Epigenetic scores for the circulating proteome as tools for disease prediction

Danni A Gadd^{1,*}, Robert F Hillary^{1,*}, Daniel L McCartney^{1,*}, Shaza B Zaghlood^{2,3*}, Anna J Stevenson¹, Yipeng Cheng¹, Chloe Fawns-Ritchie^{1,5}, Cliff Nangle¹, Archie Campbell¹, Robin Flaig¹, Sarah E Harris^{4,5}, Rosie M Walker⁶, Liu Shi⁷, Elliot M Tucker-Drob^{8,9}, Christian Gieger^{10,11,12,13}, Annette Peters^{11,12,13}, Melanie Waldenberger^{10,11,12}, Johannes Graumann^{14,15}, Allan F McRae¹⁶, Ian J Deary^{4,5}, David J Porteous¹, Caroline Hayward^{1,17}, Peter M Visscher¹⁶, Simon R Cox^{4,5}, Kathryn L Evans¹, Andrew M McIntosh^{1,18}, Karsten Suhre², Riccardo E Marioni^{1,†}

¹ Centre for Genomic and Experimental Medicine, Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, EH4 2XU

² Department of Physiology and Biophysics, Weill Cornell Medicine-Qatar, Education City, PO 24144 Doha, Qatar

³ Computer Engineering Department, Virginia Tech, Blacksburg, VA, USA.

⁴ Lothian Birth Cohorts, University of Edinburgh, Edinburgh, EH8 9JZ

⁵ Department of Psychology, University of Edinburgh, Edinburgh, EH8 9JZ

⁶ Centre for Clinical Brain Sciences, Chancellor's Building, 49 Little France Crescent, Edinburgh BioQuarter, Edinburgh, EH16 4SB

⁷ Department of Psychiatry, University of Oxford, UK

⁸ Department of Psychology, The University of Texas at Austin, United States

⁹ Population Research Center, The University of Texas at Austin, United States

¹⁰ Research Unit Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Bavaria, Germany

¹¹ Institute of Epidemiology, Helmholtz Zentrum München, German Research Center for
Environmental Health, Neuherberg, Bavaria, Germany

¹² German Center for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Munich,
Germany

¹³ German Center for Diabetes Research (DZD), Neuherberg, Germany

¹⁴ Scientific Service Group Biomolecular Mass Spectrometry, Max Planck Institute for Heart and
Lung Research, W.G. Kerckhoff Institute, Bad Nauheim, Germany

¹⁵ German Centre for Cardiovascular Research (DZHK), Partner Site Rhine-Main, Max Planck
Institute of Heart and Lung Research, Bad Nauheim, Germany

¹⁶ Institute for Molecular Bioscience, University of Queensland, Brisbane, QLD, Australia

¹⁷ Medical Research Council Human Genetics Unit, Institute of Genetics and Cancer, University of
Edinburgh, Edinburgh, EH4 2XU

¹⁸ Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, EH10 5HF,
UK

* These authors contributed equally

[†] Corresponding author: Riccardo Marioni, riccardo.marioni@ed.ac.uk

Abstract

Protein biomarkers have been identified across many age-related morbidities. However, characterising epigenetic influences could further inform disease predictions. Here, we leverage epigenome-wide data to study links between the DNAm signatures of the circulating proteome and incident diseases. Using data from four cohorts, we trained and tested epigenetic scores (EpiScores) for 953 plasma proteins, identifying 109 scores that explained between 1% and 58% of the variance in protein levels after adjusting for known protein quantitative trait loci (pQTL) genetic effects. By projecting these EpiScores into an independent sample, (Generation Scotland; n=9,537) and relating them to incident morbidities over a follow-up of 14 years, we uncovered 137 EpiScore – disease associations. These associations were largely independent of immune cell proportions, common lifestyle and health factors and biological aging. Notably, we found that our diabetes-associated EpiScores highlighted previous top biomarker associations from proteome-wide assessments of diabetes. These EpiScores for protein levels can therefore be a valuable resource for disease prediction and risk stratification.

Introduction

Chronic morbidities place longstanding burdens on our health as we age. Stratifying an individual's risk prior to symptom presentation is therefore critical (NHS England, 2016). Though complex morbidities are partially driven by genetic factors (Fuchsberger et al., 2016; Yao et al., 2018), epigenetic modifications have also been associated with disease (Lord & Cruchaga, 2014). DNA methylation (DNAm) encodes information on the epigenetic

landscape of an individual and blood-based DNAm signatures have been found to predict all-cause mortality and disease onset, providing strong evidence to suggest that methylation is an important measure of disease risk (Hillary, Stevenson, et al., 2020; Lu et al., 2019; Y. Zhang et al., 2017). DNAm can regulate gene transcription (Lea et al., 2018), and epigenetic differences can be reflected in the variability of the proteome (Hillary et al., 2019; Hillary, Trejo-Banos, et al., 2020; Zaghlool et al., 2020). Low-grade inflammation, which is thought to exacerbate many age-related morbidities, is particularly well-captured through DNAm studies of plasma protein levels (Zaghlool et al., 2020). As proteins are the primary effectors of disease, connecting the epigenome, proteome and time to disease onset may help to resolve predictive biological signatures.

Epigenetic predictors have utilised DNAm from the blood to estimate a person's 'biological age' (Lu et al., 2019), measure their exposure to lifestyle and environmental factors (McCartney, Hillary, et al., 2018; McCartney, Stevenson, Hillary, et al., 2018; Peters et al., 2021) and predict circulating levels of inflammatory proteins (A. Stevenson et al., 2020; A. J. Stevenson et al., 2021). A leading epigenetic predictor of biological aging, the GrimAge epigenetic clock incorporates methylation scores for seven proteins along with smoking and chronological age, and is associated with numerous incident disease outcomes independently of smoking (Hillary, Stevenson, et al., 2020; Lu et al., 2019). This suggests there is predictive value gained in utilising DNAm scores relevant to protein levels as intermediaries for predictions. Methylation scores also point towards the pathways that may act on health beyond the protein biomarker that they are trained on. A portfolio of methylation scores for proteins across the circulating proteome could therefore aid in the prediction of disease and offer a different, but additive signal beyond methylation or protein data alone. Generation of

an extensive range of epigenetic scores for protein levels has not been attempted to date. The capability of specific protein scores to predict a range of morbidities has also not been tested. However, DNAm scores for Interleukin-6 and C-Reactive protein have been found to associate with a range of phenotypes independently of measured protein levels, show more stable longitudinal trajectories than repeated protein measurements, and, in some cases, outperform blood-based proteomic associations with brain morphology (Conole et al., 2020; A. J. Stevenson et al., 2021). This is likely due to DNA methylation representing the accumulation of more sustained effects over a longer period of time than protein measurements, which have often been shown to be highly variable in their levels when measured at multiple time points (Koenig et al., 2003; Liu et al., 2015; Moldoveanu et al., 2000; Shah et al., 2014). DNA methylation scores for proteins could therefore be used to alert clinicians to individuals with high-risk biological signatures, many years prior to disease onset.

Here, we report a comprehensive association study of blood-based DNAm with proteomics and disease (**Figure 1**). We trained epigenetic scores – referred to as EpiScores – for 953 plasma proteins (with sample size ranging from 725 – 944 individuals) and validated them using two independent cohorts with 778 and 162 participants. We regressed out known genetic pQTL effects from the protein levels prior to generating the EpiScores to preclude the signatures being driven by common SNP data that are invariant across the lifespan. We then examined whether the most robust predictors (n=109 EpiScores) associated with the incidence of 12 major morbidities (**Table 1**), over a follow up period of up to 14 years in the Generation Scotland cohort (n = 9,537). We also tested for associations between EpiScore levels and COVID-19 disease outcomes. We regressed out the effects of age on protein levels

prior to training and testing; age was also included as a covariate in disease prediction models. We controlled for common risk factors for disease and assessed the capacity of EpiScores to identify previously reported protein-disease associations.

