently vortexed every few minutes. After incubation, the bottom closure was snapped off, and the top cap was loosened. The mini column was placed into a two mL collection tube and centrifuged at 1,000g for 2 min. The column containing the resin was discarded, and the filtrate contained the sample with albumin, IgG, and other abundant proteins removed. The BCA assay (23225; Thermo Fischer Scientific, Waltham, MA, USA) was employed to measure the protein concentration of the plasma.

Protein digestion
The mouse plasma proteins were prepared using filter-aided sample preparation (FASP) methods (Wisniewski et al., 2009). The sample was deoxidized with 20 mM DTT (95 °C, 15 min) and alkylated with 50 mM IAA (room temperature in the dark, 45 min). Then, 6X precooled-acetone precipitation was used to extract proteins at −20 °C overnight. The proteins were redissolved with 20 mM Tris, loaded onto a 30 kDa cutoff ultrafiltration unit, and centrifuged at 14,000g at 4 °C. After being washed 3 times with 20 mM Tris, the samples were digested with MS-grade trypsin (1:50) at 37 °C overnight. After centrifugation at 4 °C for 10 min, the peptide solution was collected and stored at −80 °C for later use.

LC–MS/MS
Peptides were separated with a capillary liquid chromatography (LC) column (75 μm × 500 mm, C18, 3 μm; Kyoto Monotech, Kyoto, Japan). The following 60-min gradient at a flow rate of 0.6 µL/min was used: 0–1 min, 6–11% solvent B (mobile phase A: 0.1% formic acid in H₂O; mobile phase B: 0.1% formic acid in acetonitrile); 1–9 min, 11–17% solvent B; 9–40 min, 17–29% solvent B; 40–50 min, 29–37% solvent B; 50–55 min, 37–100% solvent B; 55–60 min, 100% solvent B. The autosampler temperature was set at 4 °C, and the column was maintained at ambient temperature. An Orbitrap Q-Exactive HF (Thermo Scientific, Dreieich, Germany) mass spectrometer was employed to analyze the eluted peptides from LC. Data were acquired with data-dependent acquisition (DDA) using the following parameters: data-dependent tandem mass spectrometry (MS/MS) scans per full scan in top-speed mode for 3 s; MS scan resolution of 120,000; MS/MS scan resolution of 30,000; 30% HCD collision energy; charge-state screening for +2 to +7; dynamic exclusion duration of 30 s; maximum injection time of 45 ms.

The raw MS data files were searched using SpectroMine (version 3.0; Biognosys, Schlieren, Switzerland) with Sequest HT against the SwissProt mouse database (http://www.UniProt.org) for quantitative and qualitative analysis. A maximum of two missed cleavages for trypsin was used; cysteine carbamidomethylation was set as a fixed modification, and oxidation (M), carbamylatation, cysteine carbamidomethylation and deamidation were used as variable modifications. Protein identifications were required to have a false discovery rate (FDR) of less than 1.0% at the protein level with at least two unique peptides.
Statistical analysis
Quantitative results are expressed as the mean± S.E.M. Comparisons of parameters between groups were made by t test. A value of \( p < 0.05 \) was considered to be statistically significant. Statistical significance was evaluated with R (4.1.1).

DEP analysis
Pearson correlation tests were performed to evaluate the significance of differences in protein expression between groups with the DEP software package of R (4.1.1). After the data were standardized using the limma software package of R, we identified DEPs by comparing AAA with normal samples in the youth group (or aged group). Principal component analysis (PCA) was implemented using R. If the change factor was greater than 1.2-fold (\(|\text{fold change}| \geq 1.2\) and the \( p \) value was \( \leq 0.05 \), a protein was considered to be differentially expressed. A volcano plot, heatmap, and Venn diagram of the DEPs were generated using the ggplot2, pheatmap, and Venn software packages of R, respectively.

GO, KEGG, and MeSH enrichment analyses
Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and MeSH enrichment analyses were performed using the clusterProfiler package (Yu et al., 2012) in R (4.1.1). A hypergeometric distribution was used to analyze and calculate the significance levels of the significantly affected signaling pathways of the DEPs (\( p < 0.05 \)).

PPI network and TF analyses
The STRING database was used to construct a protein–protein interaction (PPI) network of the aging-related DEPs. The PPI file was imported into Cytoscape (3.6.5). The degree, closeness, intermediate degree of each node in the network, and average value of each protein's nodal degree were defined as the threshold of the PPI network nodes, and the proteins whose degrees were more significant than the threshold value were selected. The key nodes (the hub proteins) of the PPI network were identified by the MCC of cytoHubba and mapped, and the correlation scores of the nodes and their interacting proteins were calculated. The functions of the hub proteins were predicted by the GeneMANIA app of Cytoscape. The transcription factors (TFs) of the top 10 hub proteins were predicted by iRegulon (Chen et al., 2016).

RESULTS
Workflow of the proteome analysis
In this study, 20 samples were enrolled and divided into four groups: the normal (youth) group included mice of ten weeks of age that received saline administration for 28 days (\( n = 5 \)); the AAA (youth) group included mice of ten weeks of age that received Ang II administration for 28 days (\( n = 5 \)); the normal (aged) group included mice of 24 weeks of age that received saline administration for 28 days (\( n = 5 \)); and the AAA (aged) group included mice of 24 weeks of age that received Ang II administration for 28 days (\( n = 5 \)). Fig. 1A shows the general workflow of this study. By measuring the diameter of the abdominal aorta in mice, it was found that the AAA/normal abdominal aortic diameter
increase (as represented by the AAA to normal ratio) in the elderly group was significantly greater than that in the young group (p < 0.01, Figs. 1B and 1C).

All of the samples were assessed in two technical replicates by DDA 2D-LC/MS analysis, and the mean value of two test results was taken for each sample. In the DDA analysis, 992 proteins were detected with a protein FDR <1%. For each sample, 700 protein groups were identified by peptide-spectrum matching. Pearson correlation analysis showed a major cluster of samples in relevant groups (Fig. 2A). In total, 535 protein groups with quantitative data in more than 70% of samples in each group were selected for further analysis (Fig. 2B). Sample normalization was good, and intergroup grouping was relatively straightforward (Figs. 2C and 2D).

