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COVID-19-related publications utilizing SomaScan® Technology

Abdel-Aziz, MI *et al.* (2021) "Association of endopeptidases, involved in SARS-CoV-2 infection, with microbial aggravation in sputum of severe asthma." *Allergy* **76**(6): 1917-1921.

https://www.doi.org/10.1111/all.14731

Aid, M *et al.* (2022) "Ad26.COV2.S prevents upregulation of SARS-CoV-2 induced pathways of inflammation and thrombosis in hamsters and rhesus macaques." *PLoS Pathog* **18**(4): e1009990.

https://www.doi.org/10.1371/journal.ppat.1009990

Syrian golden hamsters exhibit features of severe disease after SARS-CoV-2 WA1/2020 challenge and are therefore useful models of COVID-19 pathogenesis and prevention with vaccines. Recent studies have shown that SARS-CoV-2 infection stimulates type I interferon, myeloid, and inflammatory signatures similar to human disease and that weight loss can be prevented with vaccines. However, the impact of vaccination on transcriptional programs associated with COVID-19 pathogenesis and protective adaptive immune responses is unknown. Here we show that SARS-CoV-2 WA1/2020 challenge in hamsters stimulates myeloid and inflammatory programs as well as signatures of complement and thrombosis associated with human COVID-19. Notably, immunization with Ad26.COV2.S, an adenovirus serotype 26 vector (Ad26)-based vaccine expressing a stabilized SARS-CoV-2 spike protein, prevents the upregulation of these pathways, such that the mRNA expression profiles of vaccinated hamsters are comparable to uninfected animals. Using proteomics profiling, we validated these findings in rhesus macaques challenged with SARS-CoV-2 WA1/2020 or SARS-CoV-2 B.1.351. Finally, we show that Ad26.COV2.S vaccination induces T and B cell signatures that correlate with binding and neutralizing antibody responses weeks following vaccination. These data provide insights into the molecular mechanisms of Ad26.COV2.S protection against severe COVID-19 in animal models.

Aid, M *et al.* (2020) "Vascular Disease and Thrombosis in SARS-CoV-2-Infected Rhesus Macaques." *Cell* **183**(5): 1354-1366 e1313.

https://www.doi.org/10.1016/j.cell.2020.10.005

The COVID-19 pandemic has led to extensive morbidity and mortality throughout the world. Clinical features that drive SARS-CoV-2 pathogenesis in humans include inflammation and thrombosis, but the mechanistic details underlying these processes remain to be determined. In this study, we demonstrate endothelial disruption and vascular thrombosis in histopathologic sections of lungs from both humans and rhesus macaques infected with SARS-CoV-2. To define key molecular pathways associated with SARS-CoV-2 pathogenesis in macaques, we performed transcriptomic analyses of bronchoalveolar lavage and peripheral blood and proteomic analyses of serum. We observed macrophage infiltrates in lung and upregulation of macrophage, complement, platelet activation, thrombosis, and proinflammatory markers, including C-reactive protein, MX1, IL-6, IL-1, IL-8, TNFalpha, and NF-kappaB. These results suggest a model in which critical interactions between inflammatory and thrombosis pathways lead to SARS-CoV-2-induced vascular disease. Our findings suggest potential therapeutic targets for COVID-19.

AlGhatrif, M *et al.* (2021) "Age-associated difference in circulating ACE2, the gateway for SARS-COV-2, in humans: results from the InCHIANTI study." *Geroscience* **43**(2): 619-627.

https://www.doi.org/10.1007/s11357-020-00314-w

Levels of angiotensin-converting enzyme 2 (ACE2), the gateway for COVID-19 virus into the cells, have been implicated in worse COVID-19 outcomes associated with aging and cardiovascular disease (CVD). Data on age-associated differences in circulating ACE2 levels in humans and the role of CVD and medications is limited. We analyzed data from 967 participants of the InCHIANTI study, a community-dwelling cohort in the Chianti region,

Italy. Relative abundance of ACE2 in plasma was assessed using a proteomics platform. CVD diagnoses, use of renin-angiotensin-aldosterone system (RAAS) antagonists: ACEi, ARBs, and aldosterone antagonists, were ascertained. Multiple linear analyses were performed to examine the independent association of ACE2 with age, CVD, and RAAS antagonist use. Age was independently associated with lower log (ACE2) in persons aged >/= 55 years (STD beta = - 0.12, p = 0.0002). ACEi treatment was also independently associated with significantly lower ACE2 levels, and ACE2 was inversely associated with weight, and positively associated with peripheral artery disease (PAD) status. There was a trend toward higher circulating ACE2 levels in hypertensive individuals, but it did not reach statistical significance. In a stratified analysis, the association between log (ACE2) and log (IL-6) was more evidenced in participants with PAD. Circulating ACE2 levels demonstrate curvilinear association with age, with older individuals beyond the sixth decade age having lower levels. ACEi was associated with greater circulating ACE2 levels. Interestingly, ACE2 was elevated in PAD and positively associated with inflammatory markers, suggesting compensatory upregulation in the setting of chronic inflammation. Further studies are needed to comprehensively characterize RAAS components with aging and disease, and assess its prognostic role in predicting COVID-19 outcomes.

