

Apolipoprotein-A1 transports and regulates MMP2 in the blood

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Synthesized in the liver and intestines, apolipoprotein A1 (APOA1) transports cholesterol in high density lipoproteins from atherosclerotic lesions to the liver, protecting against atherosclerotic plaque rupture. Here, we show that proMMP2 (zymogen of matrix metalloproteinase-2) circulates associated with APOA1 in humans and APOA1-expressing mice. This is noteworthy because MMP2 is the most abundant MMP in blood, and MMPs promote atherosclerotic plaque rupture. Artificial intelligence (AlphaFold)-based modeling suggested that APOA1 and MMP2 interact; direct interactions were confirmed using five orthogonal interaction assays, showing that APOA1 binds to MMP2 catalytic and hemopexin-like domains. APOA1 inhibited MMP2 autolysis and allosterically increased MMP2 activity—an effect specifically reproduced by plasma from humans and APOA1-expressing mice but not albumin nor plasma from APOA1 knockout mice. These function-altering interactions with APOA1 may increase MMP2 bioavailability and lay the foundation for future research on how apolipoproteins and MMPs influence atherosclerotic plaque rupture, independently of cholesterol transport.

Interorgan communication is an evolutionarily conserved mechanism in all animals that serves to maintain homeostasis in response to changing external conditions¹. Organs synthesize and release diverse molecular signals that can reach distal target organs through blood circulation. These signals include (i) metabolites, (ii) peptides, and (iii) proteins^{2–4}. Among the proteins found in the circulation are apolipoproteins and proteases.

Synthesized and secreted in the liver and intestines, apolipoprotein A1 (APOA1) is best known as the major protein component in high-density lipoproteins (HDL) and its role in the transport and redistribution of excess cholesterol from arterial foam cells (of vascular smooth muscle cell or macrophage origin) and peripheral organs to

the liver from where it is delivered into the intestines for excretion in the feces as a component of the bile⁵. This process is known as reverse cholesterol transport and may protect against atherogenesis and arterial thrombosis—the primary causes of atherothrombotic (type I) acute myocardial infarction^{5–8}. Beyond cholesterol, APOA1 interacts with proteins in the blood as well as in target cells and organs^{5,9,10}. These interactions have the potential to influence the biological functions of APOA1 and/or its interactors, serving as mechanisms that can contribute to homeostasis or the genesis of pathologies beyond atherogenesis or arterial thrombosis, such as diabetes^{2,5–10}.

Among the proteases found in the blood circulation are matrix metalloproteinases (MMPs). At least 25 different MMPs have been

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identified in vertebrates; twenty-four are present in *Homo sapiens*¹¹. MMPs are expressed as inactive zymogens (proMMPs) and generally consist of an N-terminal propeptide, catalytic domain, and C-terminal hemopexin-like (PEX) domain^{11,12}. Proteolytic removal of the propeptide by MMPs (e.g., by membrane-anchored MMP14) or non-MMP endopeptidases (e.g., plasmin) yields active MMPs¹¹. Active MMPs have many substrates, including extracellular matrix components, proinflammatory cytokines, growth factors, and cell-surface receptors^{11,13}. The diversity of substrates allows MMPs to regulate a range of processes including extracellular matrix remodeling and inflammatory signaling in the cells and organs where the MMPs are synthesized^{11,13}. MMP activity is strongly regulated by tissue inhibitors of matrix metalloproteinases (TIMPs)^{13–16}, whose N-terminus binds non-covalently to the catalytic domain of MMPs, effectively blocking substrate access¹⁷.

In normal cells and organs, small amounts of proMMPs and MMPs are constitutively synthesized and secreted into the extracellular space and blood circulation^{12,13,18,19}. In disease conditions, affected organs often become foci of high synthesis and secretion of proMMPs and MMPs—as previously documented at sites of arterial lesions and atherosclerotic plaques, failing organs, and cancer tumors^{12,20,21}. Following secretion, whether MMPs circulate free or are bound to inhibitors or to other blood-borne proteins is unclear. This is, at least in part, because the blood interactome of MMPs remains largely unknown.

Here, we discovered that proMMP2 (the zymogen of MMP2) circulates associated with APOA1/HDL in the blood of humans and APOA1-expressing mice. We found that APOA1 interacts with active MMP2 specifically and with high affinity (nanomolar K_d), protecting MMP2 from self-proteolysis (autolysis) and allosterically increasing MMP2 proteolytic activity. Through these interactions, blood-borne APOA1 may increase MMP2 bioavailability. These findings were not predictable because: (i) complexation of MMP2 by APOA1 could not be anticipated given the current knowledge about these proteins, and (ii) the active MMP2 form was not known to be an allosteric enzyme. These findings identify function-altering interactions involving APOA1 and MMP2 that could encourage future studies focused on how interactions between apolipoproteins and MMPs influence organismal homeostasis and disease pathogenesis independently of canonical cholesterol transport by apolipoproteins.

Results

Figure 1A depicts our pathway to the discovery of specific, high-affinity, allosteric interactions between blood-borne APOA1 in HDL and proMMP2/MMP2.

MMP2 circulates in the blood as a large complex with APOA1

We fractionated human serum and plasma specimens by fast-performance liquid chromatography (FPLC) using a Superose 6 10/300 gel-filtration FPLC size exclusion column (SEC) isocratically developed with NaCl (150 mM) buffer as the mobile phase, a technique routinely used to resolve and analyze lipoproteins^{9,22}. Among the lipoproteins, HDL particles (175–500 kDa, 6–12 nm) are a heterogeneous group comprising cholesterol transport proteins that generally include APOA1 (most abundant protein component, found in almost all HDL particles), APOA2 (found in 60% of all HDL particles), and APOE (half of its total amount in plasma is found in HDL)^{5,9,23}. Fractions containing the FPLC SEC eluent were analyzed for the presence of APOA1 (28 kDa), APOA2 (17 kDa) and APOE (34 kDa), cholesterol, triglycerides, proMMP2 (72 kDa), and MMP2 (62 kDa) (Fig. 1B). Gelatin zymography permits a highly sensitive detection of proMMP2 (0.5 ng) and MMP2 (0.06 ng)²⁴. ProMMP2 zymographic activity was detected in the FPLC SEC fractions containing HDL, as determined by the presence of APOA1 as well as the cholesterol and triglyceride fractionation profiles (Fig. 1B, fractions F13–F15, with retention times

<45 min and MW > 158 kDa; Supplementary Fig. 1). We did not detect proMMP2/MMP2 in FPLC SEC fractions containing complexes larger than HDL, such as low-density lipoproteins (LDL, 2.93 MDa, 22–29 nm) and very low-density lipoproteins (VLDL, 6–27 MDa, 25–90 nm) (Fig. 1B).

Studies with plasma of humans or mice showed that proMMP2/MMP2 consistently fractionate in FPLC SEC fractions containing APOA1 in both species (Fig. 1B, C). Mouse proMMP2 also comigrated with APOA1 when plasma was electrophoresed on 2D gradient Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE), which enables the separation of large protein complexes²⁵ (Supplementary Fig. 2A).

ProMMP2 and MMP2 circulate in blood at low nanomolar concentrations (~5 nM in humans serum, as determined in Fig. 1D using a quantitative MMP and TIMP 13-plex immunoreactivity-based assay), whereas APOA1 circulates at (~50000 nM)⁵; i.e., physiological concentrations of APOA1 in blood can be up to 10^3 to 10^4 times greater than those of proMMP2 and MMP2. We investigated whether other MMPs also fractionated with APOA1. We measured the quantity of all detectable MMPs in selected FPLC SEC plasma fractions (F13–F17). These fractions contained APOA1 (Fig. 1B, E) and represented a wide range of protein sizes and complexes (from <44 kDa to >158 kDa). MMP2 was most abundant in fractions F14–F15 (Fig. 1E) and was partially resolved from that of MMP9 [mostly found in high MW (HMW) fractions] and MMP1, MMP3, MMP7, and MMP10 (mostly found in low MW (LMW) fractions). As expected, TIMPs were detected in fractions containing MMPs (Fig. 1E). MMP8, MMP12, and MMP13 were not detected. Therefore, unlike MMP2, some plasma MMPs do not predominantly fractionate with APOA1, despite APOA1 being at a large molar excess over them. Together, these findings suggest that APOA1 may specifically interact with proMMP2/MMP2.

When plasma lipoproteins were subjected to the KBr density gradient ultracentrifugation technique (sometimes considered the gold standard for HDL isolation), we found that: (i) APOA1 cofractionated with HDL cholesterol (as expected) but not with proMMP2; and (ii) KBr inhibited the proteolytic activity of active human recombinant MMP2 in solution, whether APOA1 was present or not (Supplementary Fig. 2B).

Taken together, these fractionation data suggested that APOA1 and MMP2 may interact and thus cofractionate in FPLC SEC and comigrate in 2D BN-PAGE. However, the high KBr concentrations needed for density gradient ultracentrifugation may disrupt any possible interactions between APOA1 and either proMMP2 or MMP2. Disruption of APOA1 interactions with proMMP2 by KBr density gradient ultracentrifugation could explain why these interactions were not discovered earlier. Therefore, we sought to determine whether APOA1 and MMP2 interact.

Alphafold2-based modeling predicts that APOA1 interacts with active MMP2

Human APOA1 is a 243-amino-acid amphipathic α -helical protein that serves as a scaffold to pack lipids (primarily cholesterol and phospholipids), resulting in the formation of HDL particles²⁶. This helical scaffold provides flexibility, thermodynamic stability and physiological functionality to the HDL²⁷. Experimental studies of APOA1 demonstrate that the lipid-free APOA1 purified from recombinant sources can exist in multiple conformations^{27,28}. Another set of experimental structures of lipid-free APOA1 deciphered using crystallography (PDB: 1AV1, and PDB: 3R2P) offer insight into conformations of APOA1 that mimic its lipid-bound state^{29,30}. Nuclear magnetic resonance and transmission electron microscopy (PDB: 2N5E)^{26,31}, as well as molecular dynamics³² in presence of lipids have shown that APOA1 has a discoidal architecture in the nascent HDL particle and a spherical architecture in the mature HDL particle^{30,33,34}. Alternative models have been proposed using data obtained from hydrogen – deuterium exchange mass spectrometry, crosslinking and solution scattering