Figure 1. EpiScores for plasma proteins as tools for disease prediction study design. DNA methylation scores were trained on 953 circulating plasma protein levels in the KORA and LBC1936 cohorts. There were 109 EpiScores selected based on performance ($r > 0.1, P < 0.05$) in independent test sets. The selected EpiScores were projected into Generation Scotland, a cohort that has extensive data linkage to GP and hospital records. We tested whether levels of each EpiScore at baseline could predict the onset of 12 leading causes of morbidity, over a follow-up period of up to 14 years. 137 EpiScore – disease associations were identified, for 11 morbidities. We then assessed whether EpiScore associations reflected protein associations for diabetes, which is a trait that has been well-characterised using SOMAScan protein measurements. Of the 29 SOMAScan-derived EpiScore – diabetes associations, 23 reflected highlighted previously reported protein - diabetes associations.

Morbidity	N cases	N Controls	Years to event (mean, sd)	N cases	N Controls	Years to event (mean, sd)
Rheumatoid arthritis	65	9281	6.1 (3.5)	54	7736	6.4 (3.3)
Alzheimer's dementia	69	3764	8.3 (2.7)	52	3137	8.2 (2.7)
Bowel cancer	77	9398	6.4 (3.2)	65	7817	6.5 (3.2)
Depression	101	8306	3.9 (3.3)	80	6976	3.7 (3.3)
Breast cancer	129	5355	6 (3.4)	110	4401	5.9 (3.4)
Lung cancer	201	9265	5.2 (3.1)	172	7705	5.1 (3.1)
Inflammatory bowel disease	203	9083	5 (3.5)	163	7567	4.9 (3.5)
Stroke	317	9023	6.5 (3.4)	248	7546	6.4 (3.5)
COPD	346	8939	6.2 (3.4)	273	7459	6.1 (3.4)
Ischaemic heart disease	395	8646	5.8 (3.3)	309	7248	5.9 (3.3)
Diabetes	428	8756	5.7 (3.4)	322	7331	5.7 (3.4)
Pain (back/neck)	1494	5341	5.2 (3.5)	1221	4475	5.3 (3.5)

Table 1. Incident morbidities in the Generation Scotland cohort. Counts are provided for the number of cases and controls for each incident trait in the basic and fully-adjusted Cox models run in the Generation Scotland cohort (n=9,537). Mean time-to-event is summarised in years for each phenotype. Alzheimer's dementia cases and controls were restricted to those older than 65 years. Breast cancer cases and controls were restricted to females.

Results

Selecting the most robust EpiScores for protein levels

To generate epigenetic scores for a comprehensive set of plasma proteins, we ran elastic net penalised regression models using protein measurements from the SOMAscan (aptamer-based) and Olink (antibody-based) platforms. We used two cohorts: the German population-based study KORA (n=944, mean age 59 years (SD 7.8), with 793 SOMAscan proteins) and the Scottish Lothian Birth Cohort 1936 (LBC1936) study (between 725 and 875 individuals in the training cohort, with a total of 160 Olink neurology and inflammatory panel proteins). The mean age of the LBC1936 participants at sampling was 70 (SD 0.8) for inflammatory and 73 (SD 0.7) for neurology proteins. Full demographic information is available for all cohorts in **Supplementary file 1A**.

Prior to running the elastic net models, we rank-based inverse normalised protein levels and adjusted for age, sex, cohort-specific variables and, where present, *cis* and *trans* pQTL effects identified from previous analyses (Hillary et al., 2019; Hillary, Trejo-Banos, et al., 2020; Suhre et al., 2017) (**Methods**). Of a possible 793 proteins in KORA, 84 EpiScores had Pearson $r > 0.1$ and $P < 0.05$ when tested in an independent subset of Generation Scotland (The Stratifying Resilience and Depression Longitudinally [STRADL] study, n=778) (**Supplementary file 1B**). These EpiScores were selected for EpiScore-disease analyses. Of the 160 Olink proteins trained in LBC1936, there were 21 with $r > 0.1$ and $P < 0.05$ in independent test sets (STRADL, n=778, Lothian Birth Cohort 1921: LBC1921, n=162) (**Supplementary file 1C**). Independent test set data were not available for four Olink proteins. However, they were included based on their performance ($r > 0.1$ and $P < 0.05$) in a holdout sample of 150 individuals who were left out of the training set. We then retrained these four predictors on the full training sample.

A total of 109 EpiScores (84 SOMAscan-based and 25 Olink-based) were brought forward ($r > 0.1$ and $P < 0.05$) to EpiScore-disease analyses (**Figure 2 and Supplementary file 1D**). There were five EpiScores for proteins common to both Olink and SOMAscan panels, which had variable correlation strength (GZMA $r = 0.71$, MMP.1 $r = 0.46$, CXCL10 $r = 0.35$, NTRK3 $r = 0.26$, and CXCL11 $r = 0.09$). Predictor weights, positional information and *cis/trans* status for CpG sites contributing to these EpiScores are available in **Supplementary file 1F**. The number of CpG features selected for EpiScores ranged from one (Lysozyme) to 395 (Aminoacylase-1), with a mean of 96 **Supplementary file 1G**). The most frequently selected CpG was the smoking-related site cg05575921 (mapping to the *AHRR* gene), which was included in 25 EpiScores. Counts for each CpG site are summarised in **Supplementary file 1H**. This table includes the set of protein EpiScores that each CpG contributes to, along with phenotypic annotations (traits) from the MRC-IEU EWAS catalog (MRC-IEU, 2021) for each CpG site having genome-wide significance ($P < 3.6 \times 10^{-8}$) (Saffari et al., 2017). GeneSet enrichment analysis of the original proteins used to train the 109 EpiScores highlighted pathways associated with immune response and cell remodelling, adhesion and extracellular matrix function (**Supplementary File 1E**).

Figure 2. Test performance for the 109 selected protein EpiScores. Test set correlation coefficients for associations between protein EpiScores for (a) inflammatory Olink, (b) neurology Olink and (c) SOMAmer protein panel EpiScores and measured protein levels are plotted. Upper and lower confidence intervals are shown for each correlation. The 109 protein EpiScores shown achieved $r > 0.1$ and $P < 0.05$ either one or both of the GS:STRADL ($n=778$) and LBC1921 ($n=162$) test sets, wherever protein data was available for comparison. Data shown corresponds to the results included in **Supplementary files 1B-C**. Correlation heatmaps between the 109 EpiScore measures (**Figure 2 – figure supplement 1**) and between the 109 EpiScores and phenotypic traits and estimated white blood cell proportions (**Figure 2 – figure supplement 2**) are

also provided, along with a summary of the most enriched functional pathways for the genes of the 109 proteins used to train EpiScores (**Figure 2 – figure supplement 3**).

EpiScore-disease associations in Generation Scotland

The Generation Scotland dataset contains extensive electronic health data from GP and hospital records available as well as DNA methylation data for 9,537 individuals. This makes it uniquely positioned to test whether EpiScore signals can predict disease onset. We ran nested mixed effects Cox proportional hazards models (**Figure 3**) to determine whether the levels of each EpiScore at baseline associated with the incidence of 12 morbidities over a maximum of 14 years of follow up. The correlation structures for the 109 EpiScore measures used for Cox modelling are presented in Figure 2 – figure supplement 1.