**DEP analysis**

There were 70 DEPs between the AAA (youth) and normal (youth) groups. Thirty-three of them were upregulated, and 37 were downregulated in the AAA (youth) group compared to the normal (youth) group (Figs. 3A and 3B). In the AAA (aged) group compared with the normal (aged) group, 108 DEPs were identified, 67 of which were upregulated and 41 of which were downregulated in the AAA (aged) group (Figs. 3C and 3D). Moreover, we found that 138 DEPs were significantly different between the AAA (aged) group and the AAA (youth) group. Moreover, 83 of the DEPs were upregulated, while 55 were downregulated in the AAA (aged) group (Figs. 3E and 3F).
There were three independent DEP sets (Fig. 3G): AAA (youth) vs normal (youth) DEPs, AAA (aged) vs normal (aged) DEPs, and AAA (aged) vs AAA (youth) DEPs. In total, 92 DEPs that might be related to the aging progress and the formation of AAA were identified in the AAA (aged) vs AAA (youth) DEP set and the AAA (youth) vs normal
Figure 3 Differential expressed proteins analysis. The X-axis represents log2Fold Change, and Y-axis represents −log10 (p.Value). Red dots represent proteins with |foldchange| > 1.2 and −log(p.Value) < 0.05. (A) AAA (Youth) vs Normal (Youth); (C) AAA (Aged) vs Normal (Aged); (E) AAA (Aged) vs AAA (Youth). Heatmap of DEPs and the diagram presents the result of a two-way hierarchical clustering of all the DEPs and samples (B), AAA (Youth) vs Normal (Youth); (D) AAA (Aged) vs Normal (Aged); (F) AAA (Aged) vs AAA (Youth). The Veen diagrams show overlap.
DEP set (or the AAA (aged) vs normal (aged) DEP set) (Table 1). The DEPs were also divided and plotted in a Venn diagram in six differential subsets based on whether they were upregulated or downregulated in the sets (Fig. 3H). To further analyze the expression of DEPs in different sets, we generated Venn diagrams of upregulated and downregulated proteins. The upregulated Venn diagram showed 48 common DEPs in both the AAA (aged) vs normal (aged) and AAA (aged) vs AAA (youth) sets (Fig. 3I). The downregulated Venn diagram showed 18 common DEPs in both the AAA (aged) vs normal (aged) and AAA (aged) vs AAA (youth) sets (Fig. 3J).

Functional annotation of the DEPs
To explore the molecular mechanisms and biological progress of the DEPs, which might be related to aging and AAA formation, 89 proteins of the 92 DEPs (as 3 DEPs failed to match with Entrez ID proteins) were subjected to GO, KEGG, and MeSH annotation analyses. The results of the GO and KEGG pathway for DEPs are shown in Tables S1 and S2. The GO biological process (Sohrabpour, Kearns & Massari, 2016) analysis showed that the DEPs were associated with blood coagulation, hemostasis, coagulation, and hydrogen peroxide catabolic processes; the GO cellular component (CC) analysis showed that the DEPs were related to the cell cortex, platelet alpha granules, the myelin sheath, and blood microparticles; the GO molecular function (MF) analysis indicated the DEPs were related to antioxidant activity, peroxidase activity and oxidoreductase activity (Fig. 4A). The top five enriched GO terms were wound healing, response to oxidative stress, regulation of body fluid levels, ribose phosphate metabolic process and blood coagulation (Fig. 4B, Table 1). The primary enriched GO process terms were coagulation, hemostasis, and hydrogen peroxide catabolic process, which were associated with Gpx1, Prdx1, Prdx2, and Prdx6 (Fig. 4C). The DEPs showed enrichment of the KEGG pathway terms platelet activation, complement and coagulated cascades, glycolysis/gluconeogenesis, carbon metabolism, biosynthesis of amino acids, and ECM-receptor interaction (Figs. 4D and 4E, Table 1). The MeSH analysis of the DEPs showed annotation of the terms thrombosis, leukemia, lung diseases, anemia, and malaria (Fig. 4F).

Identification of hub proteins of the DEPs
The DEPs were mapped into a coexpression network obtained from previous protocols using STRING and Cytoscape. The weighted edge threshold was set as 71 nodes, and 358 edges were in the DEP coexpression network (Figs. 5A, Tables S3). The DEPs were clustered into six main clusters according to their function using STRING (MCL clusters) and mapped through Cytoscape (Fig. 5B). The most significant cluster of the DEP coexpression network detected by MCC (Cytoscape, CytoHubba) consisted of 10 genes (Fig. 5C, Table 2). Triosephosphate isomerase 1 (Tpi1, which belongs to the...
Figure 4 The significantly enriched GO, KEGG pathways, and Mesh words an analysis of the 92 DEPs which are age-related involved in AAA formation. The GO analysis on CC, BP, and MF (A), top 20 enriched GO progress of 92 DEPs (B), and top five enriched GO progresses with their related proteins were mapped (C). The KEGG pathways of 92 DEPs (D) and top five KEGG terms with their related proteins were mapped (E). The Mesh analysis of these DEPs, p < 0.05 (F).

Table 1 GO and KEGG pathways terms with top 5 count number of DEPs.

| ID     | Terms                                      | Count | p value     |
|--------|--------------------------------------------|-------|-------------|
| GO     |                                            |       |             |
| GO:0042060 | Wound healing                             | 14    | 4.21E–11    |
| GO:0006979 | Response to oxidative stress              | 14    | 2.59E–10    |
| GO:0005938 | Cell cortex                               | 14    | 7.03E–12    |
| GO:0050878 | Regulation of body fluid levels           | 13    | 6.08E–10    |
| GO:0019693 | Ribose phosphate metabolic process        | 13    | 7.19E–10    |
| KEGG   |                                            |       |             |
| mmu04611 | Platelet activation                        | 6     | 0.00011651  |
| mmu04610 | Complement and coagulation cascades        | 5     | 0.00027545  |
| mmu01200 | Carbon metabolism                          | 5     | 0.0009225   |
| mmu00010 | Glycolysis/Gluconeogenesis                 | 4     | 0.00079126  |
| mmu01230 | Biosynthesis of amino acids                | 4     | 0.00146719  |
triosephosphate isomerase family), alpha-enolase (Eno1, a multifunctional enzyme that, in addition to its role in glycolysis, plays a part in various processes, such as growth control, hypoxia tolerance, and allergic responses), and peroxiredoxin-1 (Prdx1, a protein that plays a role in cell protection against oxidative stress) were the proteins with the top three degrees in the cluster (Fig. 5C). We also analyzed the functions and networks of these hub proteins through GeneMANIA, and the main functions were peroxidase activity, purine ribonucleoside diphosphate metabolic processes, ADP metabolic processes, negative regulation of response to wounding, blood coagulation, endothelial cell apoptotic processes, and negative regulation of the extrinsic apoptotic signaling pathway (Fig. 5D). The TFs of the top 10 hub proteins were predicted by iRegulon, and the top 5 TFs were Nfe2, Srf, Epas1, Tbp, and Hoxc8 (Fig. 5E, Table 3).