Anisul, M *et al.* (2021) "A proteome-wide genetic investigation identifies several SARS-CoV-2-exploited host targets of clinical relevance." *Elife* **10**.

https://www.doi.org/10.7554/eLife.69719

Background: The virus SARS-CoV-2 can exploit biological vulnerabilities (e.g. host proteins) in susceptible hosts that predispose to the development of severe COVID-19.
Methods: To identify host proteins that may contribute to the risk of severe COVID-19, we undertook proteome-wide genetic colocalisation tests, and polygenic (pan) and cis-Mendelian randomisation analyses leveraging publicly available protein and COVID-19 datasets. Results: Our analytic approach identified several known targets (e.g. ABO, OAS1), but also nominated new proteins such as soluble Fas (colocalisation probability > 0.9, p = 1 x 10(-4)), implicating Fas-mediated apoptosis as a potential target for COVID-19 risk. The polygenic (pan) and cis-Mendelian randomisation analyses showed consistent associations of genetically predicted ABO protein with several COVID-19 phenotypes. The ABO signal is highly pleiotropic and a look-up of proteins associated with the ABO signal revealed that the strongest association was with soluble CD209. We demonstrated experimentally that CD209 directly interacts with the spike protein of SARS-CoV-2, suggesting a mechanism that could explain the ABO association with COVID-19.
Conclusions: Our work provides a prioritised list of host targets potentially exploited by SARS-CoV-2 and is a precursor for further research on CD209 and FAS as therapeutically tractable targets for COVID-19.

Arthur, L., et al. (2021) “Cellular and plasma proteomic determinants of COVID-19 and non-COVID-19 pulmonary diseases relative to healthy aging.” *Nat Aging* **1**: 535–549. https://doi.org/10.1038/s43587-021-00067-x

We examine the cellular and soluble determinants of coronavirus disease 2019 (COVID-19) relative to aging by performing mass cytometry in parallel with clinical blood testing and plasma proteomic profiling of ~4,700 proteins from 71 individuals with pulmonary disease and 148 healthy donors (25–80 years old). Distinct cell populations were associated with age ($GZMK^+CD8^+$ T cells and $CD25^{low}$ $CD4^+$ T cells) and with COVID-19 ($TBET^-EOMES^-$ $CD4^+$ T cells, $HLA\text{-}DR^+CD38^+$ $CD8^+$ T cells and $CD27^+CD38^+$ B cells). A unique population of $TBET^+EOMES^+$ $CD4^+$ T cells was associated with individuals with COVID-19 who experienced moderate, rather than severe or lethal, disease. Disease severity correlated with blood creatinine and urea nitrogen levels. Proteomics revealed a major impact of age on the disease-associated plasma signatures and highlighted the divergent contribution of hepatocyte and muscle secretomes to COVID-19 plasma proteins. Aging plasma was enriched in matrisome proteins and heart/aorta smooth muscle cell-specific proteins. These findings reveal age-specific and disease-specific changes associated with COVID-19, and potential soluble mediators of the physiological impact of COVID-19.

Accompanying News & Views: Montgomery, R.R. & Steen, H. (2021) “Using ‘big data’ to disentangle aging and COVID-19.” *Nat Aging* **1:** 496–497. https://doi.org/10.1038/s43587-021-00078-8

Chirinos, JA *et al.* (2020) "Clinical and Proteomic Correlates of Plasma ACE2 (Angiotensin-Converting Enzyme 2) in Human Heart Failure." *Hypertension* **76**(5): 1526-1536.

https://www.doi.org/10.1161/HYPERTENSIONAHA.120.15829

ACE2 (angiotensin-converting enzyme 2) is a key component of the renin-angiotensin-aldosterone system. Yet, little is known about the clinical and biologic correlates of circulating ACE2 levels in humans. We assessed the clinical and proteomic correlates of plasma (soluble) ACE2 protein levels in human heart failure. We measured plasma ACE2 using a modified aptamer assay among PHFS (Penn Heart Failure Study) participants (n=2248). We performed an association study of ACE2 against approximately 5000 other plasma proteins measured with the SomaScan platform. Plasma ACE2 was not associated with ACE inhibitor and angiotensin-receptor blocker use. Plasma ACE2 was associated with older age, male sex, diabetes mellitus, a lower estimated glomerular filtration rate, worse New York Heart Association class, a history of coronary artery bypass surgery, and higher pro-BNP (pro-B-type natriuretic peptide) levels. Plasma ACE2 exhibited associations with 1011 other plasma proteins. In pathway overrepresentation analyses, top canonical pathways associated with plasma ACE2 included clathrin-mediated endocytosis signaling, actin cytoskeleton signaling, mechanisms of viral exit from host cells, EIF2 (eukaryotic initiation factor 2) signaling, and the protein ubiquitination pathway. In conclusion, in humans with heart failure, plasma ACE2 is associated with various clinical factors known to be associated with severe coronavirus disease 2019 (COVID-19), including older age, male sex, and diabetes mellitus, but is not associated with ACE inhibitor and angiotensin-receptor blocker use. Plasma ACE2 protein levels are prominently associated with multiple cellular pathways involved in cellular endocytosis, exocytosis, and intracellular protein trafficking. Whether these have a causal relationship with ACE2 or are relevant to novel coronavirus-2 infection remains to be assessed in future studies.