The Cox proportional hazard assumption dictates that hazard ratios for EpiScore – disease associations should remain constant over time. We correlated the Schoenfeld residuals from the models with time to test this. Two associations in the basic model adjusting for age and sex failed to satisfy the global assumption (across all covariates) and were excluded. There were 294 remaining EpiScore-disease associations with a False Discovery Rate (FDR)-adjusted $P < 0.05$ in the basic model. After further adjustment for common risk factor covariates (smoking, social deprivation status, educational attainment, body mass index (BMI) and alcohol consumption), 137 of the 294 EpiScore-disease associations from the basic model had $P < 0.05$ in the fully-adjusted model (**Supplementary files 1I-J**). Eleven of the 137 fully-adjusted associations failed the Cox proportional hazards assumption for the EpiScore variable ($P < 0.05$ for the association between the Schoenfeld residuals and time; **Supplementary file 1K**). When we restricted the time-to-event/censor period by each year of possible follow-up, there were minimal differences in the EpiScore - disease hazard ratios

between follow-up periods that did not violate the assumption and those that did (Supplementary file 1L). The 137 associations were therefore retained as the primary results.

The 137 associations found in the fully-adjusted model comprised 78 unique EpiScores that were related to the incidence of 11 of the 12 morbidities studied. Diabetes and chronic obstructive pulmonary disease (COPD) had the greatest number of associations, with 33 and 41, respectively. **Figure 4** presents the EpiScore-disease relationships for COPD and the remaining nine morbidities: stroke, lung cancer, ischaemic heart disease, inflammatory bowel disease, rheumatoid arthritis, depression, bowel cancer, pain (back/neck) and Alzheimer's dementia. There were 13 EpiScores that associated with the onset of three or more morbidities. **Figure 5** presents relationships for these 13 EpiScores in the fully-adjusted Cox model results. Of note is the EpiScore for Complement 5 (C5), which associated with five outcomes: stroke, diabetes, ischaemic heart disease, rheumatoid arthritis and COPD. Of the 29 SOMAscan-derived EpiScore associations with incident diabetes, 23 replicated previously reported protein associations (Elhadad et al., 2020; Gudmundsdottir et al., 2020; Ngo et al., 2021) with incident or prevalent diabetes in one or more cohorts (**Figure 6 and Supplementary file 1M**).

Figure 3. Nested Cox proportional hazards assessment of protein EpiScore-disease prediction. Mixed effects Cox proportional hazards analyses in Generation Scotland (n = 9,537) tested the relationships between each of the 109 selected EpiScores and the incidence of 12 leading causes of morbidity (**Supplementary files 1I-J**). The basic model was adjusted for age and sex and yielded 294 associations between EpiScores and disease diagnoses, with FDR-adjusted $P < 0.05$. In the fully-adjusted model, which included common risk factors as additional covariates (smoking, deprivation, educational attainment, BMI and alcohol consumption) 137 of the basic model associations remained significant with $P < 0.05$. In a sensitivity analysis, the addition of estimated White Blood Cells (WBCs) to the fully-adjusted models led to the attenuation of 34 of the 137 associations. In a further sensitivity analysis, 81 associations remained after adjustment for both immune cell proportions and GrimAge acceleration.

Figure 4. Protein EpiScore associations with incident disease. EpiScore-disease associations for ten of the eleven morbidities with associations where $P < 0.05$ in the fully-adjusted mixed effects Cox proportional hazards models in Generation Scotland ($n=9,537$). Hazard ratios are presented with confidence intervals for 104 of the 137 EpiScore – incident disease associations reported. Models were adjusted for age, sex and common risk factors (smoking, BMI, alcohol consumption, deprivation and educational attainment). IBD: inflammatory bowel disease. IHD: ischaemic heart disease. COPD: chronic obstructive pulmonary disease. For EpiScore - diabetes associations, see **Figure 6**. Data shown corresponds to the results included in **Supplementary file 1J**.

Figure 5. Protein EpiScores that associated with the greatest number of morbidities. EpiScores with a minimum of three relationships with incident morbidities in the fully-adjusted Cox models. The network includes 13 EpiScores as dark blue (SOMAScan) and grey (Olink) nodes, with disease outcomes in black. EpiScore-disease associations with hazard ratios < 1 are shown as blue connections, whereas hazard ratios > 1 are shown in red. COPD: chronic obstructive pulmonary disease. IHD: ischaemic heart disease. Data shown corresponds to the results included in **Supplementary file 1J**.

Figure 6. Replication of known protein-diabetes associations with protein EpiScores. EpiScore – incident diabetes associations in Generation Scotland (n=9,537). The 29 SOMAscan (top panel) and four Olink (bottom panel) associations shown with $P < 0.05$ in fully-adjusted mixed effects Cox proportional hazards models. Of the 29 SOMAscan-derived EpiScores, 23 associations were consistent with protein – diabetes associations (pink) in one or more of the comparison studies that used SOMAscan protein levels. Six associations were novel (blue). Data shown corresponds to the results included in **Supplementary files 1J and 1M**.

Immune cell and GrimAge sensitivity analyses

Correlations of the 109 EpiScores with covariates suggested interlinked relationships with both immune cells and GrimAge acceleration (Figure 2 – figure supplement 2). These covariates were therefore added incrementally to the fully-adjusted Cox models (**Figure 3**). There were 103 associations that remained statistically significant (FDR $P < 0.05$ in the basic model and $P < 0.05$ in the fully-adjusted model) after adjustment for immune cell

proportions, of which 81 remained significant when GrimAge acceleration scores were added to this model (**Supplementary file 1J**). In a further sensitivity analysis, relationships between both estimated White Blood Cell (WBC) proportions and GrimAge acceleration scores with incident diseases were assessed in the Cox model structure independently of EpiScores. Of the 60 possible relationships between WBC measures and the morbidities assessed, four were statistically significant (FDR-adjusted $P < 0.05$) in the basic model and remained significant with $P < 0.05$ in the fully-adjusted model (**Supplementary file 1N**). A higher proportion of Natural Killer cells was linked to decreased risk of incident COPD, rheumatoid arthritis, diabetes and pain (back/neck). The GrimAge acceleration composite score was associated with COPD, IHD, Diabetes and Pain (back/neck) in the fully-adjusted models ($P < 0.05$) (**Supplementary file 1O**). The magnitude of the GrimAge effect sizes were comparable to the EpiScore findings.

Relationship between EpiScores and subsequent COVID-19

Two previous studies utilising pilot proteomic data from the Generation Scotland cohort (N=199) as part of wider analyses have implicated several proteins that we have trained EpiScores for as associated with COVID-19 outcomes (Demichev et al., 2021; Messner et al., 2020). These included proteins such as CRP, C9, SELL and SHBG, all of which were associated with one or more incident diseases in this study. Two subsets (N=268 and N=173) of the Generation Scotland sample who contracted COVID-19 were therefore used to test the hypothesis that EpiScores would associate with COVID-19 outcomes. No significant associations were identified that delineated differences between the risk of developing long-covid (duration > 4 weeks) or hospitalisation from COVID-19 (associations that had $P < 0.05$ did not withstand Bonferroni adjustment for multiple testing) (**Supplementary file 1P**).

Discussion

Here, we report a comprehensive DNA methylation scoring study of 953 circulating proteins. We define 109 robust EpiScores for plasma protein levels that are independent of known pQTL effects. By projecting these EpiScores into a large cohort with extant data linkage, we show that 78 EpiScores associate with the incidence of 11 leading causes of morbidity (137 EpiScore – disease associations in total), but do not associate with COVID-19 outcomes. Finally, we show that EpiScore - diabetes associations highlight previously measured protein - diabetes relationships. The bulk of EpiScore-disease associations are independent of common lifestyle and health factors, differences in immune cell composition and GrimAge acceleration. EpiScores therefore provide methylation-proteomic signatures for disease prediction and risk stratification.