### DISCUSSION

We conducted a proteome analysis of plasma from induced AAA mice and normal mice of different ages (youth group and aged group). Ninety-two DEPs in plasma were identified to be associated with aging and AAA formation. The GO analysis of these DEPs showed enrichment of the terms wound healing, response to oxidative stress, regulation of body fluid levels, ribose phosphate metabolic process and coagulation, processes that might be associated with the aging process and contribute to the formation of AAA. The main GO enrichment terms were wounding healing, response to oxidative stress, regulation of body fluid levels, ribose phosphate metabolic process and coagulation. The DEPs were associated with the KEGG pathway terms platelet activation, complement and coagulated cascades, glycolysis/gluconeogenesis, carbon metabolism, biosynthesis of amino acids, and ECM-receptor interaction. The hub protein cluster was the most significant in the DEP coexpression network selected by cytoHubba. The top 10 proteins in the cluster were Tpi1,
Eno1, Prdx1, Ppia, Prdx6, Vwf, Prdx2, Fga, Fgg, and Fgb. These proteins are potentially crucial proteins that are related to the aging process and the pathogenesis of AAA.

Arterial senescence may be associated with decreased vasodilation due to increased reactive oxygen species (ROS) production, whereas endothelial cell activation induces

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Table 3  The predicting transcription factors of Hub proteins.

| TF       | Uniport id | Full names                              |
|----------|------------|-----------------------------------------|
| Hoxc8    | P09025     | Homeobox protein Hox-C8                 |
| Tbp      | P62340     | TATA box-binding protein-like 1          |
| Epas1    | P97481     | Endothelial PAS domain-containing protein 1 |
| Srf      | Q9JM73     | Serum response factor                    |
| Nfe2     | Q07279     | Transcription factor NF-E2 45 kDa subunit |

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procoagulant changes, which are also related to the inflammation and oxidative stress of AAA (Sanchez-Infantes et al., 2021; Usui et al., 2015). ROS are produced in large amounts during inflammation and can stimulate connective tissue-degrading proteases and smooth muscle cell (SMC) apoptosis, and ROS are increased locally in AAA and lead to enhanced oxidative stress (Miller et al., 2002). ROS control extracellular matrix degradation and remodeling by upregulating proteolytic enzymes, such as MMPs, with concurrent significant elevations in oxidative stress and proteolytic enzyme expression within aneurysmal walls covered by thin thrombi (<10 mm) compared with thick thrombus-covered walls (≥25 mm) (Wiernicki et al., 2019). In our study, oxidative stress was closely related to the occurrence of AAA and aging, and we found that Prdx1, 2 and 6 also contributed to ageing and AAA formation. Prdx proteins are a ubiquitous thiol-specific antioxidant enzymes that controls the level of intracellular peroxides and are involved in oxidative stress and signal transduction; in addition, the mammalian Prdx family includes six members: Prdx1-6 (Rhee, Chae & Kim, 2005; Wood et al., 2003). Simvastatin may inhibit NF-κB activity by inhibiting free radicals and TNF-α production, improving human AAA wall tissue (Piechota-Polanczyk et al., 2012). Elevated Prdx-1 levels in the serum and tissues of AAA patients were associated with AAA size and the AAA growth rate (Martinez-Pinna et al., 2011; Ramella et al., 2018; Rasiova et al., 2019). Prdx2 plays a role as a negative regulator of the pathological process of AAA (Jeong et al., 2020; Martinez-Pinna et al., 2014). However, in other studies, Prdx2 was found to be an inducer of ruptured AAA (Urbonavicius et al., 2009), while Prdx6 expression was elevated in patients with AAA; in addition, in AAA tissue, prdx6 colocalized with neutrophils, vascular SMCs, and oxidized lipids. Prdx6 is elevated in AAA plasma, reflecting increased systemic oxidative stress, and there is a positive correlation between prdx6 levels and AAA diameter (Burillo et al., 2016). Our study found that Prdx2 was downregulated in the AAA (youth) group compared with the normal (youth) group. Furthermore, it was upregulated in the AAA (aged) group compared with the other groups, which indicates that Prdx2 might promote AAA formation in aged people, but more studies are needed.

Metabolic activities control cell fate, and glucose is the primary energy source for metabolic activities and an essential substrate for protein and lipid synthesis in mammalian cells. However, Increased glycolysis contributes to the pathogenesis of various diseases, such as cancer, degenerative diseases, metabolic syndrome, and infection (Afonso et al., 2020; Kolovou, Kolovou & Mavrogeni, 2014). Increased glycolysis is associated with many inflammatory processes and contributes to AAA formation, and macrophages produce inflammasomes (Sun et al., 2015). Recent studies have shown that the modified glucose analog 18-fluorodeoxyglucose (18F-FDG) accumulates in atherosclerosis-based AAA, suggesting an association between inflammation and glycolysis in the pathogenesis of AAA development. Intrapерitoneal injection of glycolysis inhibitors and 2-deoxyglucose significantly attenuated aortic aneurysm formation in male C57BL/6J mice with CaCl₂-induced abdominal aorta dilatation or male ApoE KO mice with decreased angiotensin II infusion. Increased glycolysis activity in the aortic wall contributes to the pathogenesis of aneurysm development (Tsuruda et al., 2012). Rescue intervention with the glycolytic inhibitor pfk15 in an AngII model showed that interfering with the glycolytic
switch prevented aneurysm formation (Gabel et al., 2021). Triosephosphate isomerase (Tpi1) can catalyze the switch between glycolysis and gluconeogenesis in the presence of dihydroxyacetone and 3-D-glyceraldehyde (Rodriguez-Almazan et al., 2008). Tpi1, a hub protein in our study, has been identified as a human ageing-related protein (de la Mora-de la Mora et al., 2015), but there has been less research in AAA. Alpha-enolase (Eno1) is a glycolytic enzyme that catalyzes the conversion of 2-phosphoglycerate into phosphoenolpyruvate, and it is involved in various processes, such as growth control, hypoxia tolerance and anaphylaxis (Huang et al., 2018). Eno1 may also function in the intravascular and pericellular fibrinolytic system due to its ability to act as a receptor and activator of plasminogen on the surface of several cell types, such as leukocytes and neurons. Eno1 is a significant facilitator of plasminogen activation on the leukocyte surface (Lopez-Alemany et al., 2003; Sugahara et al., 1992). Therefore, the roles of Tpi1 and Eno1 in the mechanism by which glycolysis contributes to AAA should be investigated.