Demidowich, AP *et al.* (2020) "Colchicine's effects on metabolic and inflammatory molecules in adults with obesity and metabolic syndrome: results from a pilot randomized controlled trial." *Int J Obes (Lond)* **44**(8): 1793-1799.

https://www.doi.org/10.1038/s41366-020-0598-3

OBJECTIVE: Recent clinical trials have demonstrated that colchicine may have metabolic and cardiovascular and benefits in at-risk patients; however, the mechanisms through which colchicine may improve outcomes are still unclear. We sought to examine colchicine's effects on circulating inflammatory and metabolic molecules in adults with obesity and metabolic syndrome (MetS).
METHODS: Blood samples were collected pre- and post-intervention during a double-blind randomized controlled trial in which 40 adults with obesity and MetS were randomized to colchicine 0.6 mg or placebo twice-daily for 3 months. Serum samples were analyzed for 1305 circulating factors using the SomaScan Platform. The Benjamini-Hochberg procedure was used to adjust the false discovery rate (FDR) for multiple testing.
RESULTS: At baseline, age (48.0 +/- 13.8 vs. 44.7 +/- 10.3 years) and BMI (39.8 +/- 6.4 vs. 41.8 +/- 8.2 kg/m(2)) were not different between groups. After controlling for the FDR, 34 molecules were significantly changed by colchicine. Colchicine decreased concentrations of multiple inflammatory molecules, including C-reactive protein, interleukin 6, and resistin, in addition to vascular-related proteins (e.g., oxidized low-density lipoprotein receptor, phosphodiesterase 5A). Conversely, relative to placebo, colchicine significantly increased concentrations of eight molecules including secreted factors associated with metabolism and anti-thrombosis.
CONCLUSIONS: In adults with obesity, colchicine significantly affected concentrations of proteins involved in the innate immune system, endothelial function and atherosclerosis, uncovering new mechanisms behind its cardiometabolic effects. Further research is warranted to investigate whether colchicine's IL-6 suppressive effects may be beneficial in COVID-19.

Deutsch, EW *et al.* (2021) "Advances and Utility of the Human Plasma Proteome." *J Proteome Res* **20**(12): 5241-5263.

https://www.doi.org/10.1021/acs.jproteome.1c00657

The study of proteins circulating in blood offers tremendous opportunities to diagnose, stratify, or possibly prevent diseases. With recent technological advances and the urgent need to understand the effects of COVID-19, the proteomic analysis of blood-derived serum and plasma has become even more important for studying human biology and pathophysiology. Here we provide views and perspectives about technological

developments and possible clinical applications that use mass-spectrometry(MS)- or affinity-based methods. We discuss examples where plasma proteomics contributed valuable insights into SARS-CoV-2 infections, aging, and hemostasis and the opportunities offered by combining proteomics with genetic data. As a contribution to the Human Proteome Organization (HUPO) Human Plasma Proteome Project (HPPP), we present the Human Plasma PeptideAtlas build 2021-07 that comprises 4395 canonical and 1482 additional nonredundant human proteins detected in 240 MS-based experiments. In addition, we report the new Human Extracellular Vesicle PeptideAtlas 2021-06, which comprises five studies and 2757 canonical proteins detected in extracellular vesicles circulating in blood, of which 74% (2047) are in common with the plasma PeptideAtlas. Our overview summarizes the recent advances, impactful applications, and ongoing challenges for translating plasma proteomics into utility for precision medicine.

Filbin, MR *et al.* (2021) "Longitudinal proteomic analysis of severe COVID-19 reveals survival-associated signatures, tissue-specific cell death, and cell-cell interactions." *Cell Rep Med* **2**(5): 100287.

https://www.doi.org/10.1016/j.xcrm.2021.100287

Mechanisms underlying severe coronavirus disease 2019 (COVID-19) disease remain poorly understood. We analyze several thousand plasma proteins longitudinally in 306 COVID-19 patients and 78 symptomatic controls, uncovering immune and non-immune proteins linked to COVID-19. Deconvolution of our plasma proteome data using published scRNA-seq datasets reveals contributions from circulating immune and tissue cells. Sixteen percent of patients display reduced inflammation yet comparably poor outcomes. Comparison of patients who died to severely ill survivors identifies dynamic immune-cell-derived and tissue-associated proteins associated with survival, including exocrine pancreatic proteases. Using derived tissue-specific and cell-type-specific intracellular death signatures, cellular angiotensin-converting enzyme 2 (ACE2) expression, and our data, we infer whether organ damage resulted from direct or indirect effects of infection. We propose a model in which interactions among myeloid, epithelial, and T cells drive tissue damage. These datasets provide important insights and a rich resource for analysis of mechanisms of severe COVID-19 disease.

Galbraith, MD *et al.* (2022) "Specialized interferon action in COVID-19." *Proc Natl Acad Sci U S A* **119**(11).

https://www.doi.org/10.1073/pnas.2116730119

The impacts of interferon (IFN) signaling on COVID-19 pathology are multiple, with both protective and harmful effects being documented. We report here a multiomics investigation of systemic IFN signaling in hospitalized COVID-19 patients, defining the multiomics biosignatures associated with varying levels of 12 different type I, II, and III IFNs. The antiviral transcriptional response in circulating immune cells is strongly associated with a specific subset of IFNs, most prominently IFNA2 and IFNG. In contrast, proteomics signatures indicative of endothelial damage and platelet activation associate with high levels of IFNB1 and IFNA6. Seroconversion and time since hospitalization associate with a significant decrease in a specific subset of IFNs. Additionally, differential IFN subtype production is linked to distinct constellations of circulating myeloid and lymphoid immune cell types. Each IFN has a unique metabolic signature, with IFNG being the most associated with activation of the kynurenine pathway. IFNs also show differential relationships with clinical markers of poor prognosis and disease severity. For example, whereas IFNG has the strongest association with C-reactive protein and other immune markers of poor prognosis, IFNB1 associates with increased neutrophil to lymphocyte ratio, a marker of late severe disease. Altogether, these results reveal specialized IFN action in COVID-19, with potential diagnostic and therapeutic implications.