The consistency between our EpiScore – diabetes associations and previously identified protein – diabetes relationships (Elhadad et al., 2020; Gudmundsdottir et al., 2020; Ngo et al., 2021) suggests that epigenetic scores identify disease-relevant biological signals. In addition to the comprehensive lookup of SOMAscan proteins with diabetes, several of the markers we identified for COPD and IHD also reflect previous associations with measured proteins (Ganz et al., 2016; Serban et al., 2021). The three studies used for the diabetes comparison represent the largest candidate protein characterisations of diabetes to date and the top markers identified included aminoacylase-1 (ACY-1), sex hormone binding globulin (SHBG), growth

hormone receptor (GHR) and Insulin-like growth factor-binding protein 2 (IGFBP-2) (Elhadad et al., 2020; Gudmundsdottir et al., 2020; Ngo et al., 2021) Our EpiScores for these top markers are also associated with diabetes, in addition to EpiScores for several other protein markers reported in these studies. A growing body of evidence suggests that type 2 diabetes is mediated by genetic and epigenetic regulators (Kwak & Park, 2016) and proteins such as ACY-1 and GHR are thought to influence a range of diabetes-associated metabolic mechanisms (Kim & Park, 2017; Pérez-Pérez et al., 2012). Proteins that we identify through EpiScore associations, such as NTR domain-containing protein 2 (WFIKKN2), have also been causally implicated in diabetes onset through Mendelian randomisation analysis (Ngo et al., 2021). In the case of diabetes, EpiScores may therefore be used as disease-relevant risk biomarkers, many years prior to onset. Validation should be tested when sufficient data become available for the remaining morbidities.

With modest test set performances (for example, SHBG $r = 0.18$ and ACY-1 $r = 0.25$), it is perhaps surprising that such strong synergy is observed between EpiScores for proteins that associated with diabetes and the trends seen with measured proteins. Nonetheless, DNA methylation scores for CRP and IL6 have previously been shown to perform modestly in test sets ($r \sim 0.2$, equivalent to $\sim 4\%$ explained variance in protein level), but augment and often outperform the measured protein related to a range of phenotypes (A. Stevenson et al., 2020; A. J. Stevenson et al., 2021). Compared to scores utilising DNAm for the prediction of singular diseases, our EpiScores enable the granular study of individual protein predictor signatures with clinical outcomes. For example, levels of the acid sphingomyelinase (ASM) EpiScore predicted onset of Alzheimer's dementia, several years prior to diagnosis. ASM (encoded by *SMPD1*) has been discussed as a therapeutic candidate for Alzheimer's disease

(Cataldo et al., 2004; Kamil et al., 2016; Lee et al., 2014; Park et al., 2020) and has been shown to disrupt autophagic protein degradation and associate with accumulation of amyloid-beta in murine models of Alzheimer's pathology (Lee et al., 2014; Park et al., 2020).

Our large-scale assessment of EpiScores provides a platform for future studies, as composite predictors for traits may be created using our EpiScore database. These should be tested in incident disease predictions when sufficient case data are available. Our results indicated that the set of 109 EpiScores are likely to be heavily enriched for inflammatory, complement system, innate immune system pathways, in addition to extracellular matrix, cell remodelling and cell adhesion pathways. This reinforces previous work linking chronic inflammation and the epigenome (Zaghlool et al., 2020). It also suggests that EpiScores could be useful in the prediction of morbidities that are characterised by pro-inflammatory states. An example of this is the EpiScore for Complement Component 5 (C5), which was associated with the onset of five morbidities, the highest number for any EpiScore (**Figure 5**). The EpiScore for C5 is likely to reflect the biological pathways occurring in individuals with heightened complement cascade activity and could be utilised to alert clinicians to individuals at high risk of multimorbidity. Elevated levels of C5 peptides have been associated with severe inflammatory, autoimmune and neurodegenerative states (Ma et al., 2019; Mantovani et al., 2014; Morgan & Harris, 2015) and a range of C5-targeting therapeutic approaches are in development (Alawieh et al., 2018; Brandolini et al., 2019; Hawksworth et al., 2017; Hernandez et al., 2017; Morgan & Harris, 2015; Ort et al., 2020).

Though EpiScores such as C5 – which occupy central hubs in the disease-prediction framework – may provide evidence of early methylation signatures common to the onset of multiple diseases, we did not observe associations between EpiScores and COVID-19

hospitalisation or long-COVID status. This is surprising, given that many of the morbidities that EpiScores predicted are also known risk factors for increased risk of death due to COVID-19 (Williamson et al., 2020). Many of the EpiScores were also found to be associated with incident diseases were trained on the levels of proteins that have been implicated in two previous studies that identified markers for COVID-19 severity and progression, using a pilot sample (N=199) from the Generation Scotland cohort at baseline (Demichev et al., 2021; Messner et al., 2020). COVID-19 likely has multiple intersecting risk factors that impact severity and recovery and the lack of associations we observe is likely to be in part due to limited COVID-19 cases available in Generation Scotland. Additionally, there is a large lag time between baseline biological measurement and COVID-19 in our analyses, whereas the two studies that found protein marker associations integrated protein measurements longitudinally and from samples extracted during COVID-19 progression. With increased power available through continued data linkage, EpiScore relationships with COVID-19 outcomes may be observed in future work.

This study has several limitations. First, we demonstrate that EpiScores carry disease-relevant signals that may be clinically meaningful to delineate early disease risk when comparing relative differences within a cohort. However, projecting a new individual onto a reference set is complicated due to absolute differences in methylation quantification resulting from batch and processing effects. Second, future studies should assess paired protein and EpiScore contributions to traits, as inference from EpiScores alone, while useful for disease risk stratification, is not sufficient to determine mechanisms. This may also highlight EpiScores that outperform the measured protein equivalent in disease. Third, the epitope nature of the protein measurement in the SOMAscan panel may incur probe cross-reactivity

and non-specific binding; there may also be differences in how certain proteins are measured across panels (Pietzner et al., 2020; Sun et al., 2018). Comparisons of both protein measurement technologies on the same samples would help to explore this in more detail. Fourth, there may be pQTLs with small effect sizes that were not regressed from the proteins prior to generating the EpiScores. Fifth, while training and testing was performed across multiple cohorts, it is likely that further development of EpiScores in larger proteomic samples with diverse ancestries will improve power to generate robust scores. Upper bounds for DNAm prediction of complex traits, such as proteins, can be estimated by variance components analyses (Hillary, Trejo-Banos, et al., 2020; Trejo Banos et al., 2020; F. Zhang et al., 2019). Finally, associations present between EpiScore measures and disease incidence may have been influenced by external factors such as prescription medications for comorbid conditions and comorbid disease prevalence.

We have shown that EpiScores for circulating protein levels predict the incidence of multiple diseases, up to 14 years prior to diagnosis. Our findings suggest that DNA methylation phenotyping approaches and data linkage to electronic health records in large, population-based studies have the potential to (1) Capture inter-individual variability in protein levels; (2) predict incident disease risk many years prior to morbidity onset; and (3) highlight disease-relevant biological signals for further exploration. The EpiScore weights are publicly available, enabling any cohort with Illumina DNAm data to generate them and to relate them to various outcomes. Given the increasingly widespread assessment of DNAm in cohort studies (McCartney et al., 2020; Min et al., 2020), EpiScores offer an affordable and consistent (i.e. array-based) way to utilise these signatures. This information is likely to be important in risk stratification and prevention of age-related morbidities.