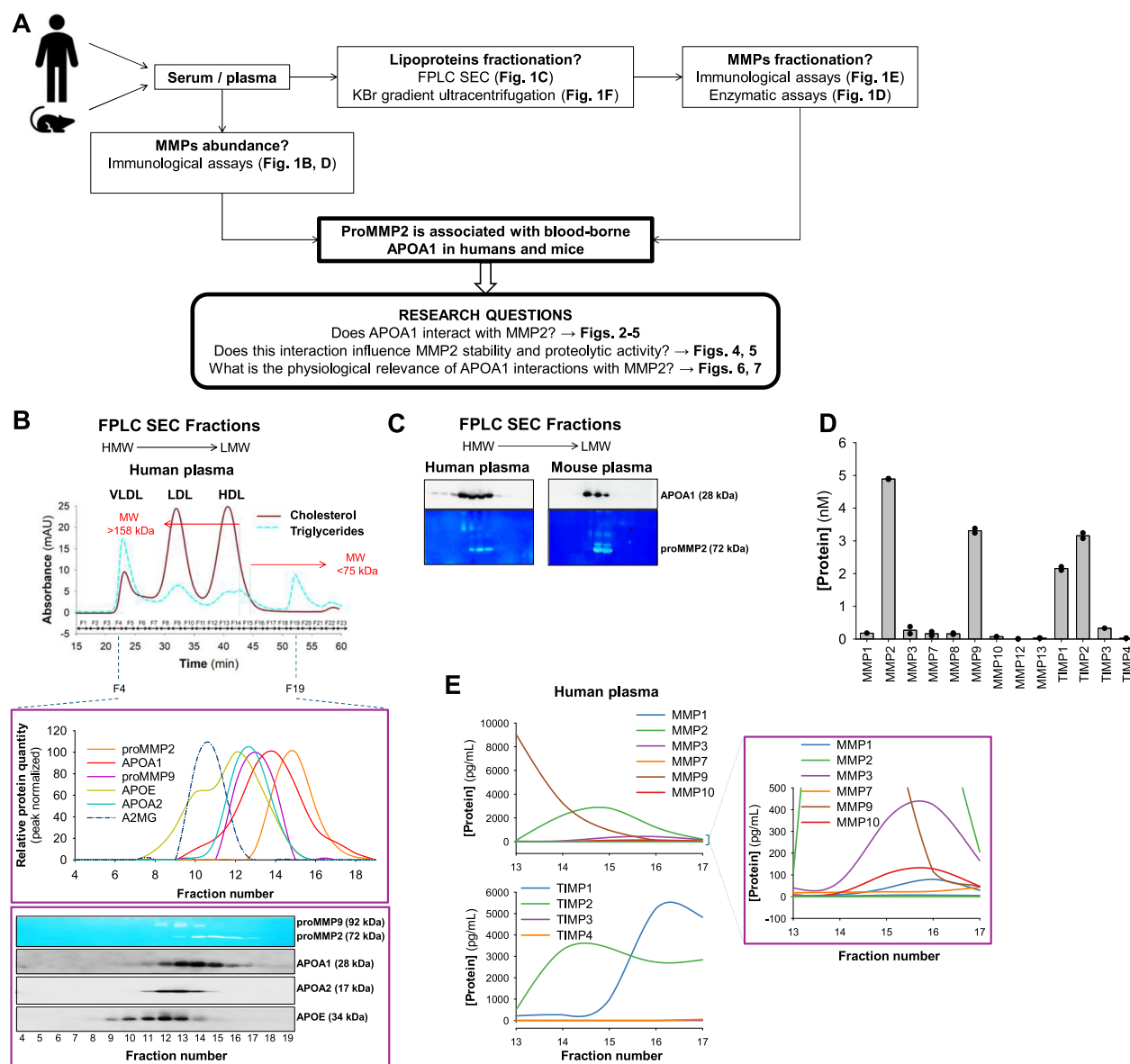


Fig. 1 | Co-fractionation of MMP2 with blood-borne APOA1 suggests research questions and new roles played by APOA1 and MMP2 in humans and mice.

A Discovery pathway. Details on human and mouse specimens tested are provided under Methods. **B** FPLC SEC fractionation profiles of human serum lipoproteins. Determination of protein (absorbance at 280 nm), triglycerides, and cholesterol (top). Data are presented as the mean value of separate analyses for $N = 4$ donors (biological replicates). Representative peak-normalized FPLC SEC profiles of human proMMP2 and proMMP9 (detected by gelatin zymography) or APOA1, APOA2, APOE, and A2MG (detected by western blotting) in plasma pooled from $N = 5$ donors (middle). Gelatin zymography and western blot traces (bottom). For the protein analyses, the FPLC SEC separation was performed isostatically at 400 μ L/min. Fractions were collected every 2 min resulting in 800 μ L fractions. Equal volumes of column eluant (20 μ L/fraction) were used within each experiment

to ensure fair comparison between fractions. **C** FPLC SEC fractionation profiles of plasma APOA1 and proMMP2 in mice, compared to humans. Plasma of a human donor and pool of $N = 9$ WT male mice (CBL57/B6) aged 12–15 weeks. APOA1 was detected by western blotting. ProMMP2 was detected by gelatin zymography. Similar co-fractionation of APOA1 and proMMP2 from human plasma is shown in Fig. 1B, E. **D** The abundance of all detectable MMPs and TIMPs in human serum pooled from $N = 14$ donors as determined using a MMP and TIMP 13-plex immunoreactivity-based assay. Mean of technical replicates of the pool ($N = 2$). **E** Representative FPLC SEC fractionation profiles of MMPs and TIMPs in plasma pooled from five humans. MMP1, 2, 3, 7, 9, and 10; and TIMP1, 2, 3, and 4 were detected, and quantitated using a MMP and TIMP 13-plex immunoreactivity-based assay. Zoom-in of the FPLC SEC traces for low abundance MMPs (MMP1, 3, 7, and 10). Mean of technical replicates of the pool ($N = 2$).

studies in the presence of specific lipids³⁵. These APOA1 structures have various shapes including horseshoe, turtle, helix-dimer hairpin, and double superhelix^{34,35}.

To determine *in silico* whether APOA1 interacts with active MMP2, we used the artificial intelligence-based software, AlphaFold2 (Supplementary Note 1 sections A.1–A.4, Supplementary Movie 1, and Supplementary Datasets 1–7 (in Source Data)). Using a crystal structure of APOA1 that ‘mimics’ its lipid-bound conformation (PDB: 1AV1)²⁹ as a template (Supplementary Note 1 section A.3), AlphaFold2 predicted

that APOA1 can form a heterodimer with proMMP2 in which residues from the proMMP2 catalytic domain and the fibronectin-like repeats interact with the N-terminal region of APOA1; this prediction had relatively low confidence scores (Supplementary Note 1 section A.4).

AlphaFold2 also predicted that APOA1 can interact with specific regions on MMP2 such as the catalytic groove and fibronectin-like repeats; this prediction had high confidence scores, when we provided a crystal structure of human APOA1 (PDB: 1AV1)²⁹ as a template for modeling (Fig. 2A–C, Supplementary Note 1 section A.4). To validate

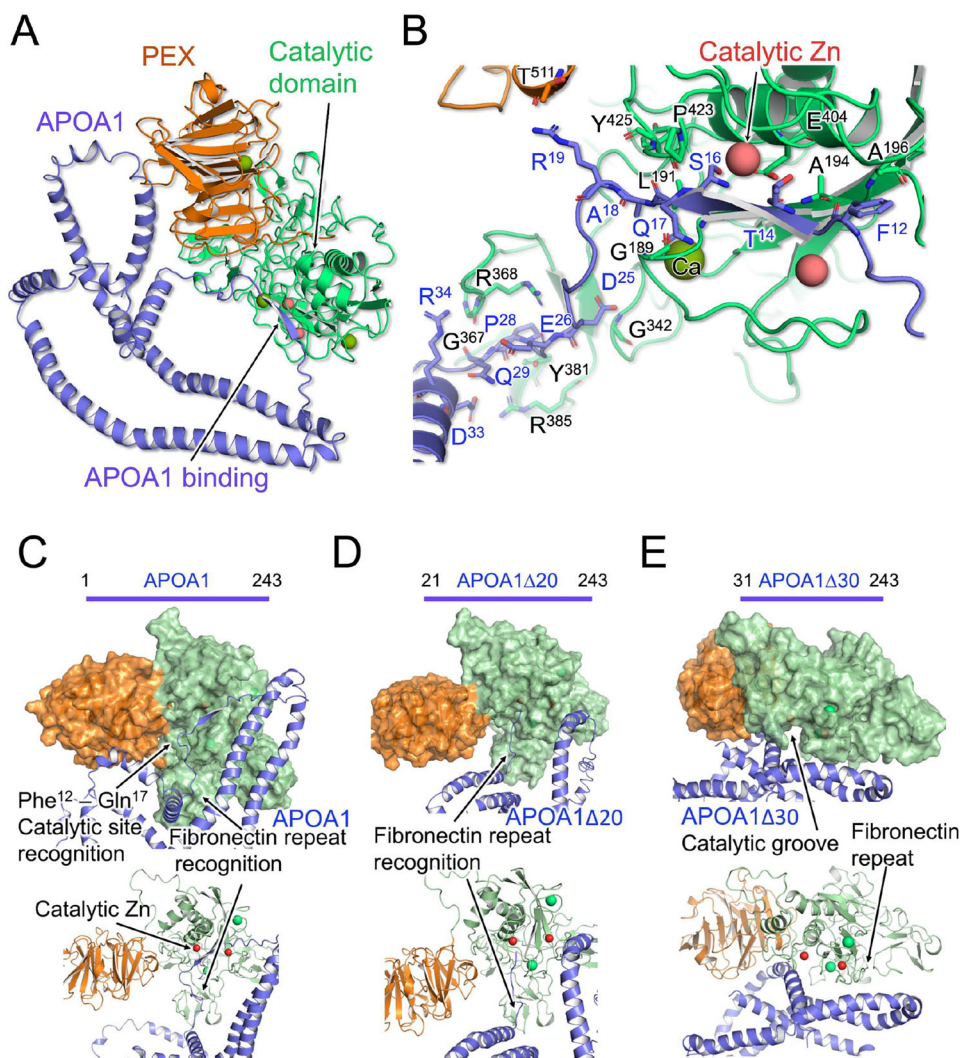


Fig. 2 | AlphaFold2-based in silico prediction of a APOA1-MMP2 complex.

A Structure of a APOA1-MMP2 complex obtained using deep-learning assisted structure calculation. **B** Interaction interface showing the residues of APOA1 (blue font) interacting with active MMP2 (black font). Interacting residues are shown in stick representation. Zoomed view of the N-terminal segment of APOA1 stabilizing the calcium coordinating loop. **(C-E)** In silico truncations of APOA1 shed light on APOA1 interactions with active MMP2. The surface (top) and cartoon (bottom)

representations of wild-type APOA1 – active MMP2 **(C)**; APOA1Δ20 – active MMP2 **(D)**; and APOA1Δ30 – active MMP2 **(E)** are shown. APOA1Δ30 does not interact with the catalytic cavity of MMP2 which is otherwise observed in the wild-type APOA1 **(C)**. Panels **(C-E)** include schematic representations of APOA1 constructs: full-length APOA1 (residues 1 – 243), APOA1Δ20 (residues: 21 – 243), and APOA1Δ30 (residues: 31 – 243). A movie showing the 3D structures of specific models is available (Supplementary Movie 1).