Galbraith, MD *et al.* (2021) "Seroconversion stages COVID19 into distinct pathophysiological states." *Elife* **10**.

https://www.doi.org/10.7554/eLife.65508

COVID19 is a heterogeneous medical condition involving diverse underlying pathophysiological processes including hyperinflammation, endothelial damage, thrombotic microangiopathy, and end-organ damage. Limited knowledge about the molecular mechanisms driving these processes and lack of staging biomarkers hamper

the ability to stratify patients for targeted therapeutics. We report here the results of a cross-sectional multi-omics analysis of hospitalized COVID19 patients revealing that seroconversion status associates with distinct underlying pathophysiological states. Low antibody titers associate with hyperactive T cells and NK cells, high levels of IFN alpha, gamma and lambda ligands, markers of systemic complement activation, and depletion of lymphocytes, neutrophils, and platelets. Upon seroconversion, all of these processes are attenuated, observing instead increases in B cell subsets, emergency hematopoiesis, increased D-dimer, and hypoalbuminemia. We propose that seroconversion status could potentially be used as a biosignature to stratify patients for therapeutic intervention and to inform analysis of clinical trial results in heterogenous patient populations.

Gaziano, L *et al.* (2021) "Actionable druggable genome-wide Mendelian randomization identifies repurposing opportunities for COVID-19." *Nat Med* **27**(4): 668-676.

https://www.doi.org/10.1038/s41591-021-01310-z

Drug repurposing provides a rapid approach to meet the urgent need for therapeutics to address COVID-19. To identify therapeutic targets relevant to COVID-19, we conducted Mendelian randomization analyses, deriving genetic instruments based on transcriptomic and proteomic data for 1,263 actionable proteins that are targeted by approved drugs or in clinical phase of drug development. Using summary statistics from the Host Genetics Initiative and the Million Veteran Program, we studied 7,554 patients hospitalized with COVID-19 and >1 million controls. We found significant Mendelian randomization results for three proteins (ACE2, P = 1.6 x 10(-6); IFNAR2, P = 9.8 x 10(-11) and IL-10RB, P = 2.3 x 10(-14)) using cis-expression quantitative trait loci genetic instruments that also had strong evidence for colocalization with COVID-19 hospitalization. To disentangle the shared expression quantitative trait loci signal for IL10RB and IFNAR2, we conducted phenome-wide association scans and pathway enrichment analysis, which suggested that IFNAR2 is more likely to play a role in COVID-19 hospitalization. Our findings prioritize trials of drugs targeting IFNAR2 and ACE2 for early management of COVID-19.

Hernandez Cordero, AI *et al.* (2021) "Multi-omics highlights ABO plasma protein as a causal risk factor for COVID-19." *Hum Genet* **140**(6): 969-979.

https://www.doi.org/10.1007/s00439-021-02264-5

SARS-CoV-2 is responsible for the coronavirus disease 2019 (COVID-19) and the current health crisis. Despite intensive research efforts, the genes and pathways that contribute to COVID-19 remain poorly understood. We, therefore, used an integrative genomics (IG) approach to identify candidate genes responsible for COVID-19 and its severity. We used Bayesian colocalization (COLOC) and summary-based Mendelian randomization to combine gene expression quantitative trait loci (eQTLs) from the Lung eQTL (n = 1,038) and eQTLGen (n = 31,784) studies with published COVID-19 genome-wide association study (GWAS) data from the COVID-19 Host Genetics Initiative. Additionally, we used COLOC to integrate plasma protein quantitative trait loci (pQTL) from the INTERVAL study (n = 3,301) with COVID-19 loci. Finally, we determined any causal associations between plasma proteins and COVID-19 using multi-variable two-sample Mendelian randomization (MR). The expression of 18 genes in lung and/or blood co-localized with COVID-19 loci. Of these, 12 genes were in suggestive loci (PGWAS < 5 x 10(-05)). LZTFL1, SLC6A20, ABO, IL10RB and IFNAR2 and OAS1 had been previously associated with a heightened risk of COVID-19 (PGWAS < 5 x 10(-08)). We identified a causal association between OAS1 and COVID-19 GWAS. Plasma ABO protein, which is associated with blood type in humans, demonstrated a significant causal relationship with COVID-19 in the MR analysis; increased plasma levels were associated with an increased risk of COVID-19 and, in particular, severe COVID-19. In summary, our study identified genes associated with COVID-19 that may be prioritized for future investigations. Importantly, this is the first study to demonstrate a causal association between plasma ABO protein and COVID-19.

Mills, RJ *et al.* (2021) "BET inhibition blocks inflammation-induced cardiac dysfunction and SARS-CoV-2 infection." *Cell* **184**(8): 2167-2182 e2122.

https://www.doi.org/10.1016/j.cell.2021.03.026

Cardiac injury and dysfunction occur in COVID-19 patients and increase the risk of mortality. Causes are ill defined but could be through direct cardiac infection and/or inflammation-induced dysfunction. To identify mechanisms and cardio-protective drugs, we use a state-of-the-art pipeline combining human cardiac organoids with phosphoproteomics and single nuclei RNA sequencing. We identify an inflammatory "cytokine-storm", a cocktail of interferon gamma, interleukin 1beta, and poly(I:C), induced diastolic dysfunction. Bromodomain-containing protein 4 is activated along with a viral response that is consistent in both human cardiac organoids (hCOs) and hearts of SARS-CoV-2-infected K18-hACE2 mice. Bromodomain and extraterminal family inhibitors (BETi) recover dysfunction in hCOs and completely prevent cardiac dysfunction and death in a mouse cytokine-storm model. Additionally, BETi decreases transcription of genes in the viral response, decreases ACE2 expression, and reduces SARS-CoV-2 infection of cardiomyocytes. Together, BETi, including the Food and Drug Administration (FDA) breakthrough designated drug, apabetalone, are promising candidates to prevent COVID-19 mediated cardiac damage.