Materials and Methods

The KORA sample population

The KORA F4 study includes 3,080 participants who reside in Southern Germany. Individuals were between 32 and 81 years of age when recruited to the study from 2006 and 2008. In the current study, there were 944 individuals with methylation, proteomics and genetic data available. The Infinium HumanMethylation450 BeadChip platform was used to generate DNA methylation data for these individuals. The Affymetrix Axiom array was used to generate genotyping data and the SOMAscan platform was used to generate proteomic measurements in the sample.

DNA methylation in KORA

Methylation data were generated for 1,814 individuals(Petersen et al., 2014); 944 also had protein and genotype measurements available. During preprocessing, 65 SNP probes were excluded and background correction was performed in minfi (Aryee et al., 2014). Samples with a detection rate of less than 95% were excluded. Next, the minfi R package was used to perform normalization on the intensity methylation measures (Aryee et al., 2014), with a method consistent with the Lumi:QN +BMIQ pipeline. After excluding non-cg sites and

CpGs on sex chromosomes or with fewer than 100 measures available, 470,837 CpGs were available for analyses.

Proteomics in KORA

The SOMAscan platform (V3.2) (Gold et al., 2010) was used to quantify protein levels in undepleted plasma for 1129 SOMAmer probes (Suhre et al., 2017). Of the 1,000 samples provided for analysis, two samples were excluded due to errors in bio-bank sampling and one based on quality control measures. Of the 997 samples available, there were 944 individuals with methylation and genotypic data. Of the 1,129 probes available, five failed the QC, leaving a total of 1,124 probes for the subsequent analysis. Protein measurements were transformed by rank-based inverse normalisation and regressed onto age, sex, known pQTLs and 20 genetic principal components of ancestry derived from the Affymetrix Axiom Array to control for population structure. pQTLs for each protein were taken from a previous GWAS in the sample (Suhre et al., 2017).

The LBC1936 and LBC1921 sample populations

The Lothian Birth Cohorts of 1921 (LBC1921; N = 550) and 1936 (LBC1936; N = 1091) are longitudinal studies of aging in individuals who reside in Scotland (Deary et al., 2012; Taylor et al., 2018). Participants completed an intelligence test at age 11 years and were recruited for these cohorts at mean ages of 79 (LBC1921) and 70 (LBC1936). They have been followed up triennially for a series of cognitive, clinical, physical and social data, along with

blood donations that have been used for genetic, epigenetic, and proteomic measurement.

DNAm, proteomic (Olink® platform) and genetic data for up to 875 individuals from Waves 1 and 2 of the LBC1936 (at mean ages 70 and 73 years) and 162 individuals at Wave 3 of the LBC1921 (at mean age 87 years).

DNAm in LBC1936 and LBC1921

DNA from whole blood was assessed using the Illumina 450 K methylation array. Details of quality control have been described elsewhere (Shah et al., 2014; Q. Zhang et al., 2018). Raw intensity data were background-corrected and normalised using internal controls. Manual inspection resulted in the removal of low quality samples that presented issues related to bisulphite conversion, staining signal, inadequate hybridisation or nucleotide extension. Probes with low detection rate <95% at $P < 0.01$ and samples with low call rates (<450,000 probes detected at $P < 0.01$) were removed. Samples were also removed if they had a poor match between genotype and SNP control probes, or incorrect DNA methylation-predicted sex.

Proteomics in LBC1936 and LBC1921

Plasma samples were analysed using either the Olink® neurology 92-plex or the Olink® inflammation 92-plex proximity extension assays (Olink® Bioscience, Uppsala Sweden). One inflammatory panel protein (BDNF) failed quality control and was removed. A further 21 proteins were removed, as over 40% of samples fell below the lowest limit of detection.

Two neurology proteins, MAPT and HAGH, were excluded due to >40% of observations being below the lower limit of detection. This resulted in 90 neurology (LBC1936 Wave 2) and 70 inflammatory (LBC1936 Wave 1) proteins in LBC1936 and 92 neurology proteins available in LBC1921. Protein levels were rank-based inverse normalised and regressed onto age, sex, four genetic components of ancestry derived from multidimensional scaling of the Illumina 610-Quadv1 genotype array and Olink® array plate. In LBC1936, pQTLs were adjusted for, through reference to GWAS in the samples (Hillary et al., 2019; Hillary, Trejo-Banos, et al., 2020).

Generation Scotland and STRADL sample populations

Generation Scotland: the Scottish Family Health Study (GS) is a large, family-structured, population-based cohort study of >24,000 individuals from Scotland (mean age 48 years)(Smith et al., 2013). Recruitment took place between 2006 and 2011 with a clinical visit where detailed health, cognitive, and lifestyle information was collected along with biological samples (blood, urine, saliva). In GS, there were 9,537 individuals with DNAm and phenotypic information available. The Stratifying Resilience and Depression Longitudinally (STRADL) cohort is a subset of 1,188 individuals from the GS cohort who undertook additional assessments approximately five years after the study baseline (Navrady et al., 2018).

DNA methylation in Generation Scotland and STRADL

In the GS cohort, blood-based DNA methylation was generated in two sets using the Illumina EPIC array. Set 1 comprised 5,190 related individuals whereas Set 2 comprised 4,583 individuals, unrelated to each other and to those in Set 1. During quality control, probes were removed based on visual outlier inspection, bead count <3 in over 5% of samples and samples with detection P value below adequate thresholds (McCartney, Stevenson, Walker, et al., 2018; Seeboth et al., 2020). Samples were removed based on sex mismatches, low detection P values for CpGs and saliva samples and genetic outliers (Amador et al., 2015). The quality-controlled dataset comprised 9,537 individuals ($n_{\text{Set1}}=5,087$, $n_{\text{Set2}}=4,450$). The same steps were also applied to process DNAm in STRADL.

Proteomics in STRADL

Measurements for 4,235 proteins in 1,065 individuals from the STRADL cohort were recorded using the SOMAscan® technology. 793 epitopes matched between the KORA and STRADL cohorts and were included for training in KORA and testing in STRADL. There were 778 individuals with proteomics data and DNAm data in STRADL. Protein measurements were transformed by rank-based inverse normalisation and regressed onto age, sex and 20 genetic principal components (derived from multidimensional scaling of genotype data from the Illumina 610-Quadv1 array).

Electronic health data linkage in Generation Scotland

Over 98% of GS participants consented to allow access to electronic health records via data linkage to GP records (Read 2 codes) and hospital records (ICD codes). Data are available prospectively from the time of blood draw, yielding up to 14 years of linkage. We considered incident data for 12 morbidities. Ten of the diseases are listed by the world health organisation (WHO) as leading causes of either morbidity or mortality (Hay et al., 2017; World Health Organization, 2018). Inflammatory bowel disease (IBD) (Kassam et al., 2014) and rheumatoid arthritis (RA) (James et al., 2018) are also included as traits as they have been reported as leading causes of disability and morbidity and the global burdens of these diseases are rising (Alatab et al., 2020; Safiri et al., 2019). Prevalent cases (ascertained via retrospective ICD and Read 2 codes or self-report from a baseline questionnaire) were excluded. For inflammatory bowel disease (IBD) prevalent cases were excluded based on data linkage alone. Included and excluded terms can be found in **Supplementary file 1Q-1B1**. Alzheimer's dementia was limited to cases/controls with age of event/censoring ≥ 65 years. Breast cancer was restricted to females only. Recurrent, major and moderate episodes of depression were included in depression. Diabetes was comprised of type 2 diabetes and more general diabetes codes such as diabetic retinopathy and diabetes mellitus with renal manifestation. Type 1 and juvenile diabetes cases were excluded.

Elastic net protein EpiScores

Penalised regression models were generated for 160 proteins in LBC1936 and 793 proteins in KORA using Glmnet (Version 4.0-2) (Friedman et al., 2010) in R (Version 4.0) (R, 2020). Protein levels were the outcome and there were 428,489 CpG features per model in the LBC1936 training and 397,630 in the KORA training. An elastic net penalty was specified

($\alpha=0.5$) and cross validation was applied. DNAm and protein measurements were scaled to have a mean of zero and variance of one.