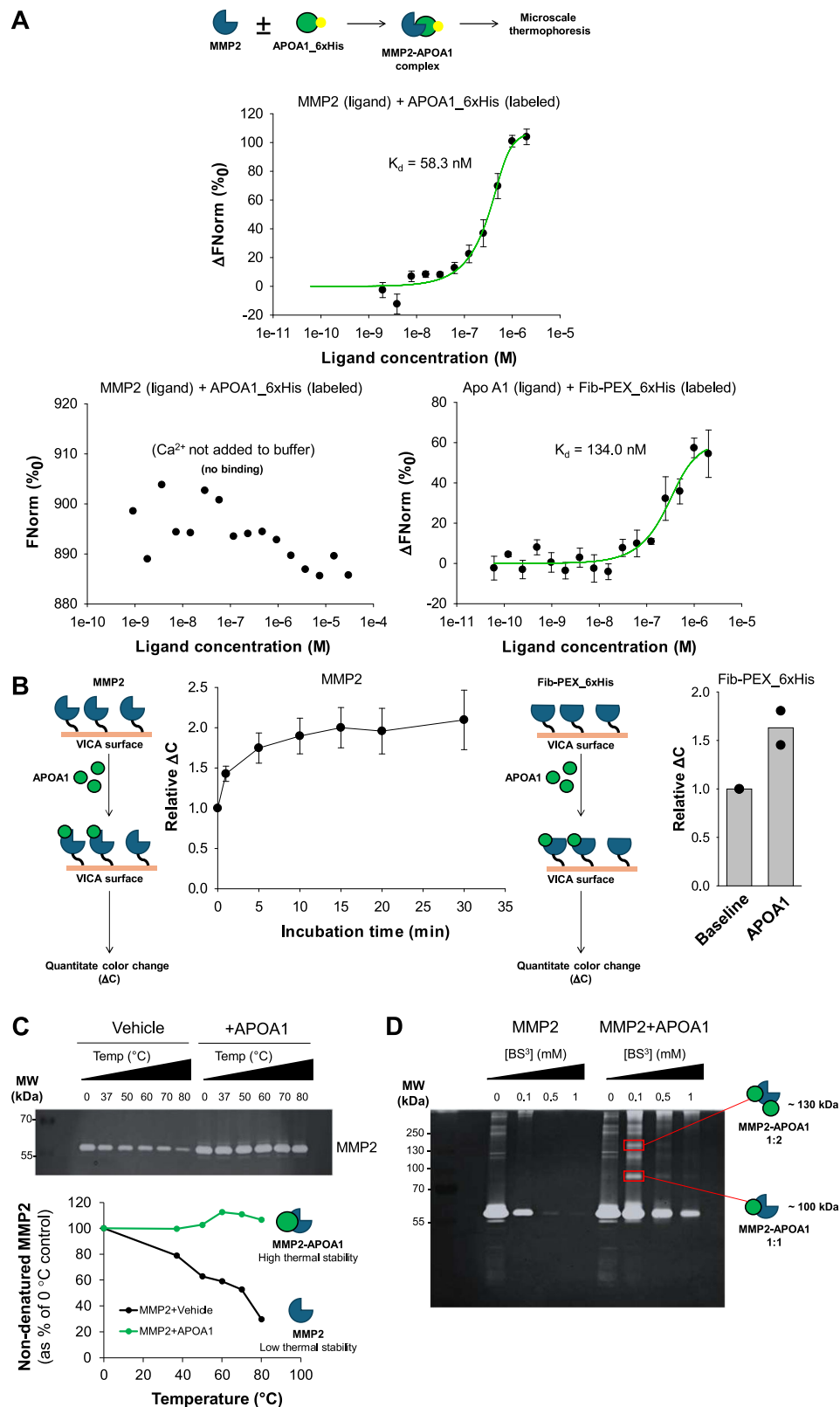
the specificity of the interaction of APOA1 residues with active MMP2, we used two in silico truncated APOA1 constructs generated via deletion of the first twenty or thirty residues (APOA1Δ20 and APOA1Δ30). The residue stretch Phe¹² – Gln¹⁷, which adopts an antiparallel beta sheet conformation in full-length APOA1 while traversing through the catalytic groove of active MMP2 was absent APOA1Δ20 (Fig. 2C). APOA1Δ20 did not enter the same groove as the wild-type (WT) APOA1, suggesting that the first 20 residues of the APOA1 N-terminal region, encompassing the residue stretch Phe¹² – Gln¹⁷, indeed interact with the catalytic cavity of MMP2. The region at which APOA1Δ20 interacts with the fibronectin-like repeats of MMP2 was the same as in the full-length APOA1 (Fig. 2D). APOA1Δ30 did not interact with the catalytic groove nor with the fibronectin-like repeats of MMP2 (Fig. 2E). APOA1Δ30 may interact with MMP2 via the groove between the PEX domain and the catalytic site. In this interaction, the helical structure of APOA1Δ30 is retained, and no change in conformation from helix to loop is observed (Fig. 2E).

A video showing the 3D structures of specific models is available (Supplementary Movie 1).

APOA1 directly interacts with MMP2

We sought to establish whether, indeed, APOA1 and MMP2 interact, specifically and directly, under controlled experimental conditions. Microscale thermophoresis (MST) analyses using active MMP2 (as ligand) and APOA1_{6xHis} (labeled with a RED-tris-NTA) confirmed that intact MMP2 directly interacts with APOA1 forming a complex (hereafter, indistinctively called APOA1-MMP2 or MMP2-APOA1) with a dissociation constant (K_d) of 58.3 nM, indicating high binding affinity (Fig. 3A). The native conformation of MMP2 is maintained by Ca²⁺ ions³⁶. MMP2 did not bind to APOA1 in the absence of Ca²⁺ ions (Fig. 3A), indicating that the interaction of MMP2 with APOA1 depends on MMP2 conformation. MST analysis revealed the interaction of APOA1 with a Fib-PEX_{6xHis} construct comprising the PEX domain along with part of fibronectin-like repeats had a K_d of 134 nM (Fig. 3A), which is greater than that of APOA1 for intact MMP2 (Fig. 3A), indicating a weaker binding affinity of APOA1 for Fib-PEX, compared to intact MMP2.

We applied three additional interaction techniques to confirm the direct interaction of APOA1 with MMP2: (i) visual interference color



assay (VICA), (ii) thermal stability assay and (iii) chemical crosslinking. In the VICA assay, the deposition of APOA1 over immobilized MMP2 reproducibly generated changes in the observed color upon binding (Fig. 3B). The VICA analyses also indicated that APOA1 binds to the Fib-PEX_{6xHis} construct (Fig. 3B).

In the thermal stability assay, the inclusion of APOA1 drastically increased the thermal stability of MMP2, indicating that MMP2 forms a

stable complex with APOA1 (Fig. 3C). To stabilize the APOA1–MMP2 complex, we incubated APOA1 and MMP2 in the presence of bis(sulfosuccinimidyl)suberate (BS³), an amine-to-amine chemical crosslinker with an 8-carbon spacer arm. Two distinct chemically crosslinked complexes (~100 kDa, and ~130 kDa) were observed. This suggested that APOA1 (28 kDa) may bind MMP2 (62 kDa) at a 1:1 or 2:1 molar ratio (Fig. 3D).

Fig. 3 | APOA1 directly interacts with MMP2. **A** Microscale thermophoresis analyses of APOA1 interactions with MMP2 and the MMP2 Fib-PEX domain. Increasing concentrations of active recombinant human MMP2 (from 0.9 nM to 30 μ M) incubated with 0.6 μ M APOA1_6xHis labeled with RED-Tris-NTA dye were analyzed in the presence or absence of calcium ions in the assay buffer. The MST analyses of the interaction between the MMP2 Fib-PEX domain and APOA1 are also presented. Data for positive interaction plots are presented as mean $\pm$ SEM. $N = 3$ independent interaction experiments (experimental replicates). **B** A visual interference color assay (VICA) analysis of APOA1 interactions with MMP2 and the MMP2 Fib-PEX domain. Active recombinant human MMP2 (75 μ g/mL) was deposited on the VICA surface and incubated with APOA1 (100 μ g/mL) for the times indicated, followed by color change (ΔC) quantitation relative to $t = 0$ (left). Mean $\pm$ SEM. $N = 3$ independent experiments. Recombinant Fib-PEX_6xHis (75 μ g/mL) was deposited on the

VICA surface, followed by incubation with APOA1 (100 μ g/mL) for 30 min (right) and color change quantitation relative to Fib-PEX_6xHis by itself on the surface. Mean of $N = 2$ independent interaction experiments (experimental replicates). **C** Thermal stability assay. Active recombinant human MMP2 (10 nM) was mixed with vehicle (enzyme assay buffer) or APOA1 (100 nM). Mixtures were heated separately at increasing temperatures (0–80 $^{\circ}$ C) for 10 min and, next, analyzed by in-gel gelatin zymography to detect and quantitate non-denatured MMP2. Representative gel image. Plot with mean value of $N = 2$ determinations (experimental replicates) of non-denatured MMP2 quantity at each temperature. **D** Representative chemical crosslinking experiments to determine the stoichiometry of APOA1–MMP2 complexes. Zymogram shows MMP2 complexes formed with BS³ crosslinker in the absence or presence of APOA1. Similar observations were made in $N = 2$ independent crosslinking experiments (see Supplementary Fig. 5).

Together, these different and orthogonal protein-protein interaction assays clearly show that APOA1 directly interacts with MMP2.

APOA1 inhibits MMP2 autolysis

We applied a 2D non-gradient BN-PAGE technique to explore whether MMP2 changes its aggregation state in the presence of APOA1-containing HDL. We mixed active recombinant human MMP2 (10 nM) with vehicle (enzyme assay buffer: 50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM CaCl₂, 1 μ M ZnCl₂) or increasing concentrations of APOA1-containing HDL purified from human plasma (2.5, 25, or 50 μ g/mL; i.e., 0, 10, 100 or 200 nM, commercial sourced and diluted in the same vehicle as MMP2). Next, we separated the mixture by BN-PAGE (1st dimension), followed by SDS-PAGE / gelatin zymography (2nd dimension, to determine the fractionation profile of MMP2) (Supplementary Fig. 3). We observed that MMP2 gelatinolytic activity migrated predominantly in two areas in the 2D gel (Supplementary Fig. 3). The activity on the left side of the 2D gel indicated that MMP2 formed HMW complexes with itself. The activity on the right side of the 2D gel was consistent with monomeric MMP2 (~62 kDa) and degradation products (<62 kDa) indicative of MMP2 autolysis. We unexpectedly found that HDL concentration-dependently inhibited MMP2 autolysis (Supplementary Fig. 3). To confirm this latter observation, we incubated active recombinant human MMP2 (10 nM) with vehicle (enzyme assay buffer), HDL (25 μ g/mL, i.e., 100 nM) or TIMP2 (100 nM) at 37 $^{\circ}$ C for 0, 15, or 30 min followed by detection of MMP2 by either gelatin zymography or western blotting to determine the extent of MMP2 autolysis under those conditions. We found that autolysis was more effectively inhibited by HDL (a noninhibitor of MMP2) than by TIMP2 (an inhibitor of MMP2) (Supplementary Fig. 4A, B). Further inspection confirmed that APOA1 and HDL inhibited MMP2 autolysis to a larger extent than other abundant blood-borne proteins, such as albumin or alpha 2 macroglobulin (A2MG) (Fig. 4A, B). The propensity to autolysis exhibited by many MMPs has been proposed to be a posttranslational reaction that limits MMP activity in vivo^{37,38}. Our data indicated that APOA1 and APOA1-containing HDL inhibit MMP2 autolysis at concentrations $\sim 10^2$ – 10^3 times lower than those of blood-borne APOA1. These data suggest that APOA1 and HDL can increase MMP2 bioavailability through inhibiting MMP2 autolysis.