Moin, ASM *et al.* (2021) "Soluble Neuropilin-1 Response to Hypoglycemia in Type 2 Diabetes: Increased Risk or Protection in SARS-CoV-2 Infection?" *Front Endocrinol (Lausanne)* **12**: 665134.

https://www.doi.org/10.3389/fendo.2021.665134

Introduction: Neuropilin-1(NRP1) is a cofactor that enhances SARS-CoV-2 coronavirus cell infectivity when co-expressed with angiotensin-converting enzyme 2(ACE2). The Renin-Angiotensin System (RAS) is activated in type 2 diabetes (T2D); therefore, the aim of this study was to determine if hypoglycaemia-induced stress in T2D would potentiate serum NRP1(sNRP1) levels, reflecting an increased risk for SARS-CoV-2 infection.
Methods: A case-control study of aged-matched T2D (n = 23) and control (n = 23) subjects who underwent a hyperinsulinemic clamp over 1-hour to hypoglycemia(<40mg/dl) with subsequent timecourse of 4-hours and 24-hours. Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement determined RAS-related proteins: renin (REN), angiotensinogen (AGT), ACE2, soluble NRP1(sNRP1), NRP1 ligands (Vascular endothelial growth factor, VEGF and Class 3 Semaphorins, SEM3A) and NRP1 proteolytic enzyme (A Disintegrin and Metalloproteinase 9, ADAM9).
Results: Baseline RAS overactivity was present with REN elevated and AGT decreased in T2D ($p<0.05$); ACE2 was unchanged. Baseline sNRP1, VEGF and ADAM9 did not differ between T2D and controls and remained unchanged in response to hypoglycaemia. However, 4-hours post-hypoglycemia, sNRP1, VEGF and ADAM9 were elevated in T2D($p<0.05$). SEMA3A was not different at baseline; at hypoglycemia, SEMA3A decreased in controls only. Post-hypoglycemia, SEMA3A levels were higher in T2D versus controls. sNRP1 did not correlate with ACE2, REN or AGT. T2D subjects stratified according to ACE inhibitor (ACEi) therapies showed no difference in sNRP1 levels at either glucose normalization or hypoglycaemia.
Conclusion: Hypoglycemia potentiated both plasma sNRP1 level elevation and its ligands VEGF and SEMA3A, likely through an ADAM9-mediated mechanism that was not associated with RAS overactivity or ACEi therapy; however, whether this is protective or promotes increased risk for SARS-CoV-2 infection in T2D is unclear.

Moin, ASM *et al.* (2021) "Type 2 Diabetes Coagulopathy Proteins May Conflict With Biomarkers Reflective of COVID-19 Severity." *Front Endocrinol (Lausanne)* **12**: 658304.

https://www.doi.org/10.3389/fendo.2021.658304

Objective: Detailed proteomic analysis in a cohort of patients with differing severity of COVID-19 disease identified biomarkers within the complement and coagulation cascades as biomarkers for disease severity has been reported; however, it is unclear if these proteins differ sufficiently from other conditions to be considered as biomarkers.
Methods: A prospective, parallel study in T2D (n = 23) and controls (n = 23). A hyperinsulinemic clamp was performed and normoglycemia induced in T2D [4.5 +/- 0.07 mmol/L (81 +/- 1.2 mg/dl)] for 1-h, following which blood glucose was decreased to </=2.0 mmol/L (36 mg/dl). Proteomic analysis for the complement and coagulation cascades were measured using Slow Off-rate Modified Aptamer (SOMA)-scan.
Results: Thirty-four proteins were measured. At baseline, 4 of 18 were found to differ in T2D versus controls for platelet degranulation [Neutrophil-activating peptide-2 (p = 0.014), Thrombospondin-1 (p = 0.012), Platelet factor-4 (p = 0.007), and Kininogen-1 (p = 0.05)], whilst 3 of 16 proteins differed for complement and coagulation

cascades [Coagulation factor IX ($p < 0.05$), Kininogen-1 ($p = 0.05$), and Heparin cofactor-2 ($p = 0.007$)]; STRING analysis demonstrated the close relationship of these proteins to one another. Induced euglycemia in T2D showed no protein changes versus baseline. At hypoglycemia, however, four proteins changed in controls from baseline [Thrombospondin-1 ($p < 0.014$), platelet factor-4 ($p < 0.01$), Platelet basic protein ($p < 0.008$), and Vitamin K-dependent protein-C ($p < 0.00003$)], and one protein changed in T2D [Vitamin K-dependent protein-C, ($p < 0.0002$)].
Conclusion: Seven of 34 proteins suggested to be biomarkers of COVID-19 severity within the platelet degranulation and complement and coagulation cascades differed in T2D versus controls, with further changes occurring at hypoglycemia, suggesting that validation of these biomarkers is critical. It is unclear if these protein changes in T2D may predict worse COVID-19 disease for these patients.