In the KORA analyses, 10-fold cross validation was applied and EpiScores were tested in STRADL ($n=778$). Of 480 EpiScores that generated ≥ 1 CpG features, 84 had Pearson $r > 0.1$ and $P < 0.05$ in STRADL. As test set comparisons were not available for every protein in the LBC1936 analyses, a holdout sample was defined, with two folds set aside as test data and 10-fold cross validation carried out on the remaining data ($n_{\text{train}}=576$, $n_{\text{test}}=130$ for neurology and $n_{\text{train}}=725$, $n_{\text{test}}=150$ for inflammatory proteins). We retained 36 EpiScores with Pearson $r > 0.1$ and $P < 0.05$. New predictors for these 36 proteins were then generated using 12-fold cross validation and tested externally in STRADL ($n=778$) and LBC1921 ($n=162$, for the neurology panel). 21 EpiScores had $r > 0.1$ and $P < 0.05$ in at least one of the external test sets. Four EpiScores did not have external comparisons and were included based on holdout performance.

Functional annotations for each of the proteins used to train the finalised set of 109 EpiScores were sourced from the STRING database (Jensen et al., 2009). GeneSet enrichment analysis against protein-coding genes was performed using the FUMA database, to quantify which canonical pathways were most commonly implicated across the 109 genes corresponding to the proteins used to train the 109 EpiScores (Watanabe et al., 2017). The background gene-set was specified as protein coding genes and a threshold of FDR $P < 0.05$ was applied for enrichment status, with the minimum overlapping genes with gene-sets set to greater than or equal to two.

The 109 selected EpiScores were then applied to Generation Scotland ($n=9,537$). DNAm at each CpG site was scaled to have a mean of zero and variance of one, with scaling performed separately for GS Sets.

Associations with health linkage phenotypes in Generation Scotland

Mixed effects Cox proportional hazards regression models adjusting for age, sex, and methylation set were used to assess the relationship between 109 EpiScores and 12 morbidities in Generation Scotland. Models were run using `coxme` (Therneau, 2020b) (Version 2.2-16) with a kinship matrix accounting for relatedness in Set 1. Cases included those diagnosed after baseline who had died, in addition to those who received a diagnosis and remained alive. Controls were censored if disease free at time of death, or at the end of the follow-up period. EpiScore levels were rank-base inverse normalised. Fully-adjusted models included: the following additional covariates measured at baseline: alcohol consumption (units consumed in the previous week); deprivation (assessed by the Scottish Index of Multiple Deprivation (GovScot, 2016)); body mass index (kg/m^2); educational attainment (an 11-category ordinal variable) and a DNAm-based score for smoking status (Bollepalli et al., 2019). A false discovery rate multiple testing correction $P < 0.05$ was applied to the 1306 EpiScore-disease associations (109 EpiScores by 12 incident disease traits, with 2 associations excluded for failing the global proportional hazards assumption). Proportional hazards assumptions were checked through Schoenfeld residuals (global test and a test for the protein-EpiScore variable) using the `coxph` and `cox.zph` functions from the survival package (Therneau, 2020a) (Version 3.2-7). For each association failing to meet the assumption (Schoenfeld residuals $P < 0.05$), a sensitivity analysis was run across yearly follow-up intervals.

Fully-adjusted Cox proportional hazards models were run with Houseman-estimated White Blood Cell (WBC) proportions as covariates (Houseman et al., 2012). A further sensitivity

analyses added GrimAge acceleration (Lu et al., 2019) as an additional covariate. Basic and fully-adjusted Cox models were also run with estimated Monocyte, Bcell, CD4T, CD8T and Natural Killer cell proportions as predictors.

Correlation structures for EpiScores, DNAm-based white cell proportions and phenotypic information were assessed using Pearson correlations and pheatmap (Kolde, 2019) (Version 1.0.12) and ggcorrplot packages (Version 0.1.3) (Kassambara, 2019). The psych package (Version 2.0.9) (Revelle, 2020) was used to perform principal components analysis on EpiScores. Figures 1 and 2 were created with BioRender.com. Associations for EpiScores that were related to a minimum of three morbidities were subset from the fully-adjusted Cox proportional hazards results and were visualised using the ggraph package (Version 2.0.5) (Pedersen, 2021). This network representation was used (**Figure 5**) to highlight protein EpiScores that were connected with multiple morbidities.

Consistency of disease associations between EpiScores and measured proteins

Comparisons were conducted between EpiScore – diabetes associations and diabetes associations with measured proteins using three previous large-scale proteomic studies (Elhadad et al., 2020; Gudmundsdottir et al., 2020; Ngo et al., 2021) In these studies, six cohorts were included (Study 1: KORA n= 993, HUNT n= 940 (Elhadad et al., 2020), Study 2: AGES-Reykjavik n=5,438 and QMDiab n=356 (Gudmundsdottir et al., 2020), Study 3: Framingham Heart Study n=1,618 and the Malmo Diet and Cancer Study n=1,221). Study 1 included the KORA dataset, which we use in this study to generate SOMAscan EpiScores. We characterised which SOMAscan-based EpiScore – diabetes associations from our fully-

adjusted results reflected those observed with measured protein levels. We included basic (nominal $P < 0.05$) and fully adjusted results (with either FDR or Bonferroni-corrected $P < 0.05$), wherever available, across the lookup cohorts (**Supplementary file 1M**).

Relationship between EpiScores and COVID-19 outcomes

Associations between each robust protein EpiScore and subsequent long-COVID or COVID-19 hospitalisation were tested in the Generation Scotland population. A binary variable was used for long-COVID based on self-reported COVID-19 duration from the CovidLife study survey 3 questionnaire (N=2,399 participating individuals) (Fawns-Ritchie et al., 2021). Participants were asked about the total overall time they experienced symptoms in their first/only episode of illness, as well as the whole of their COVID-19 illness and the dataset is correct as of February 2021, when the survey 3 was administered. Of the 9,537 individuals with DNAm that were included in incident disease analyses, 173 indicated that they had COVID-19 and 56 of these individuals reported having long-COVID (> 4 weeks duration of symptoms after infection). Hospitalisation information, derived from the Scottish Morbidity Records (SMR01), was used to obtain COVID-19 hospital admissions using ICD10 codes U07.1 (lab-confirmed COVID-19 diagnosis), and U07.2 (clinically-diagnosed COVID-19). This data linkage identified 268 of the 9,537 individuals that had COVID-19 diagnoses and 29 had been recorded as being hospitalised due to COVID-19. Generalised linear models with either hospitalisation or long-COVID status as binary outcomes were used, with the 109 scaled protein EpiScores as the independent variables. Sex and Age at COVID testing were included as covariates. The latter was defined as the age at positive COVID-19 test or 1st January 2021 if COVID-19 test data were not available.

Acknowledgements

We are grateful to all study participants of KORA, LBC1936, LBC1921 and GS for their invaluable contributions to this study.

Ethics

All KORA participants have given written informed consent and the study was approved by the Ethics Committee of the Bavarian Medical Association.

All components of GS received ethical approval from the NHS Tayside Committee on Medical Research Ethics (REC Reference Number: 05/S1401/89). GS has also been granted Research Tissue Bank status by the East of Scotland Research Ethics Service (REC Reference Number: 20/ES/0021), providing generic ethical approval for a wide range of uses within medical research.

Ethical approval for the LBC1921 and LBC1936 studies was obtained from the Multi-Centre Research Ethics Committee for Scotland (MREC/01/0/56) and the Lothian Research Ethics committee (LREC/1998/4/183; LREC/2003/2/29). In both studies, all participants provided written informed consent. These studies were performed in accordance with the Helsinki declaration.