In our study, the age-related DEPs in Ang II-induced AAA models involved in AAA formation were found to be significantly enriched in the coagulation and platelet activation pathways. Coagulation and platelet activation play a crucial role in the occurrence, progression, and rupture of AAA. By detecting the plasma concentrations of thrombin–antithrombin (TAT) complexes, platelet factor 4 (PF4), and D-dimers in AAA patients, it was found that an increase in D-dimers and TAT complex levels can predict progression of the disease and the growth of aneurysms in patients with AAA or subaortic dilatation (Sundermann et al., 2018). Inhibition of FXa/FIIa can downregulate the activation of the Smad2/3 signaling pathway and MMP2 expression mediated by protease-activated receptor 2 (PAR2) to limit the severity of aortic aneurysm and atherosclerosis (Moran et al., 2017). Intraluminal thrombus (ILT) is a common pathological condition in AAA and is closely related to the formation and expansion of aneurysms. Some studies have suggested that ILT can promote the progression of AAA (Michel et al., 2011). ILT seems to have a biomechanical function by reducing peak wall stress, but it also has biological activity. In addition to increasing adventitia inflammation, ILT may enhance the degradation of the aortic wall. With the prominent accumulation of leukocytes in the ILT layer of the cavity, red blood cells and platelets release oxidative and proteolytic molecules that promote AAA wall destruction. In addition, they secrete chemotaxis-related molecules, which may increase the number and type of recruited cells (Cameron, Russell & Owens, 2018). Platelet activation and thrombus regeneration are key to the development of endovascular thrombosis and AAA. Inhibition of platelet activation limits the biological activity of thrombi in the cavity, thereby inhibiting the development of aneurysms (Dai et al., 2009). Platelet aggregation may be responsible for thrombus turnover in AAA, with luminal thrombi releasing markers of platelet activation that can be readily detected in plasma (Touat et al., 2006). The increased platelet numbers with age and increased risk of arterial thrombosis may be related to the increased interactions between endothelial cell-attached leukocytes and platelets. Antithrombotic drugs, such as low-molecular-weight heparin, rivaroxaban, and other anticoagulation drugs, also inhibit the formation of abdominal aortic aneurysms (Grzela et al., 2008).
Von Willebrand factor (VWF) is a multisubunit protein that anchors platelets to subendothelial collagen. It serves as a carrier protein for the VIII factor in plasma, and endothelial cell release of VWF plays an essential role in the formation of platelet aggregates and thrombosis and is closely associated with aging (Alavi, Rathod & Jahroudi, 2021). We found that VWF was upregulated in the AAA (aged) group (compared with the normal (aged) group or the AAA (aged) group), suggesting that it might be associated with aging and AAA formation. In previous studies, VWF was found to be expressed in aneurysm tissue and colocalized with hepatocyte growth factor (HGF) in the most severe part of the aneurysm-injured wall (Shintani et al., 2011). As age increases, VWF levels increase significantly, and thrombosis events increase significantly (Alavi, Rathod & Jahroudi, 2021). Fibrinogen is a vital protein involved in the processes of coagulation and hemostasis and plays a crucial role in the process of AAA formation, and fibrinogen has been proven to be a plasma biomarker of AAA formation and progression (Sundermann et al., 2018). A specific anti-fibrinogen antibody can induce AAA in mice through complement lectin pathway activation, and circulating antibodies in a subset of AAA patients react against fibrinogen or fibrinogen-associated epitopes in human aneurysmal tissues (Zhou et al., 2013). However, in our study, we found that fibrinogen alpha (Fga), fibrinogen beta (Fgb), and fibrinogen gamma (Fgg), which are the subunits of fibrinogen, were downregulated in the AAA (aged) group (compared with the normal (aged) group or the AAA (aged) group). However, they were not differentially expressed between the AAA (youth) group and the normal (youth) group. Thus, coagulation, platelet activation and ILT affect AAA formation through inflammation and ROS, which might be highly related to Vwf, Fga, Fgb and Fgg.

The complement system is a part of innate immunity that has been proven to play a critical role in many inflammatory diseases and plays an essential role in the pathogenesis of AAA (Wu et al., 2010). The age-related DEPs in plasma that were associated with AAA formation in this study showed similar enrichment of complement activation cascade. The innate immune response to autoantigens activates the complement system and activates the inflammatory cascade in AAA, and the activation of the complement system promotes AAA (Zhou et al., 2012). Alternative complement pathway involvement is not limited to this experimental model but is also evident in human AAA (Pagano et al., 2009). Compared with those in healthy controls, the serum C5a levels in AAA patients were significantly increased, and the serum C5a level was also significantly correlated with the maximal AAA diameter (Zagrapan et al., 2021). The complement system is a crucial mechanism involved in vascular remodeling and plays an essential role in the pathogenesis of AAA (Martin-Ventura et al., 2019; Wu et al., 2010). The alternative complement pathway also plays a vital role in the development of AAA: natural IgG antibodies direct alternative pathway-mediated complement activation, and C3 deposition is present in elastase-perfused aortic walls (Zhou et al., 2012).

Peptidyl-prolyl cis-trans isomerase A (Ppia) catalyzes the cis-trans isomerization of proline imine peptide bonds in oligopeptides, exerts strong chemotactic effects on leukocytes, activates endothelial cells in a proinflammatory manner, promotes endothelial cell chemotaxis and apoptosis, and plays a vital role in platelet activation and aggregation.
Cyclophilin A (CyPA; encoded by PPIA) is a ubiquitously expressed protein secreted in response to inflammatory stimuli that stimulates vascular SMC migration and proliferation, endothelial cell adhesion molecule expression, and inflammatory cell chemotaxis, thereby promoting atherosclerosis (Nigro et al., 2011). CyPA is highly expressed in vascular SMCs, secreted in response to ROS, and promotes inflammation, and ApoE−/−Ppia−/− mice are entirely protected from AngII-induced AAA formation because they have reduced inflammatory cytokine expression, elastic lamina degradation, and aortic dilation (Satoh et al., 2009).

Our study predicted Nfe2, Srf, Epa1, Tbp, and Hoxc8 as potential TFs of the hub genes. Nuclear factor erythroid 2 (Nfe2) is a TF that plays a vital role in the response to oxidative stress and can bind to antioxidant response elements present in the promoter regions of many cytoprotective genes and promote their expression, thereby functioning as an antioxidant response protein (Chaohui et al., 2018; Tan & de Haan, 2014). Serum response factor (Srf) is a mouse TF required for cardiac differentiation and maturation and is involved in regulating atherosclerotic disease processes in vascular SMCs. Endothelial Per-Arnt-Sim domain protein 1 (Epa1) can regulate VEGF expression and appears to be involved in the development of the vascular and pulmonary tubular systems (Stavik et al., 2016; Yang et al., 2021). TATA-box-binding protein (Tbp) is a general TF that functions at the core of the DNA-binding multiprotein factor TFIID, which is also a crucial factor in the perivascular adipose tissue of AAA (Piacentini, Chiesa & Colombo, 2020). The homeobox protein Hox-C8 (Hoxc8), a sequence-specific TF that is part of a developmental regulatory system that provides cells with specific positional identities along the anteroposterior axis and is related to proinflammatory and immunological genes and pathways in coronary artery disease (Tan et al., 2020). Although these TFs have not been confirmed to contribute to AAA formation (except Tbp), they have been shown to play a crucial role in vascular diseases in previous studies. Hence, these TFs might be potential regulators and treatment targets of AAA.