Moin, ASM *et al.* (2020) "Pro-fibrotic M2 macrophage markers may increase the risk for COVID19 in type 2 diabetes with obesity." *Metabolism* **112**: 154374.

https://www.doi.org/10.1016/j.metabol.2020.154374

Moin, ASM *et al.* (2021) "Vitamin D Association With Macrophage-Derived Cytokines in Polycystic Ovary Syndrome: An Enhanced Risk of COVID-19 Infection?" *Front Endocrinol (Lausanne)* **12**: 638621.

https://www.doi.org/10.3389/fendo.2021.638621

Background: Women with polycystic ovary syndrome (PCOS) often have vitamin D deficiency, a known risk factor for severe COVID-19 disease. Alveolar macrophage-derived cytokines contribute to the inflammation underlying pulmonary disease in COVID-19. We sought to determine if basal macrophage activation, as a risk factor for COVID-19 infection, was present in PCOS and, if so, was further enhanced by vitamin D deficiency.
Methods: A cross-sectional study in 99 PCOS and 68 control women who presented sequentially. Plasma levels of a macrophage-derived cytokine panel were determined by Slow Off-rate Modified Aptamer (SOMA)-scan plasma protein measurement. Vitamin D was measured by tandem mass spectroscopy.
Results: Vitamin D was lower in PCOS women ($p<0.0001$) and correlated negatively with body mass index (BMI) in PCOS ($r=0.28$, $p=0.0046$). Basal macrophage activation markers CXCL5, CD163 and MMP9 were elevated, whilst protective CD200 was decreased ($p<0.05$); changes in these variables were related to, and fully accounted for, by BMI. PCOS and control women were then stratified according to vitamin D concentration. Vitamin D deficiency was associated with decreased CD80 and IFN-gamma in PCOS and IL-12 in both groups ($p<0.05$). These factors, important in initiating and maintaining the immune response, were again accounted for by BMI.
Conclusion: Basal macrophage activation was higher in PCOS with macrophage changes related with increased infection risk associating with vitamin D; all changes were BMI dependent, suggesting that obese PCOS with vitamin D deficiency may be at greater risk of more severe COVID-19 infection, but that it is obesity-related rather than an independent PCOS factor.

Pietzner, M *et al.* (2020) "Genetic architecture of host proteins involved in SARS-CoV-2 infection." *Nat Commun* **11**(1): 6397.

https://www.doi.org/10.1038/s41467-020-19996-z

Understanding the genetic architecture of host proteins interacting with SARS-CoV-2 or mediating the maladaptive host response to COVID-19 can help to identify new or repurpose existing drugs targeting those proteins. We present a genetic discovery study of 179 such host proteins among 10,708 individuals using an aptamer-based technique. We identify 220 host DNA sequence variants acting in cis (MAF 0.01-49.9%) and explaining 0.3-70.9% of the variance of 97 of these proteins, including 45 with no previously known protein quantitative trait loci (pQTL) and 38 encoding current drug targets. Systematic characterization of pQTLs across the phenome identified protein-drug-disease links and evidence that putative viral interaction partners such as MARK3 affect immune response. Our results accelerate the evaluation and prioritization of new drug development programmes and repurposing of trials to prevent, treat or reduce adverse outcomes. Rapid sharing and detailed interrogation of results is facilitated through an interactive webserver (https://omicscience.org/apps/covidpgwas/).

Ramaswamy, A *et al.* (2021) "Immune dysregulation and autoreactivity correlate with disease severity in SARS-CoV-2-associated multisystem inflammatory syndrome in children." *Immunity* **54**(5): 1083-1095 e1087.

https://www.doi.org/10.1016/j.immuni.2021.04.003

Multisystem inflammatory syndrome in children (MIS-C) is a life-threatening post-infectious complication occurring unpredictably weeks after mild or asymptomatic SARS-CoV-2 infection. We profiled MIS-C, adult COVID-19, and healthy pediatric and adult individuals using single-cell RNA sequencing, flow cytometry, antigen receptor repertoire analysis, and unbiased serum proteomics, which collectively identified a signature in MIS-C patients that correlated with disease severity. Despite having no evidence of active infection, MIS-C patients had elevated S100A-family alarmins and decreased antigen presentation signatures, indicative of myeloid dysfunction. MIS-C patients showed elevated expression of cytotoxicity genes in NK and CD8(+) T cells and expansion of specific IgG-expressing plasmablasts. Clinically severe MIS-C patients displayed skewed memory T cell TCR repertoires and autoimmunity characterized by endothelium-reactive IgG. The alarmin, cytotoxicity, TCR repertoire, and plasmablast signatures we defined have potential for application in the clinic to better diagnose and potentially predict disease severity early in the course of MIS-C.

Richardson, TG *et al.* (2021) "Evaluating the effects of cardiometabolic exposures on circulating proteins which may contribute to severe SARS-CoV-2." *EBioMedicine* **64**: 103228.