Availability of data and materials

Datasets generated in this study are made available in Supplementary file 1; this file includes the protein EpiScore weights for the 109 EpiScores we provide for future studies to use. All results datasets used to create figures are included in Supplementary file 1 and specific locations for these are noted in figure legends and provided here as follows: Figure 2:

Supplementary files 1B-C, Figures 3-4: Supplementary files 1J, Figure 5: Supplementary files 1J and 1M.

All code used in the analyses is available with open access at the following Github repository: <https://github.com/DanniGadd/EpiScores-for-protein-levels>.

The source datasets from the cohorts that were analysed during the current study are not publicly available due to them containing information that could compromise participant consent and confidentiality. Data can be obtained from the data owners. Instructions for Lothian Birth Cohort data access can be found here:

<https://www.lothianbirthcohort.ed.ac.uk/content/collaboration>. Dr Simon Cox must be contacted to obtain a Lothian Birth Cohort ‘Data Request Form’ by email:

simon.cox@ed.ac.uk. Instructions for accessing Generation Scotland data can be found here:

<https://www.ed.ac.uk/generation-scotland/for-researchers/access>; the ‘GS Access Request Form’ can be downloaded from this site. Completed request forms must be sent to

access@generationscotland.org to be approved by the Generation Scotland access committee.

Data from the KORA study can be requested from KORA-gen: [http://epi.helmholtz-](http://epi.helmholtz-muenchen.de/kora-gen)

[muenchen.de/kora-gen](http://epi.helmholtz-muenchen.de/kora-gen). Requests are submitted online and are subject to approval by the

KORA board.

Competing interests

R.E.M has received a speaker fee from Illumina and is an advisor to the Epigenetic Clock Development Foundation. All other authors declare no competing interests.

References

- Alatab, S., Sepanlou, S. G., Ikuta, K., Vahedi, H., Bisignano, C., Safiri, S., Sadeghi, A., Nixon, M. R., Abdoli, A., Abolhassani, H., Alipour, V., Almadi, M. A. H., Almasi-Hashiani, A., Anushiravani, A., Arabloo, J., Atique, S., Awasthi, A., Badawi, A., Baig, A. A. A., ... Naghavi, M. (2020). The global, regional, and national burden of inflammatory bowel disease in 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *The Lancet Gastroenterology and Hepatology*, 5(1), 17–30. [https://doi.org/10.1016/S2468-1253\(19\)30333-4](https://doi.org/10.1016/S2468-1253(19)30333-4)
- Alawieh, A., Farris Langley, E., & Tomlinson, S. (2018). Targeted complement inhibition salvages stressed neurons and inhibits neuroinflammation after stroke in mice. *Science Translational Medicine*, 10(441), 6459. <https://doi.org/10.1126/scitranslmed.aa06459>
- Amador, C., Huffman, J., Trochet, H., Campbell, A., Porteous, D., Wilson, J. F., Hastie, N., Vitart, V., Hayward, C., Navarro, P., Haley, C. S., & Generation Scotland. (2015). Recent genomic heritage in Scotland. *BMC Genomics*, 16(1), 437. <https://doi.org/10.1186/s12864-015-1605-2>
- Aryee, M. J., Jaffe, A. E., Corrada-Bravo, H., Ladd-Acosta, C., Feinberg, A. P., Hansen, K. D., & Irizarry, R. A. (2014). Minfi: A flexible and comprehensive Bioconductor package for the analysis of Infinium DNA methylation microarrays. *Bioinformatics*, 30(10),

1363–1369. <https://doi.org/10.1093/bioinformatics/btu049>

Bollepalli, S., Korhonen, T., Kaprio, J., Anders, S., & Ollikainen, M. (2019). EpiSmokEr: A robust classifier to determine smoking status from DNA methylation data. *Epigenomics*, 11(13), 1469–1486. <https://doi.org/10.2217/epi-2019-0206>

Brandolini, L., Grannonico, M., Bianchini, G., Colanardi, A., Sebastiani, P., Paladini, A., Piroli, A., Allegretti, M., Varrassi, G., & Di Loreto, S. (2019). The Novel C5aR Antagonist DF3016A Protects Neurons Against Ischemic Neuroinflammatory Injury. *Neurotoxicity Research*, 36(1), 163–174. <https://doi.org/10.1007/s12640-019-00026-w>

Cataldo, A. M., Petanceska, S., Terio, N. B., Peterhoff, C. M., Durham, R., Mercken, M., Mehta, P. D., Buxbaum, J., Haroutunian, V., & Nixon, R. A. (2004). A β localization in abnormal endosomes: Association with earliest A β elevations in AD and Down syndrome. *Neurobiology of Aging*, 25(10), 1263–1272. <https://doi.org/10.1016/j.neurobiolaging.2004.02.027>

Conole, E. L. S., Stevenson, A. J., Green, C., Harris, S. E., Muñoz, S., Valdés-hernández, M. C., Harris, M. A., Bastin, M. E., & Joanna, M. (2020). An epigenetic proxy of chronic inflammation outperforms serum levels as a biomarker of brain ageing. *medRxiv*. <https://doi.org/https://doi.org/10.1101/2020.10.08.20205245>

Deary, I. J., Gow, A. J., Pattie, A., & Starr, J. M. (2012). Cohort profile: The lothian birth cohorts of 1921 and 1936. *International Journal of Epidemiology*, 41(6), 1576–1584.

Demichev, V., Tober-Lau, P., Lemke, O., Nazarenko, T., Thibeault, C., Whitwell, H., Röhl, A., Freiwald, A., Szyrwił, L., Ludwig, D., Correia-Melo, C., Aulakh, S. K., Helbig, E. T., Stubbemann, P., Lippert, L. J., Grüning, N. M., Blyuss, O., Vernardis, S., White, M., ... Kurth, F. (2021). A time-resolved proteomic and prognostic map of COVID-19. *Cell Systems*, 12(8), 780. <https://doi.org/10.1016/J.CELS.2021.05.005>

780 Elhadad, M. A., Jonasson, C., Huth, C., Wilson, R., Gieger, C., Matias, P., Grallert, H.,
 781 Graumann, J., Gailus-Durner, V., Rathmann, W., Toerne, C. von, Hauck, S. M., Koenig,
 782 W., Sinner, M. F., Oprea, T. I., Suhre, K., Thorand, B., Hveem, K., Peters, A., &
 783 Waldenberger, M. (2020). Deciphering the plasma proteome of type 2 diabetes.
 784 *Diabetes*, 69(12), 2766–2778. <https://doi.org/10.2337/db20-0296>
 785 Fawns-Ritchie, C., Altschul, D. M., Campbell, A., Huggins, C., Nangle, C., Dawson, R.,
 786 Edwards, R., Flaig, R., Hartley, L., Levein, C., McCartney, D. L., Bell, D., Douglas, E.,
 787 Deary, I. J., Hayward, C., Marioni, R. E., McIntosh, A. M., Sudlow, C., & Porteous, D.
 788 J. (2021). CovidLife: a resource to understand mental health, well-being and behaviour
 789 during the COVID-19 pandemic in the UK. *Wellcome Open Research* 2021 6:176, 6,
 790 176. <https://doi.org/10.12688/wellcomeopenres.16987.1>
 791 Friedman, J., Hastie, T., & Tibshirani, R. (2010). Regularization Paths for Generalized Linear
 792 Models via Coordinate Descent. *Journal of Statistical Software*, 33(1), 1–22.
 793 Fuchsberger, C., Flannick, J., Teslovich, T. M., Mahajan, A., Agarwala, V., Gaulton, K. J.,
 794 Ma, C., Fontanillas, P., Moutsianas, L., McCarthy, D. J., Rivas, M. A., B Perry, J. R.,
 795 Sim, X., Blackwell, T. W., Robertson, N. R., William Rayner, N., Cingolani, P., Locke,
 796 A. E., Fernandez Tajos, J., ... McCarthy, M. I. (2016). The genetic architecture of type 2
 797 diabetes. *Nature Publishing Group*, 536, 41–47.
 798 Ganz, P., Heidecker, B., Hveem, K., Jonasson, C., Kato, S., Segal, M. R., Sterling, D. G., &
 799 Williams, S. A. (2016). Development and validation of a protein-based risk score for
 800 cardiovascular outcomes among patients with stable coronary heart disease. *JAMA*,
 801 315(23), 2532–2541. <https://doi.org/10.1001/jama.2016.5951>
 802 Gold, L., Ayers, D., Bertino, J., Bock, C., Bock, A., Brody, E. N., Carter, J., Dalby, A. B.,
 803 Eaton, B. E., Fitzwater, T., Flather, D., Forbes, A., Foreman, T., Fowler, C., Gawande,