In this study, the results suggested that there is a quantity of circulation differences between aged and youth AAA mice models, which are age-related proteins involved in AAA formation. There are several limitations of this study. Firstly, this is a single animal experiment which had not been validated on human study. Secondly, the results lack of further validation with some other experimental techniques or a large sample. Nevertheless, this study is an exploratory study in which we utilized proteomics to attempt to discovery novel circulation varies, which might contribute to AAA formation in aged people. In the future, to verify the clinical application of these proteins, a large-sample, multicenter, prospective clinical validation study is needed.

**CONCLUSIONS**

Our present study revealed circulatory environment changes that were associated with age and involved in AAA formation, and these changes were related to the response to oxidative stress, coagulation and platelet activation, complement, and inflammation. Prdx1, Prdx6, Tpi1, Ppia, Eno1, and Vwf might be circulatory factors that induce AAA in aged people, while Prdx2, Ffa, Ffb, and Ffg were differentially expressed based on
comparisons with other studies. Nfe2, Srf, Epas1, Tbp, and Hoxc8 might be crucial TFs that influence AAA formation in the circulatory system of aged people. These circulatory system proteins and TFs might be treatment targets or predictors of AAA, but more research is needed.

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**Author Contributions**
- Jinrui Ren conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Jianqiang Wu conceived and designed the experiments, performed the experiments, authored or reviewed drafts of the paper, and approved the final draft.
- Xiaoyue Tang analyzed the data, prepared figures and/or tables, and approved the final draft.
- Siliang Chen analyzed the data, prepared figures and/or tables, and approved the final draft.
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- Lianglin Wu performed the experiments, analyzed the data, prepared figures and/or tables, and approved the final draft.
- Dan Yang conceived and designed the experiments, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Yuehong Zheng conceived and designed the experiments, authored or reviewed drafts of the paper, and approved the final draft.

**Animal Ethics**
The following information was supplied relating to ethical approvals (*i.e.*, approving body and any reference numbers):
All animal studies were approved by the Institutional Animal Care and Use Committee of Peking Union Medical College Hospital (JS-2629).

Data Availability
The following information was supplied regarding data availability:

The data is available at ProteomeXchange: PXD033725.

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REFERENCES
Afonso J, Santos LL, Longatto-Filho A, Baltazar F. 2020. Competitive glucose metabolism as a target to boost bladder cancer immunotherapy. Nature Reviews Urology 17(2):77–106 DOI 10.1038/s41585-019-0263-6.

Alavi P, Rathod AM, Jahroudi N. 2021. Age-associated increase in thrombogenicity and its correlation with von Willebrand factor. Journal of Clinical Medicine 10(18):4190 DOI 10.3390/jcm10184190.

Bourantas CV, Crake T, Zhang YJ, Ozkor M, Ahmed J, Garcia-Garcia HM, Serruys PW. 2018. Intravascular imaging in cardiovascular ageing. Experimental Gerontology 109:31–37 DOI 10.1016/j.exger.2017.05.011.

Burillo E, Jorge I, Martinez-Lopez D, Camafeita E, Blanco-Colio LM, Trevisan-Herraz M, Ezkurdia I, Egido J, Michel JB, Meilhac O, Vazquez J, Martin-Ventura JL. 2016. Quantitative HDL proteomics identifies peroxiredoxin-6 as a biomarker of human abdominal aortic aneurysm. Scientific Reports 6(1):38477 DOI 10.1038/srep38477.

Cameron SJ, Russell HM, Owens AP III. 2018. Antithrombotic therapy in abdominal aortic aneurysm: beneficial or detrimental? Blood 132(25):2619–2628 DOI 10.1182/blood-2017-08-743237.

Chaohui C, Wei H, Hongfeng W, Yueliang Z, Xiaoqin P, Pingli Z, Zhibing A. 2018. iRhom2 promotes atherosclerosis through macrophage inflammation and induction of oxidative stress. Biochemical and Biophysical Research Communications 503(3):1897–1904 DOI 10.1016/j.bbrc.2018.07.133.

Chen HZ, Wang F, Gao P, Pei JF, Liu Y, Xu TT, Tang X, Fu WY, Lu J, Yan YF, Wang XM, Han L, Zhang ZQ, Zhang R, Zou MH, Liu DP. 2016. Age-associated sirtuin 1 reduction in vascular smooth muscle links vascular senescence and inflammation to abdominal aortic aneurysm. Circulation Research 119(10):1076–1088 DOI 10.1161/CIRCRESAHA.116.308895.

Chichester Aneurysm Screening Group. 2001. Viborg Aneurysm Screening Study. 2001. Western Australian Abdominal Aortic Aneurysm Program. 2001. Multicentre Aneurysm Screening Study. 2001. A comparative study of the prevalence of abdominal aortic aneurysms in the United Kingdom, Denmark, and Australia. Journal of Medical Screening 8(1):46–50 DOI 10.1136/jms.8.1.46.

Dai J, Louedec L, Philippe M, Michel JB, Houard X. 2009. Effect of blocking platelet activation with AZD6140 on development of abdominal aortic aneurysm in a rat aneurysmal model. Journal of Vascular Surgery 49(3):719–727 DOI 10.1016/j.jvs.2008.09.057.

Daugherty A, Cassis LA. 2002. Mechanisms of abdominal aortic aneurysm formation. Current Atherosclerosis Reports 4(3):222–227 DOI 10.1007/s11883-002-0023-5.
de la Mora de la Mora I, Torres-Larios A, Enríquez-Flores S, Mendez ST, Castillo-Villanueva A, Gomez-Manzo S, Lopez-Velazquez G, Marcial-Quino J, Torres-Arroyo A, Garcia-Torres I, Reyes-Vivas H, Oria-Hernandez J. 2015. Structural effects of protein aging: terminal marking by deamidation in human triosephosphate isomerase. PLOS ONE 10(4):e0123379 DOI 10.1371/journal.pone.0123379.