https://www.doi.org/10.1016/j.ebiom.2021.103228

BACKGROUND: Developing insight into the pathogenesis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is of critical importance to overcome the global pandemic caused by coronavirus disease 2019 (covid-19). In this study, we have applied Mendelian randomization (MR) to systematically evaluate the effect of 10 cardiometabolic risk factors and genetic liability to lifetime smoking on 97 circulating host proteins postulated to either interact or contribute to the maladaptive host response of SARS-CoV-2.
METHODS: We applied the inverse variance weighted (IVW) approach and several robust MR methods in a two-sample setting to systemically estimate the genetically predicted effect of each risk factor in turn on levels of each circulating protein. Multivariable MR was conducted to simultaneously evaluate the effects of multiple risk factors on the same protein. We also applied MR using cis-regulatory variants at the genomic location responsible for encoding these proteins to estimate whether their circulating levels may influence severe SARS-CoV-2.
FINDINGS: In total, we identified evidence supporting 105 effects between risk factors and circulating proteins which were robust to multiple testing corrections and sensitivity analyzes. For example, body mass index provided evidence of an effect on 23 circulating proteins with a variety of functions, such as inflammatory markers c-reactive protein (IVW Beta=0.34 per standard deviation change, 95% CI=0.26 to 0.41, P = 2.19 x 10(-16)) and interleukin-1 receptor antagonist (IVW Beta=0.23, 95% CI=0.17 to 0.30, P = 9.04 x 10(-12)). Further analyzes using multivariable MR provided evidence that the effect of BMI on lowering immunoglobulin G, an antibody class involved in protection from infection, is substantially mediated by raised triglycerides levels (IVW Beta=-0.18, 95% CI=-0.25 to -0.12, P = 2.32 x 10(-08), proportion mediated=44.1%). The strongest evidence that any of the circulating proteins highlighted by our initial analysis influence severe SARS-CoV-2 was identified for soluble glycoprotein 130 (odds ratio=1.81, 95% CI=1.25 to 2.62, P = 0.002), a signal transductor for interleukin-6 type cytokines which are involved in inflammatory response. However, based on current case samples for severe SARS-CoV-2 we were unable to replicate findings in independent samples.
INTERPRETATION: Our findings highlight several key proteins which are influenced by established exposures for disease. Future research to determine whether these circulating proteins mediate environmental effects onto risk of SARS-CoV-2 infection or covid-19 progression are warranted to help elucidate therapeutic strategies for severe covid-19 disease.

Sacco, K *et al.* (2022) "Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19." *Nat Med*. **28**(5):1050-1062.

https://www.doi.org/10.1038/s41591-022-01724-3

Pediatric Coronavirus Disease 2019 (pCOVID-19) is rarely severe; however, a minority of children infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) might develop multisystem inflammatory

syndrome in children (MIS-C), with substantial morbidity. In this longitudinal multi-institutional study, we applied multi-omics (analysis of soluble biomarkers, proteomics, single-cell gene expression and immune repertoire analysis) to profile children with COVID-19 ($n = 110$) and MIS-C ($n = 76$), along with pediatric healthy controls (pHCs; $n = 76$). pCOVID-19 was characterized by robust type I interferon (IFN) responses, whereas prominent type II IFN-dependent and NF-κB-dependent signatures, matrisome activation and increased levels of circulating spike protein were detected in MIS-C, with no correlation with SARS-CoV-2 PCR status around the time of admission. Transient expansion of *TRBV11-2* T cell clonotypes in MIS-C was associated with signatures of inflammation and T cell activation. The association of MIS-C with the combination of HLA A*02, B*35 and C*04 alleles suggests genetic susceptibility. MIS-C B cells showed higher mutation load than pCOVID-19 and pHC. These results identify distinct immunopathological signatures in pCOVID-19 and MIS-C that might help better define the pathophysiology of these disorders and guide therapy.

Snider, JM *et al.* (2021) "Group IIA secreted phospholipase A2 is associated with the pathobiology leading to COVID-19 mortality." *J Clin Invest* **131**(19).

https://www.doi.org/10.1172/JCI149236

There is an urgent need to identify the cellular and molecular mechanisms responsible for severe COVID-19 that results in death. We initially performed both untargeted and targeted lipidomics as well as focused biochemical analyses of 127 plasma samples and found elevated metabolites associated with secreted phospholipase A2 (sPLA2) activity and mitochondrial dysfunction in patients with severe COVID-19. Deceased COVID-19 patients had higher levels of circulating, catalytically active sPLA2 group IIA (sPLA2-IIA), with a median value that was 9.6-fold higher than that for patients with mild disease and 5.0-fold higher than the median value for survivors of severe COVID-19. Elevated sPLA2-IIA levels paralleled several indices of COVID-19 disease severity (e.g., kidney dysfunction, hypoxia, multiple organ dysfunction). A decision tree generated by machine learning identified sPLA2-IIA levels as a central node in the stratification of patients who died from COVID-19. Random forest analysis and least absolute shrinkage and selection operator-based (LASSO-based) regression analysis additionally identified sPLA2-IIA and blood urea nitrogen (BUN) as the key variables among 80 clinical indices in predicting COVID-19 mortality. The combined PLA-BUN index performed significantly better than did either one alone. An independent cohort (n = 154) confirmed higher plasma sPLA2-IIA levels in deceased patients compared with levels in plasma from patients with severe or mild COVID-19, with the PLA-BUN index-based decision tree satisfactorily stratifying patients with mild, severe, or fatal COVID-19. With clinically tested inhibitors available, this study identifies sPLA2-IIA as a therapeutic target to reduce COVID-19 mortality.

Steffen, BT *et al.* (2022) "Proteomic Profiling Identifies Novel Proteins for Genetic Risk of Severe COVID-19: the Atherosclerosis Risk in Communities Study." *Hum Mol Genet*., *epub ahead of print.*

https://www.doi.org/10.1093/hmg/ddac024

Background Genome-wide association studies have identified six genetic variants associated with severe COVID-19, yet the mechanisms through which they may affect disease remains unclear. We investigated proteomic signatures related to COVID-19 risk variants rs657152 (*ABO*), rs10735079 (*OAS1*/*OAS2*/*OAS3*), rs2109069 (*DPP9*), rs74956615 (*TYK2*), rs2236757 (*IFNAR2*) and rs11385942 (*SLC6A20/LZTFL1/CCR9/FYCO1/CXCR6/XCR1*) as well as their corresponding downstream pathways that may promote severe COVID-19 in risk allele carriers and their potential relevancies to other infection outcomes.
Methods A DNA aptamer-based array measured 4870 plasma proteins among 11 471 participants. Linear regression estimated associations between the COVID-19 risk variants and proteins with correction for multiple comparisons, and canonical pathway analysis was conducted. Cox regression assessed associations between proteins identified in the main analysis and risk of incident hospitalized respiratory infections (2570 events) over a 20.7-year follow-up.
Results The *ABO* variant rs657152 was associated with 84 proteins in 7241 white participants with 24 replicated in 1671 Black participants. The *TYK2* variant rs74956615 was associated with ICAM-1 and -5 in white participants with ICAM-5 replicated in Black participants. Of the 84 proteins identified in the main analysis, seven were significantly associated with incident hospitalized respiratory infections including Ephrin type-A receptor 4 (hazard ratio (HR): 0.87; $P = 2.3 \times 10^{-11}$) and von Willebrand factor type A (HR: 1.17; $P = 1.6\text{x}10^{-13}$).