APOA1 increases the proteolytic activity of MMP2

The best-known interactors of MMP2 in blood (TIMPs, A2MG, fibronogen) are inhibitors^{14,16,17}. We found that both >95% pure HDL (which contains lipid-bound APOA1) and >85% pure delipidated APOA1 concentration-dependently increased MMP2 proteolytic activity (Fig. 5A, B). Control studies using >95% pure lipid-free recombinant APOA1 (which is particularly free of plasma lipids) confirmed the ability of lipid-free APOA1 to increase MMP2 activity (Supplementary Fig. 5). These studies showed that both HDL and APOA1 increase MMP2 proteolytic activity but lipidation status of APOA1 is not critical for increasing the proteolytic activity of MMP2.

We tested whether APOA1 increased MMP2 activity solely by inhibiting autolysis. We found that APOA1 inhibited MMP2 autolysis at APOA1:MMP2 molar ratios >1 and increased MMP2 proteolytic activity at APOA1:MMP2 molar ratios >5. Therefore, the increase in MMP2 activity induced by APOA1 cannot be entirely explained by an inhibition of MMP2 autolysis (Fig. 5C).

We tested whether non-APOA1 components in HDL influenced MMP2 activity. Like HDL and APOA1, APOA2 and APOE, two apolipoproteins present in HDL and involved in cholesterol transport^{5,23,39}, formed complexes with MMP2 (Supplementary Fig. 6), and increased MMP2 activity, although APOA2 was not as effective as APOA1 or APOE (Fig. 5D).

Our MST analyses suggest that the catalytic domain (which is present in intact MMP2 but absent in the Fib-PEX construct) contains residues that increase APOA1 affinity for intact MMP2, compared to the Fib-PEX construct. Consistent with this notion, specific interactions between the first 20 residues of the APOA1 N-terminal region and the catalytic cavity of MMP2 were suggested by AlphaFold2 modeling (Fig. 2). We compared the effects of HDL with TIMP2 (a canonical MMP inhibitor) on the proteolytic activity of a catalytic domain construct (which excludes the PEX domain and fibronectin-like repeats). As expected, TIMP2 did not increase the rate of the FITC-gelatin cleavage reaction by the catalytic domain, whereas HDL did (Fig. 5E). APOA1 increased the proteolytic activity of the catalytic domain by ~50% (Fig. 5F), showing that interactions with the catalytic domain indeed contribute to the increase in MMP2 activity induced by APOA1.

APOA1 allosterically increases the proteolytic activity of MMP2

To elucidate how APOA1 increases MMP2 activity, we measured MMP2 proteolytic activity in the presence of APOA1 or vehicle (enzyme assay buffer) with increasing concentrations of a substrate (FITC-gelatin) and fit the data into allosteric sigmoidal kinetics mode (Fig. 6) and Michaelis–Menten kinetics models (Supplementary Fig. 7). In both models, the $V_{\max}$ was elevated ~300% by APOA1, compared to vehicle. However, we observed no change in the K_m or K_{half} (a measure of the affinity of MMP2 for the substrate). The sigmoidal kinetics model was a better fit for the data (i.e., higher confidence levels for $V_{\max}$ and K_m or K_{half} determinations) than was the Michaelis–Menten kinetics model. This experiment revealed that APOA1 and MMP2 engage in allosteric interactions (Fig. 6).

Plasma APOA1 increases the proteolytic activity of MMP2

We sought to determine whether plasma APOA1 possessed the capacity to increase MMP2 activity. Since the FITC-gelatin cleavage assay detects no gelatinolytic activity in either whole plasma (which predominantly contains proMMP2 in addition to MMP inhibitors) or FPLC SEC fractions (which contained highly diluted proMMP2), we devised a bioassay for this experiment. We fractionated human plasma by FPLC SEC and collected fractions. Next, we added MMP2 to the FPLC SEC fractions, immediately followed by the addition of FITC-gelatin. To determine the rate of FITC-gelatin cleavage (i.e., MMP2 activity), we

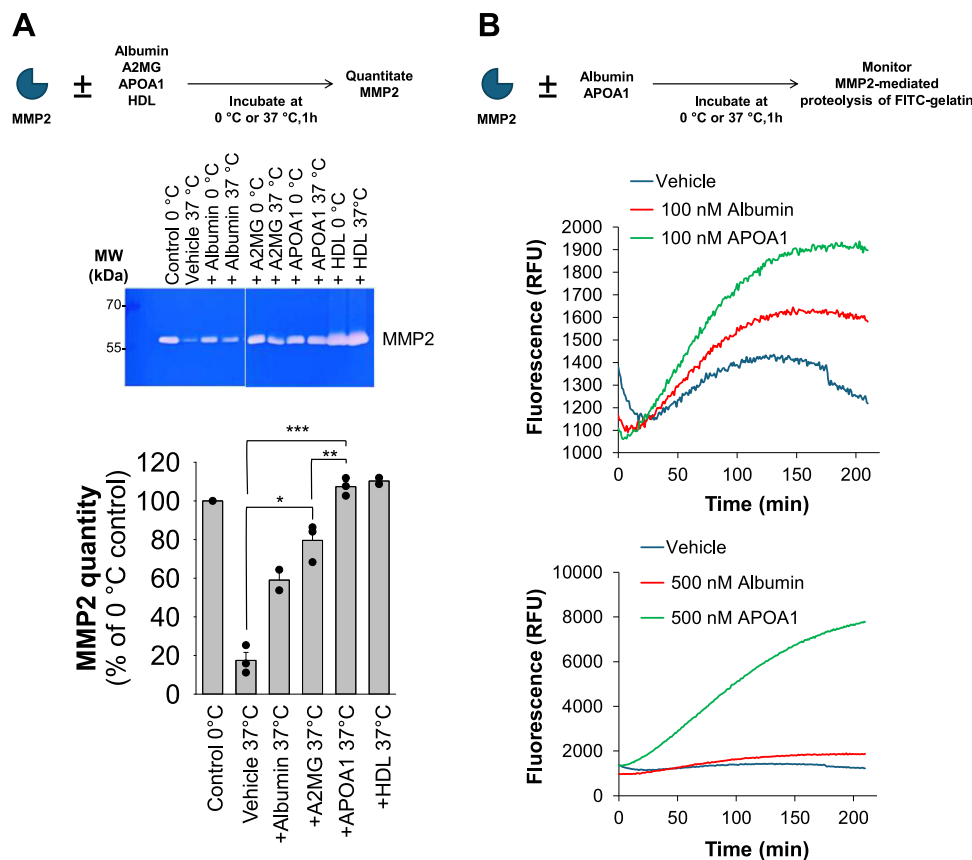


Fig. 4 | APOA1 inhibits MMP2 autolysis. A Active recombinant human MMP2 (10 nM) was incubated with vehicle (enzyme assay buffer) or albumin (100 nM), A2MG (100 nM), APOA1 (100 nM) or HDL (25 µg/mL; ~100 nM) at 0 °C or 37 °C for 1 h, after which MMP2 was detected via in-gel gelatin zymography. MMP2 quantity at 37 °C was compared to MMP2 quantity at 0 °C (no autolysis, negative control) and plotted as a bar graph. Mean ± SEM. $N = 3$ independent reactions (experimental replicates). * $p = 0.001$, ** $p = 0.005$, *** $p = 0.001$, one way ANOVA (Holm-Sidak

method). **B** Active recombinant human MMP2 (10 nM) was incubated with vehicle (enzyme assay buffer) or albumin or APOA1 at the indicated concentrations at 37 °C for 1 h, after which MMP2 proteolytic activity was measured using the FITC-gelatin cleavage assay. MMP2 activity was plotted as line graphs of fluorescence against time. Data are presented as mean value of $N = 2$ independent reactions (experimental replicates).

monitored fluorescence development. We found that MMP2 activity was the highest in fractions F13 and F14, which also had the highest content of APOA1 (Fig. 7A).

To determine whether it is indeed APOA1 in the plasma fractions that induced this upregulation of MMP2 activity, we subjected plasma from APOA1 knockout (APOA1 KO) mice and WT mice by FPLC SEC to the same bioassay. Only the fractions from WT mice, but not those from APOA1 KO mice, increased the proteolytic activity of added active MMP2 (Fig. 7B).

Further inspection of the APOA1 KO plasma revealed no difference in total proMMP2 levels, compared to WT plasma (Supplementary Fig. 8) while the fractionation profiles of APOE and cholesterol differed (Supplementary Fig. 9). As in humans (Fig. 7A), the greatest content of APOA1 in plasma of WT mice was in fraction F14 (Fig. 7B). Interestingly, there was an observable decrease in the quantity of proMMP2 in fraction F14 and a shift to the right in the fractionation profile of proMMP2 in plasma of APOA1 KO mice, compared to plasma of WT mice (Fig. 7C). The relative quantity of proMMP2 in fraction F14 (relative to F16, MW < 75 kDa) was larger in WT plasma, compared to APOA1 KO plasma (Fig. 7C). The proportion of proMMP2 comigrating with APOA1 (fraction F14, MW > 158 kDa) was ~51.1% in WT plasma and ~32.6% in APOA1 KO plasma.

We conclude that APOA1 can account for, at least most of, the observed increase in the proteolytic activity of active MMP2 in human and mouse plasma. However, proMMP2 does not interact only with APOA1. Plasma proMMP2 and MMP2 also have non-APOA1 interactors

in blood; among which TIMP2 (Fig. 1E) or other apolipoproteins (Fig. 5D) are likely candidates.

Discussion

The identification of MMPs as a family of Zn^{2+} - and Ca^{2+} -dependent proteinases began with the discovery of the tadpole collagenase (MMP1)^{40,41} and the subsequent identification of other MMPs, including MMP2⁴², and their intracellular, extracellular matrix, and cell surface substrates^{43–46} and tissue inhibitors^{45,47,48}. Likely because of the low abundance of proMMPs and MMPs in the blood circulation, the blood interactome of MMPs has remained largely unknown. Plausibly, this blood interactome participates in the regulation of MMPs proteolytic activity as well as the transport and redistribution of proMMPs and MMPs away from synthesis and secretion foci—such as normal cells and organs, sites of arterial lesion, failing organs, and cancer tumors^{12,20,21}.

In this contribution, (i) we report a serendipitous observation: blood-borne proMMP2 circulates bound to APOA1 in HDL; (ii) we applied the artificial intelligence-based AlphaFold2 software to model and predict interaction interfaces between APOA1 and MMP2, leading to the identification of APOA1 interactions with the catalytic domain and part of fibronectin-like repeats of MMP2; (iii) we utilized a diversity of interaction assays and functional experiments to confirm the AlphaFold2-based predictions; and, importantly, (iv) we discovered that interactions with APOA1 inhibit MMP2 autolysis and allosterically increase MMP2 activity, which may provide a two-pronged biochemical mechanism to increase MMP2 bioavailability.