Dominic A, Banerjee P, Hamilton DJ, Le NT, Abe JI. 2020. Time-dependent replicative senescence vs. disturbed flow-induced pre-mature aging in atherosclerosis. Redox Biology 37(63):101614 DOI 10.1016/j.redox.2020.101614.

Gabel G, Northoff BH, Balboa A, Becirovic-Agic M, Petri M, Busch A, Maegdefessel L, Mahlmann A, Ludwig S, Teupser D, de Waard V, Gollellie J, Wanhainen A, Wagsater D, Holdt LM, Lindeman JHN. 2021. Parallel murine and human aortic wall genomics reveals metabolic reprogramming as key driver of abdominal aortic aneurysm progression. Journal of The American Heart Association 10(17):e020231 DOI 10.1161/JAHA.120.020231.

George J, Rapsomaniki E, Pujades-Rodriguez M, Shah AD, Denaxas S, Herrett E, Smeeth L, Timmis A, Hemingway H. 2015. How does cardiovascular disease first present in women and men? Incidence of 12 cardiovascular diseases in a contemporary cohort of 1,937,360 people. Circulation 132(14):1320–1328 DOI 10.1161/CIRCULATIONAHA.114.013797.

Grzela T, Brawura-Biskupski-Samaha R, Jelenska MM, Szmidt J. 2008. Low molecular weight heparin treatment decreases MMP-9 plasma activity in patients with abdominal aortic aneurysm. European Journal of Vascular and Endovascular Surgery 35(2):159–161 DOI 10.1016/j.ejvs.2007.09.008.

Huang H, Tang S, Ji M, Tang Z, Shimada M, Liu X, Qi S, Locasale JW, Roeder RG, Zhao Y, Li X. 2018. p300-Mediated lysine 2-hydroxyisobutyrylation regulates glycolysis. Molecular Cell 70(4):663–678 e666 DOI 10.1016/j.molcel.2018.04.011.

Jeong SJ, Cho MJ, Ko NY, Kim S, Jung IH, Min JK, Lee SH, Park JG, Oh GT. 2020. Deficiency of peroxiredoxin 2 exacerbates angiotensin II-induced abdominal aortic aneurysm. Experimental and Molecular Medicine 52(9):1587–1601 DOI 10.1038/s12276-020-00498-3.

Kolovou GD, Kolovou V, Mavrogeni S. 2014. We are ageing. Biomed Research International 2014(9):808307 DOI 10.1155/2014/808307.

Lopez-Alemany R, Longstaff C, Hawley S, Mirshahi M, Fabregas P, Jardi M, Merton E, Miles LA, Felez J. 2003. Inhibition of cell surface mediated plasminogen activation by a monoclonal antibody against alpha-Enolase. American Journal of Hematology 72(4):234–242 DOI 10.1002/ajh.10299.

Maegdefessel L, Dalman RL, Tsoa PS. 2014. Pathogenesis of abdominal aortic aneurysms: microRNAs, proteases, genetic associations. Annual Review of Medicine 65(1):49–62 DOI 10.1146/annurev-med-101712-174206.

Martin-Ventura JL, Martinez-Lopez D, Roldan-Montero R, Gomez-Guerrero C, Blanco-Colio LM. 2019. Role of complement system in pathological remodeling of the vascular wall. Molecular Immunology 114(9):207–215 DOI 10.1016/j.molimm.2019.06.016.

Martinez-Pinna R, Burillo E, Madrigal-Matute J, Lopez JA, Camafeita E, Torres-Fonseca MM, Llamas-Granda P, Egidio J, Michel JB, Blanco-Colio LM, Martin-Ventura JL. 2014. Label-free proteomic analysis of red blood cell membrane fractions from abdominal aortic aneurysm patients. Proteomics Clinical Applications 8(7–8):626–630 DOI 10.1002/prca.201400035.

Martinez-Pinna R, Ramos-Mozo P, Madrigal-Matute J, Blanco-Colio LM, Lopez JA, Calvo E, Camafeita E, Lindholt JS, Meilhac O, Delbosc S, Michel JB, Vega de Ceniga M, Egidio J, Martin-Ventura JL. 2011. Identification of peroxiredoxin-1 as a novel biomarker of abdominal
aortic aneurysm. *Arteriosclerosis, Thrombosis, and Vascular Biology* **31**(4):935–943 DOI 10.1161/ATVBAHA.110.214429.

Michel JB, Martin-Ventura JL, Egido J, Sakalihasan N, Treska V, Lindholt J, Allaire E, Thorsteinsdottir U, Cockerill G, Swedenborg J, Consortium FE. 2011. Novel aspects of the pathogenesis of aneurysms of the abdominal aorta in humans. *Cardiovascular Research* **90**(4):18–27 DOI 10.1093/cvr/cvq337.

Miller FJ Jr, Sharp WJ, Fang X, Oberley LW, Oberley TD, Weintraub NL. 2002. Oxidative stress in human abdominal aortic aneurysms: a potential mediator of aneurysmal remodeling. *Arteriosclerosis, Thrombosis, and Vascular Biology* **22**(4):560–565 DOI 10.1161/01.ATV.0000013778.72404.30.

Moran CS, Seto SW, Krishna SM, Sharma S, Jose RJ, Biros E, Wang Y, Morton SK, Golledge J. 2017. Parenteral administration of factor Xa/IIa inhibitors limits experimental aortic aneurysm and atherosclerosis. *Scientific Reports* **7**(1):43079 DOI 10.1038/srep43079.

Nigro P, Satoh K, O’Dell MR, Soe NN, Cui Z, Mohan A, Abe J, Alexis JD, Sparks JD, Berk BC. 2011. Cyclophilin A is an inflammatory mediator that promotes atherosclerosis in apolipoprotein E-deficient mice. *Journal of Experimental Medicine* **208**(1):53–66 DOI 10.1084/jem.20101174.

Nordon IM, Hinchliffe RJ, Loftus IM, Thompson MM. 2011. Pathophysiology and epidemiology of abdominal aortic aneurysms. *Nature Reviews Cardiology* **8**(2):92–102 DOI 10.1038/nrcardio.2010.180.

Pagano MB, Zhou HF, Ennis TL, Wu X, Lambris JD, Atkinson JP, Thompson RW, Hourcade DE, Pham CT. 2009. Complement-dependent neutrophil recruitment is critical for the development of elastase-induced abdominal aortic aneurysm. *Circulation* **119**(13):1805–1813 DOI 10.1161/CIRCULATIONAHA.108.832972.

Parvizi M, Franchi F, Arendt BK, Ebtehaj S, Rodriguez-Porcel M, Lanza IR. 2021. Senolytic agents lessen the severity of abdominal aortic aneurysm in aged mice. *Experimental Gerontology* **151**:111416 DOI 10.1016/j.exger.2021.111416.