Conclusions Novel proteomics signatures and pathways for COVID-19-related risk variants *TYK2* and *ABO* were identified. A subset of these proteins predicted greater risk of incident hospitalized pneumonia and respiratory infections. Further studies to examine these proteins in COVID-19 patients are warranted.

Sullivan, KD *et al.* (2021) "The COVIDome Explorer researcher portal." *Cell Rep* **36**(7): 109527.

https://www.doi.org/10.1016/j.celrep.2021.109527

COVID-19 pathology involves dysregulation of diverse molecular, cellular, and physiological processes. To expedite integrated and collaborative COVID-19 research, we completed multi-omics analysis of hospitalized COVID-19 patients, including matched analysis of the whole-blood transcriptome, plasma proteomics with two complementary platforms, cytokine profiling, plasma and red blood cell metabolomics, deep immune cell phenotyping by mass cytometry, and clinical data annotation. We refer to this multidimensional dataset as the COVIDome. We then created the COVIDome Explorer, an online researcher portal where the data can be analyzed and visualized in real time. We illustrate herein the use of the COVIDome dataset through a multi-omics analysis of biosignatures associated with C-reactive protein (CRP), an established marker of poor prognosis in COVID-19, revealing associations between CRP levels and damage-associated molecular patterns, depletion of protective serpins, and mitochondrial metabolism dysregulation. We expect that the COVIDome Explorer will rapidly accelerate data sharing, hypothesis testing, and discoveries worldwide.

Zhou, S *et al.* (2021) "A Neanderthal OAS1 isoform protects individuals of European ancestry against COVID-19 susceptibility and severity." *Nat Med* **27**(4): 659-667.

https://www.doi.org/10.1038/s41591-021-01281-1

To identify circulating proteins influencing Coronavirus Disease 2019 (COVID-19) susceptibility and severity, we undertook a two-sample Mendelian randomization (MR) study, rapidly scanning hundreds of circulating proteins while reducing bias due to reverse causation and confounding. In up to 14,134 cases and 1.2 million controls, we found that an s.d. increase in OAS1 levels was associated with reduced COVID-19 death or ventilation (odds ratio (OR) = 0.54, P = 7 x 10(-8)), hospitalization (OR = 0.61, P = 8 x 10(-8)) and susceptibility (OR = 0.78, P = 8 x 10(-6)). Measuring OAS1 levels in 504 individuals, we found that higher plasma OAS1 levels in a non-infectious state were associated with reduced COVID-19 susceptibility and severity. Further analyses suggested that a Neanderthal isoform of OAS1 in individuals of European ancestry affords this protection. Thus, evidence from MR and a case-control study support a protective role for OAS1 in COVID-19 adverse outcomes. Available pharmacological agents that increase OAS1 levels could be prioritized for drug development.

Zimmermann, T *et al.* (2021) "Influence of renin-angiotensin-aldosterone system inhibitors on plasma levels of angiotensin-converting enzyme 2." *ESC Heart Fail* **8**(2): 1717-1721.

https://www.doi.org/10.1002/ehf2.13249

Aims: Concern has been raised that treatment with angiotensin-converting enzyme inhibitors and angiotensin receptor blockers may increase the expression of angiotensin-converting enzyme 2 (ACE2), which acts as the entry receptor for SARS-CoV-2, and lead to an increased risk of death from SARS-CoV-2. We aimed to address this concern by evaluating the in vivo relationship of treatment with ACE inhibitors and angiotensin receptor blockers (ARB) with circulating plasma concentrations of ACE2 in a large cohort of patients with established cardiovascular disease (n = 1864) or cardiovascular risk factors (n = 2144) but without a history of heart failure. **Methods and results:** Angiotensin-converting enzyme 2 was measured in 4008 patients (median age 68, 33% women, 31% on ACE-inhibitors, 31% on ARB) using the SOMAscan proteomic platform (SomaLogic Inc, Colorado, USA). Plasma concentration of ACE2 was comparable in 1250 patients on ACE inhibitors (mean 5.99) versus patients without ACE inhibitors (mean 5.98, P = 0.54). Similarly, plasma concentration of ACE2 was comparable in 1260 patients on ARB (mean 5.99) versus patients without ARB (mean 5.98, P = 0.50). Plasma concentration of ACE2 was comparable in 2474 patients on either ACE inhibitors or ARB (mean 5.99) versus patients without ACE inhibitors or ARB (mean 5.98, P = 0.31). Multivariable quantile regression model analysis confirmed the lack of association between treatment with ACE inhibitors or ARB and ACE2

concentrations. Body mass index showed the only positive association with ACE2 plasma concentration (effect 0.015, 95% confidence interval 0.002 to 0.028, P = 0.024).
Conclusions: In a large cohort of patients with established cardiovascular disease or cardiovascular risk factors but without heart failure, ACE inhibitors and ARB were not associated with higher plasma concentrations of ACE2.

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