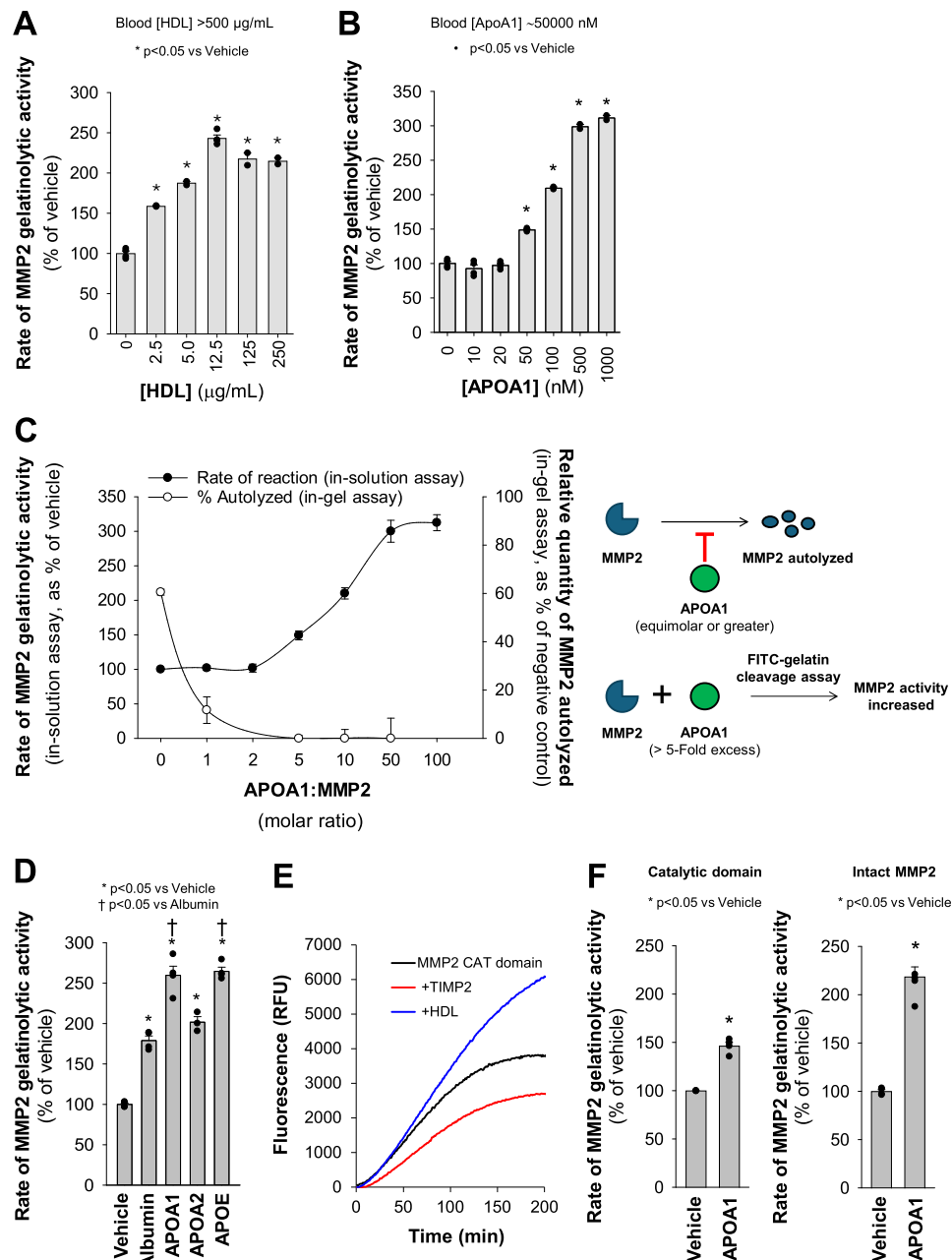


Fig. 5 | APOA1 increases MMP2 proteolytic activity. **A** APOA1-containing HDL (0–250 $\mu\text{g/mL}$; i.e., 0, 10, 20, 50, 100, and 1000 nM) was added to active recombinant human MMP2 (10 nM). MMP2 activity was determined using the FITC-gelatin cleavage assay. Mean $\pm$ SEM of independent reactions (experimental replicates). $N = 4$ (0 and 12.5 $\mu\text{g/mL}$ HDL); $N = 2$ (2.5, 5, 125 and 250 $\mu\text{g/mL}$ HDL). * $p = 0.05$ vs vehicle (enzyme assay buffer), one way ANOVA (Holm-Sidak method). **B** Delipidated APOA1 (0–1000 nM) was added to MMP2 (10 nM). MMP2 activity was determined using the FITC-gelatin cleavage assay. Mean $\pm$ SEM of independent reactions (experimental replicates). $N = 4$ (molar ratios 1:0, 1:1, 1:2 and 1:5); $N = 2$ (molar ratios 1:10, 1:50 and 1:100). * $p = 0.001$ vs vehicle, one way ANOVA (Holm-Sidak method). **C** APOA1 effects on MMP2 (10 nM) proteolytic activity (left 'y' axis, data from panel (B)) and autolysis (right 'y' axis). MMP2 was kept at 0 $^{\circ}\text{C}$ (negative control, i.e., initial amount of MMP2 used in the experiment) or 37 $^{\circ}\text{C}$ for 30 min in the absence of APOA1 (vehicle) or presence of APOA1, at indicated molar ratios. MMP2 activity was determined using the FITC-gelatin cleavage assay. In the autolysis assay, non-autolyzed MMP2 was quantitated using the in-gel gelatin zymography. Mean $\pm$ SEM of $N = 3$ independent reactions (experimental replicates).

Diagram illustrating the observations. **D** MMP2 was mixed with vehicle (enzyme assay buffer) or albumin, APOA1, APOA2, or APOE at a 1:10 molar ratio. MMP2 activity was determined using the FITC-gelatin cleavage assay. Mean $\pm$ SEM for independent reactions (experimental replicates). $N = 4$ (vehicle, albumin, APOA1 and APOE), $N = 3$ (APOA2). * $p = 0.001$ albumin vs vehicle, $p = 0.001$ APOA1 vs vehicle, $p = 0.001$ APOA2 vs vehicle, $p = 0.001$ APOE vs vehicle. † $p = 0.001$ APOA1 vs albumin, $p = 0.001$ APOE vs albumin, one way ANOVA (Holm-Sidak method). **E** MMP2 catalytic domain (10 nM) was mixed with vehicle (enzyme assay buffer) or TIMP2 (20 nM) or HDL (25 $\mu\text{g/mL}$; ~100 nM). Time plots of proteolytic activity measured using the FITC-gelatin cleavage assay. Mean of $N = 2$ independent reactions (experimental replicates). **F** Activity of MMP2 catalytic domain or intact MMP2 (10 nM) in the presence of APOA1 (100 nM). Mean $\pm$ SEM for independent reactions (experimental replicates): $N = 3$ (catalytic domain+vehicle), $N = 4$ (catalytic domain+APOA1 and intact MMP2+vehicle), $N = 5$ (intact MMP2+APOA1). * $p = 0.000160$ APOA1 vs vehicle for catalytic domain, $p = 0.0000190$ APOA1 vs vehicle for intact MMP2, unpaired two-tailed Student's t -test.

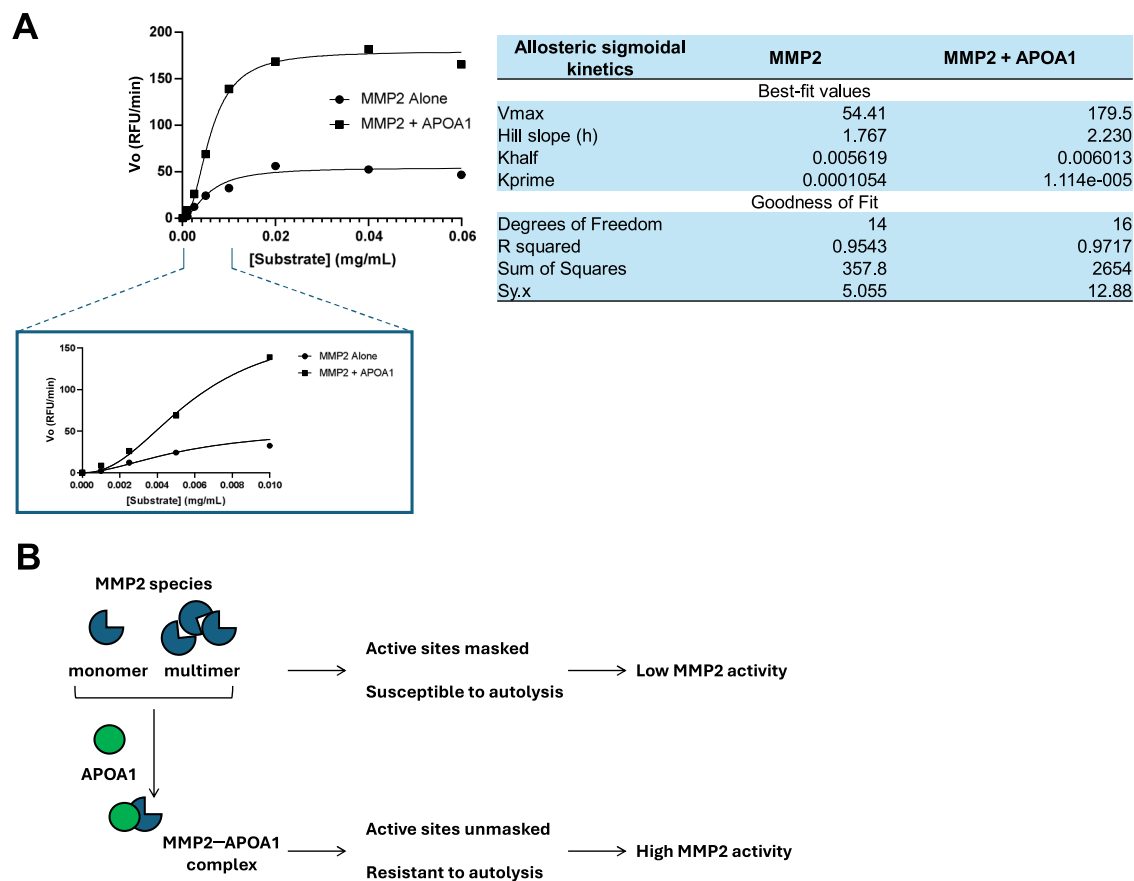


Fig. 6 | Kinetic analysis of the allosteric effect of APOA1 on MMP2 activity.