Piacentini L, Chiesa M, Colombo GI. 2020. Gene regulatory network analysis of perivascular adipose tissue of abdominal aortic aneurysm identifies master regulators of key pathogenetic pathways. *Biomedicines* **8**(8):288 DOI 10.3390/biomedicines8080288.

Piechota-Polanczyk A, Goraca A, Demyanets S, Mittlboeck M, Domenig C, Neumayer C, Wojta J, Nanobachvili J, Huk I, Klinger M. 2012. Simvastatin decreases free radicals formation in the human abdominal aortic aneurysm wall via NF-kappaB. *European Journal of Vascular and Endovascular Surgery* **44**(2):133–137 DOI 10.1016/j.ejvs.2012.04.020.

Ramella M, Bernardi P, Fusaro L, Manfredi M, Casella F, Porta CM, Nicolai L, Galeazzi E, Boldorini R, Settembrini AM, Settembrini P, Marengo E, Cannas M, Boccafoschi F. 2018. Relevance of inflammation and matrix remodeling in abdominal aortic aneurysm (AAA) and popliteal artery aneurysm (PAA) progression. *American Journal of Translational Research* **10**:3265–3275.

Rasiova M, Farkasova L, Kosco M, Moscovic M, Spak L, Petrasova D, Tkac I. 2019. Positive association between abdominal aortic diameter and serum collagen XVIII levels. *International Angiology* **38**:410–417 DOI 10.23736/S0392-9590.19.04222-6.

Ren H, Li F, Tian C, Nie H, Wang L, Li HH, Zheng Y. 2015. Inhibition of proteasome activity by low-dose bortezomib attenuates angiotensin II-induced abdominal aortic aneurysm in apo E (−/−) mice. *Scientific Reports* **5**(1):15730 DOI 10.1038/srep15730.
Rhee SG, Chae HZ, Kim K. 2005. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. *Free Radical Biology and Medicine* 38(12):1543–1552 DOI 10.1016/j.freeradbiomed.2005.02.026.

Rodriguez-Almazan C, Arreola R, Rodriguez-Larrea D, Aguirre-Lopez B, de Gomez-Puyou MT, Perez-Montfort R, Costas M, Gomez-Puyou A, Torres-Larios A. 2008. Structural basis of human triosephosphate isomerase deficiency: mutation E104D is related to alterations of a conserved water network at the dimer interface. *Journal of Biological Chemistry* 283(34):23254–23263 DOI 10.1074/jbc.M802145200.

Sakalihasan N, Limet R, Defawe OD. 2005. Abdominal aortic aneurysm. *Lancet* 365(9470):1577–1589 DOI 10.1016/S0140-6736(05)66459-8.

Sanchez-Infantes D, Nus M, Navas-Madronal M, Fite J, Perez B, Barros-Membrilla AJ, Soto B, Martinez-Gonzalez J, Camacho M, Rodriguez C, Mallat Z, Galan M. 2021. Oxidative stress and inflammatory markers in abdominal aortic aneurysm. *Antioxidants* 10(4):602 DOI 10.3390/antiox10040602.

Satoh K, Nigro P, Matoba T, O’Dell MR, Cui Z, Shi X, Mohan A, Yan C, Abe J, Illig KA, Berk BC. 2009. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. *Nature Medicine* 15(6):649–656 DOI 10.1038/nm.1958.

Shintani Y, Aoki H, Nishihara M, Ohno S, Furusho A, Hiromatsu S, Akashi H, Imaizumi T, Aoyagi S. 2011. Hepatocyte growth factor promotes an anti-inflammatory cytokine profile in human abdominal aortic aneurysm tissue. *Atherosclerosis* 216(2):307–312 DOI 10.1016/j.atherosclerosis.2011.02.025.

Sohrabpour Z, Kearns PM, Massari AM. 2016. Vibrational sum frequency generation spectroscopy of fullerene at dielectric interfaces. *Journal of Physical Chemistry C* 120(3):1666–1672 DOI 10.1021/acs.jpcc.5b10918.

Song F, Zhang X, Ren XB, Zhu P, Xu J, Wang L, Li YF, Zhong N, Ru Q, Zhang DW, Jiang JL, Xia B, Chen ZN. 2011. Cyclophilin A (CyPA) induces chemotaxis independent of its peptidylprolyl cis-trans isomerase activity: direct binding between CyPA and the ectodomain of CD147. *Journal of Biological Chemistry* 286(10):8197–8203 DOI 10.1074/jbc.C110.181347.

Stavik B, Espada S, Cui XY, Iversen N, Holm S, Mowinkel MC, Halvorsen B, Skretting G, Sandset PM. 2016. EPAS1/HIF-2 alpha-mediated downregulation of tissue factor pathway inhibitor leads to a pro-thrombotic potential in endothelial cells. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* 1862(4):670–678 DOI 10.1016/j.bbadis.2016.01.017.

Sugahara T, Nakajima H, Shirahata S, Murakami H. 1992. Purification and characterization of immunoglobulin production stimulating factor-II beta derived from Namalwa cells. *Cytotechnology* 10(2):137–146 DOI 10.1007/BF00570890.

Sun W, Pang Y, Liu Z, Sun L, Liu B, Xu M, Dong Y, Feng J, Jiang C, Kong W, Wang X. 2015. Macrophage inflammasome mediates hyperhomocysteinemia-aggravated abdominal aortic aneurysm. *Journal of Molecular and Cellular Cardiology* 81:96–106 DOI 10.1016/j.yjmcc.2015.02.005.

Sundermann AC, Saum K, Conrad KA, Russell HM, Edwards TL, Mani K, Björck M, Wanhainen A, Owens AP III. 2018. Prognostic value of D-dimer and markers of coagulation for stratification of abdominal aortic aneurysm growth. *Blood Advances* 2(22):3088–3096 DOI 10.1182/bloodadvances.2017013359.

Taimour S, Zarrouk M, Holst J, Melander O, Engström G, Smith JG, Gottsäter A. 2017. No relation between biomarkers at age 47–49 and aortic diameter after 14–19 years of follow-up-a
population-based study. Vasa-European Journal of Vascular Medicine 46(4):291–295 DOI 10.1024/0301-1526/a000632.

Tan SM, de Haan JB. 2014. Combating oxidative stress in diabetic complications with Nrf2 activators: how much is too much? Redox Report 19(3):107–117 DOI 10.1179/1351000214Y.0000000087.

Tan L, Xu Q, Wang Q, Shi R, Zhang G. 2020. Identification of key genes and pathways affected in epicardial adipose tissue from patients with coronary artery disease by integrated bioinformatics analysis. PeerJ 8(Suppl. 4):e8763 DOI 10.7717/peerj.8763.