A MMP2 proteolytic activity was measured in the presence of vehicle (enzyme assay buffer) or APOA1 (100 nM) (1:10 MMP2:APOA1 molar ratio) with increasing concentrations of the substrate (FITC-gelatin). The data is presented as line/scatter plots. Mean value for independent reactions (experimental replicates): $N = 3$ for [substrate] = 0 and 0.001 mg/mL, $N = 2$ for [substrate] = 0.0025, 0.01, 0.02 and

0.04 mg/mL, $N = 4$ for [substrate] = 0.05 mg/mL, $N = 1$ for [substrate] = 0.06 mg/mL. K_{half}: concentration of substrate that produces a half-maximal enzyme velocity (V_{max}). K_{prime}: K_{half}·h. Hill slope (h): When $h > 1.0$, the curve is said to be sigmoidal due to positive cooperativity. **B** Illustration of the proposed mechanism by which APOA1 increases MMP2 activity in solution.

These findings were not predictable because: (a) complexation of MMP2 by APOA1 could not be anticipated by current knowledge about these proteins (e.g., APOA1 interactions with the catalytic domain or part of fibronectin-like repeats of MMP2 have not previously been reported), and (b) the effects of APOA1 on MMP2 such as the allosteric increase in active MMP2 proteolytic activity could not be hypothesized as active MMP2 was not previously known to be an allosteric enzyme.

What is the role of lipids in mediating the proposed allosteric regulation of MMP2 by APOA1? Synthesized in the liver and intestines, APOA1 participates in the sequestration and transport of cholesterol from peripheral organs and atherosclerotic lesions to the liver⁵. This homeostatic interorgan communication strategy may protect against atherogenesis by sequestering cholesterol from atherosclerotic plaques⁵. We found that APOA1-containing fractions isolated from plasma of humans or WT mice (but not APOA1 KO mice) shared their ability to increase the proteolytic activity of active MMP2 with purified APOA1-containing HDL, as well as with purified delipidated APOA1 from human plasma, and lipid-free recombinant APOA1. Therefore, interactions between APOA1 and MMP2 occur at physiological levels of plasma lipids, but lipids are not needed for these interactions. Our experimental findings are consistent with various scenarios, including that proMMP2 and MMP2 may interact with: (i) lipid-free APOA1 leaked from HDL, (ii) poorly lipidated APOA1 in HDL, (iii) lipidated APOA1 in HDL (at least via the APOA1 protein-binding N-terminal stretch predicted by AlphaFold), and (iv) non-APOA1 components (apolipoproteins and non-apolipoproteins) associated with HDL. Currently, there

are no experimental structures of the heterocomplexes of APOA1 with proMMP2 and with active MMP2. Based on our experimental data, the experimental structure of lipid-free APOA1 (which is thought to reflect a conformation that mimics lipid-bound APOA1 on a nascent, discoidal HDL particle)^{28,29} utilized for our AlphaFold-based predictions provides a valid approximation of the heterocomplexes of APOA1 with proMMP2 and MMP2 for at least some of these scenarios.

We noticed that MMP2 has a propensity to form complexes with itself. Homomultimerization of MMPs, including MMP2 dimerization, has been suggested to bring protomers closer to active sites, influencing autolysis and substrate accessibility, two determinants of MMP activity^{14,49–51}. We think that: (i) APOA1 may directly interact with and dissociate MMP2 homomultimers; thus, increasing the number of active sites exposed to substrates (the kinetics data supports this notion: in the presence of APOA1 we measured a ~3-fold increase in V_{max} in the rate of MMP2-mediated cleavage of FITC-gelatin). (ii) APOA1 binds to the PEX domain along with part of the catalytic groove and fibronectin-like repeats of MMP2, masking autolysis sites to further inhibit autolysis (the MST and VICA data supports this). (iii) APOA1 interactions with the catalytic groove of active MMP2 may stabilize the complex without impeding entry of the substrate or nucleophilic activity of the catalytic zinc (as suggested by AlphaFold2-based modeling).

Are the identified interactions between APOA1 and MMP2 likely in vivo? In normal physiology^{5,9}, APOA1 concentration in blood is ~50 μM, which is a 5000-fold molar excess over the levels of MMP2

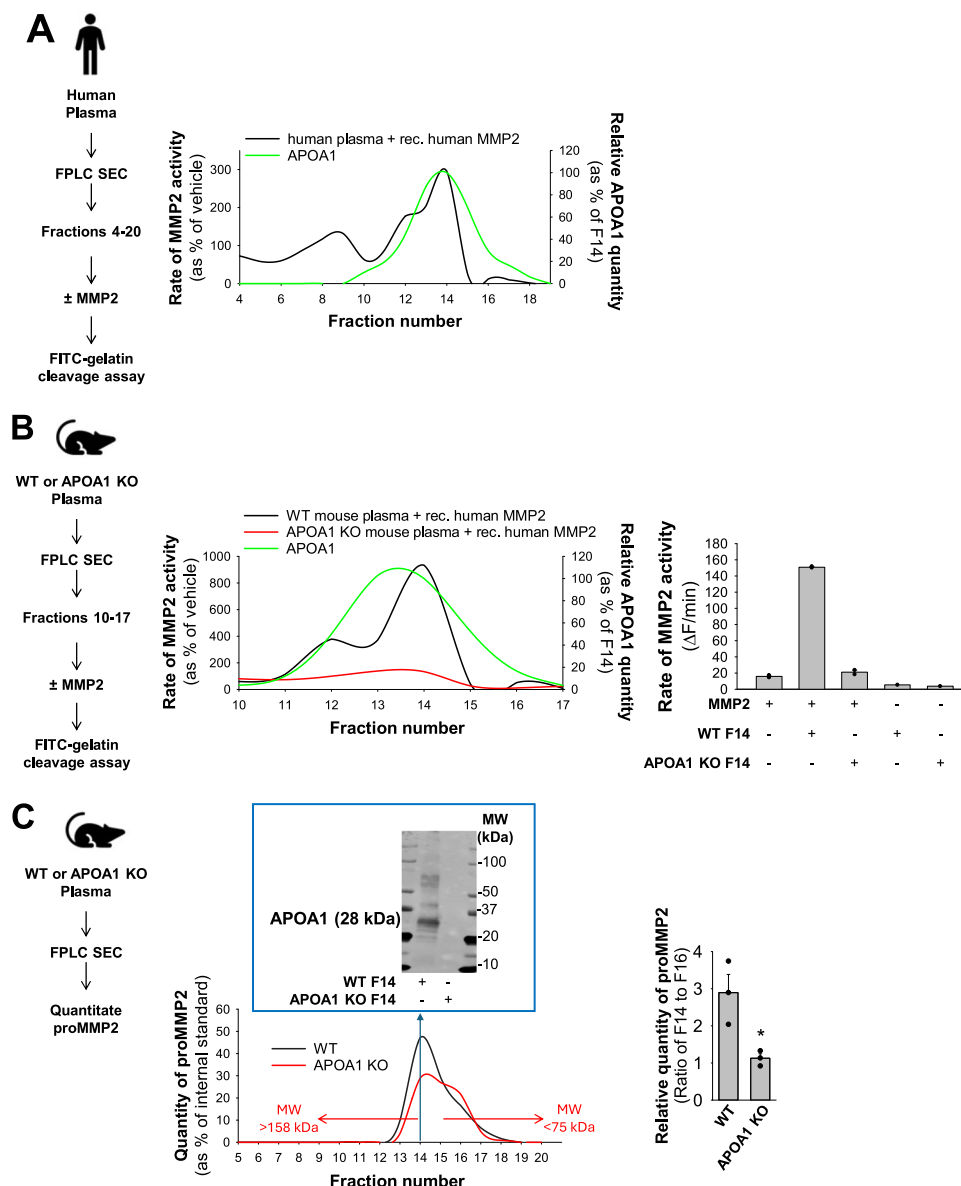


Fig. 7 | Influence of blood-borne APOA1 on proMMP2 fractionation and MMP2 proteolytic activity. **A** Plasma was pooled from five human donors and fractionated using FPLC SEC. Fractions were collected and active recombinant human MMP2 (rec. human MMP2) was added to each fraction (final MMP2 concentration was 10 nM). Next, MMP2 proteolytic activity was measured using the FITC-gelatin cleavage assay. Rates of MMP2 activity were plotted against the corresponding fraction numbers as a line/scatter plot. Maximum of MMP2 activity coincided in fractions F13-F15 with maximum of APOA1 immunoreactivity, as observed with human plasma proMMP2 (Fig. 1B). **B** Plasma was pooled from six WT mice or six age-matched APOA1 KO mice and fractionated using FPLC SEC. Fractions were collected and active recombinant human MMP2 was added to each fraction (final MMP2 concentration was 10 nM). Next, MMP2 proteolytic activity was measured using the FITC-gelatin cleavage assay. Rates of reaction were plotted against fraction number as a line/scatter plot. The bar diagram compares the rate of MMP2

proteolytic activity in fraction F14 from WT and APOA1 KO plasma. Data are presented as mean value for independent reactions (experimental replicates). $N = 4$ for MMP2 alone, $N = 2$ for MMP2 + WT F14 and MMP2 + APOA1 KO F14, $N = 1$ for negative controls (WT F14 and APOA1 KO F14 alone). Similar co-fractionation of APOA1 and proMMP2 from mouse plasma is shown in Fig. 1C. **C** Plasma was pooled from six WT mice or six age-matched APOA1 KO mice and fractionated using FPLC SEC. Fractions were collected. Plasma proMMP2 in each fraction was detected by in-gel gelatin zymography, quantitated, and plotted as scatter/line plot. The inset shows a western blot trace highlighting the presence of APOA1 in F14 of WT plasma and absence in APOA1 KO plasma. The bar diagram compares the abundance of the complexed proMMP2 in fraction F14, relative to the uncomplexed proMMP2 in fraction F16 in WT and APOA1 KO mouse plasma. Mean $\pm$ SEM. $N = 3$ independent experiments (experimental replicates). $*p = 0.0251$ vs. WT, unpaired two-tailed Student's t -test.

and between 10- and 50-fold greater than the highest concentration of APOA1 tested in our biochemical/interaction studies. Thus, the identified interactions between MMP2 and APOA1 are likely favorable in vivo, as confirmed in two different species (humans and mice).

How important is APOA1 as an interactor of MMP2 under physiological conditions? Plasma from healthy humans and APOA1-expressing mice (but not plasma of APOA1 KO mice) exhibited the

ability to increase the proteolytic activity of active MMP2. Therefore, APOA1 can account for, at least most of, the observed increase in the proteolytic activity of active MMP2 in human and mouse plasma.