Teti G, Chiarini F, Mazzotti E, Ruggeri A, Carano F, Falconi M. 2021. Cellular senescence in vascular wall mesenchymal stromal cells, a possible contribution to the development of aortic aneurysm. Mechanisms of Ageing and Development 197(15):111515 DOI 10.1016/j.mad.2021.111515.

Touat Z, Ollivier V, Dai J, Huisset MG, Bezeaud A, Sebag U, Palombi T, Rossignol P, Meilhac O, Guillou MC, Michel JB. 2006. Renewal of mural thrombus releases plasma markers and is involved in aortic abdominal aneurysm evolution. American Journal of Pathology 168(3):1022–1030 DOI 10.2353/ajpath.2006.050868.

Tsuruda T, Hatakeyama K, Nagamachi S, Sekita Y, Sakamoto S, Endo GJ, Nishimura M, Matsuyama M, Yoshimura K, Sato Y, Onitsuka T, Imamura T, Asada Y, Kitamura K. 2012. Inhibition of development of abdominal aortic aneurysm by glycolysis restriction. Arteriosclerosis, Thrombosis, and Vascular Biology 32(6):1410–1417 DOI 10.1161/ATVBAHA.111.237065.

Urbonavicius S, Lindholt JS, Vorum H, Urbonaviciene G, Henneberg EW, Honore B. 2009. Proteomic identification of differentially expressed proteins in aortic wall of patients with ruptured and nonruptured abdominal aortic aneurysms. Journal of Vascular Surgery 49(2):455–463 DOI 10.1016/j.jvs.2008.08.097.

Usui F, Shirasuna K, Kimura H, Tatsumi K, Kawashima A, Karasawa T, Yoshimura K, Aoki H, Tsutsui H, Noda T, Sagara J, Taniguchi S, Takahashi M. 2015. Inflammation activation by mitochondrial oxidative stress in macrophages leads to the development of angiotensin II-induced aortic aneurysm. Arteriosclerosis, Thrombosis, and Vascular Biology 35(1):127–136 DOI 10.1161/ATVHA.114.303763.

Wiernicki I, Parafinik M, Kolasa-Wolosiuk A, Gutowska I, Kazimierczak A, Clark J, Baranowska-Bosiacka I, Szymilowicz P, Gutowski P. 2019. Relationship between aortic wall oxidative stress/proteolytic enzyme expression and intraluminal thrombus thickness indicates a novel pathomechanism in the progression of human abdominal aortic aneurysm. FASEB Journal 33(1):885–895 DOI 10.1096/fj.201800633R.

Wisniewski JR, Zouman G, Nagaraj N, Mann M. 2009. Universal sample preparation method for proteome analysis. Nature Methods 6(5):359–362 DOI 10.1038/nmeth.1322.

Wong YY, Golledge J, Flicker L, McCaul KA, Hankey GJ, van Bockxmeer F, Yeap BB, Norman PE. 2013. Plasma total homocysteine is associated with abdominal aortic aneurysm and aortic diameter in older men. Journal of Vascular Surgery 58(2):364–370.

Wood ZA, Schroder E, Robin Harris J, Poole LB. 2003. Structure, mechanism and regulation of peroxiredoxins. Trends in Biochemical Sciences 28(1):32–40 DOI 10.1016/S0968-0004(02)00003-8.

Wu G, Chen T, Shahsaee A, Hu W, Bronson RT, Shi GP, Halperin JA, Aktas H, Qin X. 2010. Complement regulator CD59 protects against angiotensin II-induced abdominal aortic aneurysms in mice. Circulation 121(11):1338–1346 DOI 10.1161/CIRCULATIONAHA.108.844589.
Xie Y, Li X, Ge J. 2019. Cyclophilin A-FoxO1 signaling pathway in endothelial cell apoptosis. *Cellular Signalling* 61(7181):57–65 DOI 10.1016/j.cellsig.2019.04.014.

Yang Y, Gao C, Yang T, Sha Y, Cai Y, Wang X, Yang Q, Liu C, Wang B, Zhao S. 2021. Vascular characteristics and expression of hypoxia genes in Tibetan pigs’ hearts. *Veterinary Medicine and Science* 8(1):177–186 DOI 10.1002/vms3.639.

Yeap BB, Chubb SA, McCaul KA, Flicker L, Ho KK, Golledge J, Hankey GJ, Norman PE. 2012. Associations of IGF1 and its binding proteins with abdominal aortic aneurysm and aortic diameter in older men. *European Journal of Endocrinology* 166(2):191–197 DOI 10.1530/EJE-11-0725.

Yu G, Wang LG, Han Y, He QY. 2012. ClusterProfiler: an R package for comparing biological themes among gene clusters. *OMICS: A Journal of Integrative Biology* 16(5):284–287 DOI 10.1089/omi.2011.0118.

Zagrapan B, Eilenberg W, Scheuba A, Klopf J, Brandau A, Story J, Dosch K, Hayden H, Domenig CM, Fuchs I, Schernthaner R, Ristl R, Huk I, Neumayer C, Brostjan C. 2021. Complement factor C5a is increased in blood of patients with abdominal aortic aneurysm and has prognostic potential for aneurysm growth. *Journal of Cardiovascular Translational Research* 14(4):761–769 DOI 10.1007/s12265-020-10086-5.

Zhang X, Li F, Wang W, Ji L, Sun B, Xiao X, Wang X, Chen Y, Liu B, Ye W, Tian C, Wang H, Zheng Y. 2020. Macrophage pyroptosis is mediated by immunoproteasome subunit β5i (LMP7) in abdominal aortic aneurysm. *Biochemical and Biophysical Research Communications* 533(4):1012–1020 DOI 10.1016/j.bbrc.2020.09.082.

Zhou HF, Yan H, Bertram P, Hu Y, Springer LE, Thompson RW, Curci JA, Hourcade DE, Pham CT. 2013. Fibrinogen-specific antibody induces abdominal aortic aneurysm in mice through complement lectin pathway activation. *Proceedings of the National Academy of Sciences of the United States of America* 110(46):E4335–E4344 DOI 10.1073/pnas.1315512110.

Zhou HF, Yan H, Stover CM, Fernandez TM, Rodriguez de Cordoba S, Song WC, Wu X, Thompson RW, Schwaebel WJ, Atkinson JP, Hourcade DE, Pham CT. 2012. Antibody directs properdin-dependent activation of the complement alternative pathway in a mouse model of abdominal aortic aneurysm. *Proceedings of the National Academy of Sciences of the United States of America* 109(7):E415–E422 DOI 10.1073/pnas.1119000109.