APOA1, APOA2, and APOE are major protein components in HDL^{5,9,23} and their genes have a common evolutionary origin (due to duplication and diversification of a basic genetic motif encoding a periodic 22 amino acid sequence with a characteristic α -amphipathic helix signature in these three apolipoproteins)⁵². Through chemical

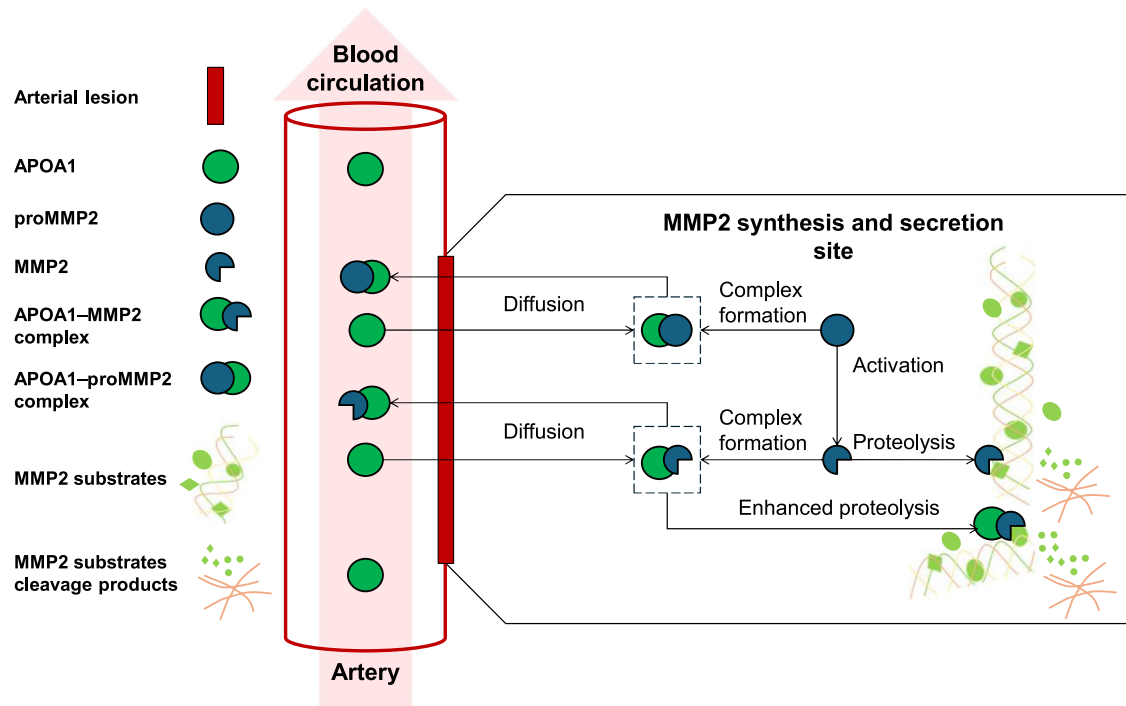


Fig. 8 | The physiological significance of the interactions between blood-borne APOA1 and proMMP2/MMP2, as suggested by this research. Blood-borne APOA1 engages in a high-affinity complex with proMMP2 and MMP2. This complex is stable and driven by blood circulation. The proteolytic activity of active MMP2 is

enhanced when it is engaged with APOA1 leading to enhanced proteolysis of MMP2 substrates. The interactions identified in this research could be relevant in MMP2 synthesis and secretion sites (illustrated for the case of an arterial lesion).

crosslinking, we could stabilize and detect complexes of MMP2 with APOA2, and (to some extent) with APOE. However, in plasma of APOA1 KO mice, the presence of APOA2 and APOE did not translate into a compensatory increase in MMP2 activity, reinforcing the notion that APOA1 is required in this function.

Over 100 cholesterol molecules can be transported in an HDL particle⁵. Fourteen types of apolipoproteins and over 100 different proteins associate with HDL^{5,9,53}, resulting in heterogeneous particles ranging from 175 to 500 kDa (6–12.5 nm in diameter)^{5,9,10}. We reason that MMP2 is a new component of the APOA1 and HDL interactomes (illustrated in Supplementary Fig. 10)^{9,10}. In addition to canonical interactors of MMPs (such as TIMP2^{45,47,48}), APOA1, APOA2, APOE, and other proteins (including albumin) could influence the biological potential of MMP2 in the blood. Hence, we propose a new term, transporter and regulator of the activity potential (TRAP) of MMPs, to define multiprotein complexes that transport MMPs in the blood and may additionally cause MMP activity to increase (e.g., as observed with APOA1, APOE, and to a lesser extent with APOA2), decrease (as confirmed here with TIMP2) or remain relatively unchanged (as we found to be the case with albumin). In this sense, APOA1 is a candidate TRAP of MMP2. Given that proMMP2 and MMP2 circulate at nanomolar concentrations, it stands to reason that the population of APOA1 functioning as a TRAP of proMMP2 (and/or MMP2) circulates at similarly low concentrations in the blood. Low physiological concentrations of proMMP2 and MMP2 and endogenous MMP inhibitors (such as TIMPs, A2MG, and fibrinogen) in the blood might have precluded the detection and functional assessment of these interactions prior to our study. In addition, technicalities (such as the interference of high KBr concentrations needed for density gradient ultracentrifugation and isolation of HDL with interactions between blood-borne APOA1 and proMMP2/MMP2) might have also contributed to preventing these findings from being made earlier.

How important are these identified interactions in disease? In disease conditions, affected organs often become foci of high

synthesis and secretion of proMMPs and MMPs—as previously documented at sites of arterial lesions and atherosclerotic plaques, failing organs, and cancer tumors^{12,20,21}. The biological consequences of an increase in secreted or circulating MMP2 levels in disease states could be amplified by an allosteric (i.e., non-linear) increase in MMP2 proteolytic activity resulting from MMP2 complexation with APOA1, as we have found; an analogous proposal was previously made for another MMP2 interactor, type I collagen³⁷. ProMMP2 and, particularly, active MMP2 are elevated in atherosclerotic lesions where the collective activity of many MMPs, including MMP2 and MMP9 (from vascular smooth muscle) and MMP8 (from macrophages), contributes to the rupture of vulnerable atherosclerotic plaques^{20,21}. Plaque rupture recruits blood platelets, which aggregate, forming thrombi²⁰—a primary cause of atherothrombotic (type I) acute myocardial infarction^{5–8}. Active MMP2 (but not its proteolytically inactive precursor, proMMP2) mediates a pathway of platelet aggregation²⁴ and thrombosis⁵⁴. Previous studies show that some apolipoproteins (e.g., APOA1, APOE, and APOA4) may exert anti-platelet and anti-thrombotic activity via specific mechanisms^{55–57}. For example, APOA1 inhibits platelet aggregation and protects from arterial thrombosis in vivo, at least in part, via the interaction of APOA1 with scavenger receptor class B type I in platelets⁵⁵. The usefulness of apolipoproteins as biomarkers of risk or therapeutic agents for atherosclerosis and arterial thrombosis remains, however, debated^{5–8}. Whereas APOA1^{5,58} and APOA4⁵⁹ may be protective, APOA2 may or may not protect^{58,60}. APOE has allele- and gender-dependent effects on platelet aggregation⁶¹ with its ε4 allele increasing the risk of coronary artery disease⁶¹. Our intriguing findings open questions for future research such as whether a high-affinity complex between blood-borne apolipoproteins and MMP2 forms in vivo to: (i) increase MMP2-mediated proteolysis of substrates (which may exacerbate atherosclerotic plaque rupture and thrombosis), or (ii) decrease MMP2-mediated proteolysis (independently of the presence of endogenous MMP2 inhibitors) because blood-borne apolipoproteins can sequester and redistribute MMP2 away from foci of

MMP2 synthesis and secretion (illustrated in Fig. 8, Supplementary Fig. 11).

The AlphaFold models generated here helped identify the MMP2 domains that specifically interact with APOA1 and helped guide some of our biochemical studies targeting those domains, but they are no replacement for experimental structures. Future acquisition of experimental cryo-electron microscopy structures of the APOA1–MMP2 complex should help clarify how these proteins interact at the atomic level and guide rational interventions to either mimic or disrupt these interactions for precise therapeutic purposes.

The function-altering interactions identified in this research are independent of canonical transport of cholesterol and phospholipids by APOA1 and lay the ground for future explorations on new potential ways to interpret, diagnose, and treat the influences of apolipoproteins and MMPs on organismal homeostasis, atherosclerosis, and thrombosis. New knowledge derived from this research could stimulate the identification of new biomarkers of risk or therapeutic agents for atherosclerosis and arterial thrombosis as well as the design of precision medicines for individuals with apolipoprotein variants that increase the risk of atherothrombosis through enhancing MMP activity.

Methods

Ethics

Approval for these studies was obtained from the University of Alberta Human and Animal Research Ethics Boards (Study IDs: Pro00089845, Pro00068611, AUP00000253). All animal procedures were approved by the University of Alberta's Animal Care and Use Committee and were in accordance with guidelines of the Canadian Council on Animal Care.

Blood specimens

To assess the influence of APOA1 on the fractionation and activity potential of proMMP2 and MMP2 in humans, these studies used human specimens described under Supplementary Table 3.

To assess the fractionation profiles of MMP2 and APOA1, these studies used EDTA-treated plasma from $N = 9$ WT male mice (CBL57/B6) aged 12–15 weeks. These mice were purchased from The Jackson Laboratory (Maine, US), and housed three to five per cage, and exposed to a 12 h light/dark cycle beginning with light at 8:00 a.m. Housing temperature was 20–24 °C, humidity was 40–70%. Mice were fed ad libitum a chow diet and had free access to demineralized UV filtered water.

To assess the influence of plasma APOA1 on the fractionation and activity potential of proMMP2, and MMP2, these studies used EDTA-treated plasma from a total of $N = 6$ wild type (WT) male mice (CBL57/B6) and $N = 6$ APOA1 KO male mice aged 7–8 weeks. Plasma samples from six APOA1 KO mice and age-matched WT mice were purchased from The Jackson Laboratory (Maine, US).

Proteins

The studies used the following protein reagents: 95% pure HDL (High Density Lipoproteins, Human Plasma, Cat# 437641-10MG, Sigma-Aldrich, USA); >85% pure, delipidated human plasma APOA1 (Apolipoprotein A1, Cat# 178452-500 µg, Sigma-Aldrich, USA; manufacturer's note: this product is delipidated during its purification process from human plasma. Although it is not routinely tested for final lipid contamination, previous lots have been tested by SPIFE Vis Cholesterol Gel against a cholesterol profile standard and no visible lipid contamination had been found); 95% pure, lipid-free recombinant human APOA1 (Human APOA1 6x_His Tag (Cat# AP1-H5225, Acrobiosystems, USA)). 98% pure active recombinant human MMP2 (Cat# SRP3118-10 µg, Sigma-Aldrich, USA). ≥90% pure MMP2 (catalytic domain) (Cat# BML-SE237-0010, Enzo life sciences, USA). 1.0 > 95% Fib-PEX (residues 359-660, Cat# PDEH100254, ElabScience, USA). 95% pure MMP2 PEX domain (residues 467-660, Cat# G04MP02H,

GiottoBiotech, Italy). ≥95% pure TIMP2 (Cat# SRP3174-10 µg, Sigma-Aldrich, USA).

Fractionation profiles of plasma lipoproteins, triglycerides, and cholesterol by Fast Protein Liquid Chromatography (FPLC) with a size exclusion column (SEC)

Plasma lipoproteins. Fractionations were performed at the University of Alberta Faculty of Medicine & Dentistry Lipidomics Core (RRID: SCR_019176). 100 µL of EDTA-treated plasma was injected by auto-sampler into an Agilent 1200 high-performance liquid chromatography instrument equipped with a Superose 6 Increase 10/300 gel-filtration FPLC column (Cytiva Life Sciences), which separates the intact lipoproteins by size. Separation was performed isocratically at 400 µL/min using 150 mM NaCl with 3 mM NaN₃ as the mobile phase. The effluent was monitored in real-time by measuring absorbance at 280 nm and the data collected and analyzed using Agilent Chemstation software. Two-minute fractions (800 µL/fraction) were collected and stored at 4 °C within 20 min, followed by analysis.

Plasma lipoprotein triglycerides. 24 µL of EDTA-treated plasma was injected by autosampler into an Agilent 1200 high-performance liquid chromatography instrument equipped with a Superose 6 Increase 10/300 gel-filtration FPLC column (Cytiva Life Sciences), which separates the intact lipoproteins by size. Separation was performed isocratically at 400 µL/min using 150 mM NaCl with 3 mM NaN₃ as the mobile phase. Detection was performed by inline post-column reaction at 37 °C with Sekisui Triglyceride-SL reagent (Sekisui Diagnostics, Charlottetown, Canada) at a rate of 200 µL/min. Effluent was monitored in real-time at 505 nm, and the data collected and analyzed using Agilent Chemstation software.

Plasma lipoprotein cholesterol. 12 µL of EDTA-treated plasma was injected by autosampler into an Agilent 1200 high-performance liquid chromatography instrument equipped with a Superose 6 Increase 10/300 gel-filtration FPLC column (Cytiva Life Sciences), which separates the intact lipoproteins by size. Separation was performed isocratically at 400 µL/min using 150 mM NaCl with 3 mM NaN₃ as the mobile phase. Detection was performed by inline post-column reaction at 37 °C with Sekisui Cholesterol reagent (Sekisui Diagnostics, Charlottetown, Canada) at a rate of 200 µL/min. Effluent was monitored in real-time at 505 nm, and the data collected and analyzed using Agilent Chemstation software.

KBr gradient ultracentrifugation of plasma lipoproteins. The method was essentially as described earlier^{62,63}. Briefly, the pool of human plasma was adjusted to a density of 1.21 g/mL with KBr and ultracentrifuged in a step gradient made of four NaCl/KBr solutions with densities ranging from 1.006 (to float off the chylomicrons and very low-density lipoproteins) to 1.24 g/mL (to precipitate out the albumin and other plasma proteins). Nineteen fractions were collected. In each fraction, we determined total cholesterol (cholesterol-SL assay), APOA1 (western blotting with APOA1 antibody), and MMP2 (gelatinolytic activity).

Modeling

To predict *in silico* whether APOA1 interacts with active MMP2, we used the artificial intelligence-based software, AlphaFold2 (Supplementary Note 1 sections A.1–A.4, Supplementary Movie 1 and Supplementary Datasets 1–7 (in Source Data)).

Microscale thermophoresis (MST) to study APOA1–MMP2 interaction

For the MST experiments, a concentration series of MMP2 was prepared using a 1:1 serial dilution in buffer supplemented with 20 mM

Hepes (pH 7.5), 50 mM NaCl, 3 mM CaCl₂, and 0.05% Tween 20. The range of MMP2 concentration was from 4 μM to a final 1.95 nM, over 11 serial diluted Monolith NT.115 premium capillaries (NanoTemper) with 10 μL samples. The APOA1 was labeled using His-tag labeling kit RED-tris-NTA 2nd generation. The interaction for MST experiments was initiated by adding 10 μL of 0.6 μM His tag – labeled APOA1. To determine the role of calcium in the interactions between APOA1 and MMP2, similar binding experiments were carried out with a dilution buffer without calcium. The MMP2 concentration range used for these control experiments was 30 μM to 0.9 nM. The measurements were performed on a Monolith NT.115 (NanoTemper) using standard capillaries at 25 °C with 90% Excitation power, high MST power. Data were analyzed by MO.Control software (NanoTemper) and MO.Affinity Analysis software (NanoTemper). The experiments were performed in triplicate.

Visual interference color assay to study APOA1–MMP2 interaction

Protein deposition onto anodized alumina surface. Lyophilized MMP2 or MMP2 hemopexin-like domain were reconstituted in deionized water and, next, diluted to the indicated concentrations. Typically, drops (18 μL) of protein solution at varying concentrations were deposited onto 7 mm diameter circles on the surface of the thin films (Cat# STA-0023, Pavonis Diagnostics, CANADA) and incubated under 100% relative humidity at 4 °C overnight to prevent MMP2 autolysis. The slide was rinsed thoroughly with deionized water. APOA1 was diluted in deionized water as indicated and binding to the immobilized MMP2 was analyzed by incubation under 100% relative humidity at room temperature (for 1–30 min).

Color detection. The detection of binding was photographed with the use of a polarizing film to eliminate p-polarized light off the device surface and viewed at an incidence angle of 75° to generate the strongest color contrast by matching s-polarized light reflection intensities off the alumina and underlying surfaces. The red, green, and blue (RGB) coordinate system was used to quantitatively define the visible colors. The color difference or distance (ΔC) was calculated according to the formula: $(\Delta C)^2 = (R2 - R1)^2 + (G2 - G1)^2 + (B2 - B1)^2$.

Detection and quantitation of MMP2 protein and quantitation of MMP2 proteolytic activity

MMP2 protein and/or activity were detected and quantitated using (i) western blotting, (ii) in-gel gelatin zymography assay, (iii) in-solution gelatinase assay (fluorescein-conjugated gelatin as substrate, Cat# D12054, Thermofisher Scientific, USA), or (iv) multiplex protein detection assays following protocols previously described by us^{16,64}. MMP2 lysis bands in zymograms were quantitated by densitometry scanning using the ImageJ software (NIH, USA). For whole plasma/serum specimens, the volume analyzed ranged from 0.1 to 1 μL, depending on the experiment. For analysis of FPLC SEC fractions, the volume analyzed was 20 μL/fraction. Uncropped and unprocessed scans of the most important blots in the Supplementary Dataset 8 (in Source Data).

Detection and quantitation of apolipoproteins

APOA1, APOA2, and APOE were detected by western blotting following electrophoretic separation by SDS-PAGE. The immunoblot protein bands were quantitated by densitometry scanning using the ImageJ software (NIH, USA). For whole plasma specimens, the volume analyzed ranged from 0.1 to 1 μL, depending on the experiment. For analysis of FPLC SEC fractions, the volume analyzed was 20 μL/fraction. Uncropped and unprocessed scans of the most important blots in the Supplementary Dataset 8 (under Source Data).

2D BN-PAGE technique to examine the aggregation state of MMP2 in the absence and presence of HDL

Sample preparation. Recombinant human active MMP2 at a final concentration of 0.4 μM was added to increasing concentrations (0 μM, 0.4 μM, 4 μM, and 8 μM) of HDL in separate tubes. The final volume was brought up to 20 μL with 1x enzyme assay buffer (50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 13 mM CaCl₂, 3 mM Na₂N₃). The samples were incubated at 25 °C for 30 min. The loading buffer (75 mM 6-aminocaproic acid, 15 mM BisTris, 0.3% Coomassie blue G-250) was then added to the samples before loading onto the gel.

Non-gradient Blue-Native PAGE. The protocol was adapted from the 10% BN-PAGE technique described by Thangthaeng et al.⁶⁵. Gels were prepared with 4.7 mL gel buffer solution (500 mM 6-aminocaproic acid, 50 mM BisTris (pH 7.1)), 1.2 mL of 40% Acrylamide/bisacrylamide (crosslinking ratio: 29:1), 45 μL 10% (w/v) ammonium persulfate, 3 μL TEMED. The anode buffer was composed of 50 mM BisTris (pH 7.1), and the cathode buffer was composed of 50 mM Tricine, 15 mM BisTris (pH 7.1), and 0.025% Coomassie blue G-250. Then the electrophoretic separation of the proteins/complexes was performed. The individual lanes were excised and placed on a 10% acrylamide SDS-PAGE-gelatin zymography gel for a second-dimensional separation (to detect MMP2) or on a 10% acrylamide SDS-PAGE followed by western blotting (with APOA1 antibody (Cat# Ab7614, Abcam, UK) to detect APOA1-containing HDL).

Gradient Blue-Native PAGE. The protocol was adapted from the gradient BN-PAGE protocols described by Wittig et al.²⁵ and our lab^{66,67} and used discontinuous 3-step (5%, 10%, and 15%) gradient manually cast using glycerol to increase the gel's mechanical strength. These gradient gels covered a protein mass range from 10 kDa to 3 MDa²⁵ and were used to examine the comigration of MMP2 with APOA1 in HDL from plasma samples.

Chemical cross-linking studies

Active recombinant human MMP2 was pre-incubated with vehicle (1x PBS with 500 μM O-phenanthroline) or interactor proteins including APOA1, APOA2, APOE, Albumin, α-2-macroglobulin or TIMP2 at a 1:5 molar ratio (MMP2:interactor) for 60 min on ice followed by addition of freshly prepared 5 mM BS3 (bis(sulfo-succinimidyl)suberate) solution (in 1x PBS) and incubation at 25 °C for 30 min. After the incubation, the reaction was quenched by adding 50 mM Tris-HCl (pH 7.4). The reaction mixtures were analyzed by gelatin zymography to detect MMP2 and MMP2 complexes.

Statistical analyses

SigmaPlot 14.0 (Systat Software, San Jose, CA) was used to conduct statistical analysis on the results and plot graphs. We performed unpaired two-tailed Student's *t*-test and one-way ANOVA, where appropriate, to determine statistical significance in the difference when comparing two or more conditions. For all experiments, the N value presented in the figure legends refers to distinct and independent measurements. Data are presented as mean ± standard error of mean (SEM).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The data supporting the findings of this study are included in the manuscript and its supplementary files. The Source Data contains: Supplementary Datasets 1–9. The Supplementary Datasets 1–7 contain the source files for the AlphaFold2 models. Dataset 8 contains

uncropped images of gels and blots. Dataset 9 contains experimental data and statistics. Source data are provided with this paper.

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Author contributions

This study was conceived and directed by C.F.P.; H.S., R.P., A.L.C., T.M., X.L.Z., E.H., and C.F.P. participated in the design of experiments. H.S., R.P., A.L.C., K.R., V.K., E.A., R.P., and S.H.A. conducted experiments and collected the data. H.S. conducted most biochemical determinations and interaction proteomics studies, supported by three undergraduate students: K.R., V.K., and E.A.; under the supervision of C.F.P.; R.P. conducted the in silico and MST determinations under the supervision of J.N.M.G.; A.L.C. conducted the VICA studies and validated interactions under the supervision of T.M.; S.H.A. assisted in the first observation of an association between APOA1 and proMMP2/MMP2 in humans and mice, under the supervision of C.F.P.; X.L.Z. and T.M. also contributed with important materials. H.S., R.P., and C.F.P. wrote the manuscript. E.H. and C.F.P. edited the manuscript. All the authors participated in the data analysis, performed critical revisions of the manuscript, and approved the final version of the manuscript.

Competing interests

Todd McMuellen is the founder of Pavonis Diagnostics. The other authors declare no competing interests.

Additional information

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