B., Goss, M., Gunn, M., Gupta, S., Halladay, D., ... Zichi, D. (2010). Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS ONE*, 5(12), e15004. <https://doi.org/10.1371/journal.pone.0015004>

GovScot. (2016). *Scottish Government. The Scottish Index of Multiple Deprivation (SIMD); 1-20. (2016). Available from: <http://www.gov.scot/Resource/0050/00504809.pdf>. Accessed April 2021.*

Gudmundsdottir, V., Zaghlool, S. B., Emilsson, V., Aspelund, T., Ilkov, M., Gudmundsson, E. F., Jonsson, S. M., Zilhão, N. R., Lamb, J. R., Suhre, K., Jennings, L. L., & Gudnason, V. (2020). Circulating protein signatures and causal candidates for type 2 diabetes. *Diabetes*, 69(8), 1843–1853. <https://doi.org/10.2337/db19-1070>

Hawksworth, O. A., Li, X. X., Coulthard, L. G., Wolvetang, E. J., & Woodruff, T. M. (2017). New concepts on the therapeutic control of complement anaphylatoxin receptors. *Molecular Immunology*, 89, 36–43. <https://doi.org/10.1016/j.molimm.2017.05.015>

Hay, S. I., Abajobir, A. A., Abate, K. H., Abbafati, C., Abbas, K. M., Abd-Allah, F., Abdulle, A. M., Abebo, T. A., Abera, S. F., Aboyans, V., Abu-Raddad, L. J., Ackerman, I. N., Adedeji, I. A., Adetokunboh, O., Afshin, A., Aggarwal, R., Agrawal, S., Agrawal, A., Kiadaliri, A. A., ... Bryane, C. E. G. (2017). Global, regional, and national disability-adjusted life-years (DALYs) for 333 diseases and injuries and healthy life expectancy (HALE) for 195 countries and territories, 1990-2016: A systematic analysis for the Global Burden of Disease Study 2016. *The Lancet*, 390(10100), 1260–1344. [https://doi.org/10.1016/S0140-6736\(17\)32130-X](https://doi.org/10.1016/S0140-6736(17)32130-X)

Hernandez, M. X., Jiang, S., Cole, T. A., Chu, S. H., Fonseca, M. I., Fang, M. J., Hohsfield, L. A., Torres, M. D., Green, K. N., Wetsel, R. A., Mortazavi, A., & Tenner, A. J. (2017). Prevention of C5aR1 signaling delays microglial inflammatory polarization, favors

clearance pathways and suppresses cognitive loss. *Molecular Neurodegeneration*, 12(1),
66. <https://doi.org/10.1186/s13024-017-0210-z>

Hillary, R. F., McCartney, D. L., Harris, S. E., Stevenson, A. J., Seebboth, A., Zhang, Q.,
Liewald, D. C., Evans, K. L., Ritchie, C. W., Tucker-Drob, E. M., Wray, N. R., McRae,
A. F., Visscher, P. M., Deary, I. J., & Marioni, R. E. (2019). Genome and epigenome
wide studies of neurological protein biomarkers in the Lothian Birth Cohort 1936.
Nature Communications, 10(1), 3160. <https://doi.org/10.1038/s41467-019-11177-x>

Hillary, R. F., Stevenson, A. J., McCartney, D. L., Campbell, A., Walker, R. M., Howard, D.
M., Ritchie, C. W., Horvath, S., Hayward, C., McIntosh, A. M., Porteous, D. J., Deary,
I. J., Evans, K. L., & Marioni, R. E. (2020). Epigenetic measures of ageing predict the
prevalence and incidence of leading causes of death and disease burden. *Clin Epigenet*,
12(115). <https://doi.org/10.1186/s13148-020-00905-6>

Hillary, R. F., Trejo-Banos, D., Kousathanas, A., McCartney, D. L., Harris, S. E., Stevenson,
A. J., Patxot, M., Ojavee, S. E., Zhang, Q., Liewald, D. C., Ritchie, C. W., Evans, K. L.,
Tucker-Drob, E. M., Wray, N. R., McRae, A. F., Visscher, P. M., Deary, I. J., Robinson,
M. R., & Marioni, R. E. (2020). Multi-method genome- And epigenome-wide studies of
inflammatory protein levels in healthy older adults. *Genome Medicine*, 12, 60.
<https://doi.org/10.1186/s13073-020-00754-1>

Houseman, E. A., Accomando, W. P., Koestler, D. C., Christensen, B. C., Marsit, C. J.,
Nelson, H. H., Wiencke, J. K., & Kelsey, K. T. (2012). DNA methylation arrays as
surrogate measures of cell mixture distribution. *BMC Bioinformatics*, 13, 86.
<https://doi.org/10.1186/1471-2105-13-86>

James, S. L., Abate, D., Abate, K. H., Abay, S. M., Abbafati, C., Abbasi, N., Abbastabar, H.,
Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe,

- Z., Abera, S. F., Abil, O. Z., Abraha, H. N., Abu-Raddad, L. J., Abu-Rmeileh, N. M. E., Accrombessi, M. M. K., ... Murray, C. J. L. (2018). Global, regional, and national incidence, prevalence, and years lived with disability for 354 Diseases and Injuries for 195 countries and territories, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet*, 392(10159), 1789–1858.
[https://doi.org/10.1016/S0140-6736\(18\)32279-7](https://doi.org/10.1016/S0140-6736(18)32279-7)
- Jensen, L. J., Kuhn, M., Stark, M., Chaffron, S., Creevey, C., Muller, J., Doerks, T., Julien, P., Roth, A., Simonovic, M., Bork, P., & von Mering, C. (2009). STRING 8--a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Research*, 37(Database issue). <https://doi.org/10.1093/NAR/GKN760>
- Kamil, B., Anna, F., Anna, S., Sławomir, P., & Halina, C. (2016). Regulation of sphingomyelin metabolism. *Pharmacological Reports*, 68(3), 570–581.
<https://doi.org/10.1016/j.pharep.2015.12.008>
- Kassam, Z., Belga, S., Roifman, I., Hirota, S., Jijon, H., Kaplan, G. G., Ghosh, S., & Beck, P. L. (2014). Inflammatory bowel disease cause-specific mortality: A primer for clinicians. *Inflammatory Bowel Diseases*, 20(12), 2483–2492.
<https://doi.org/10.1097/MIB.0000000000000173>
- Kassambara, A. (2019). *ggcorrplot: Visualization of a Correlation Matrix using “ggplot2”*. R package (Version 0.1.3). <https://cran.r-project.org/web/packages/ggcorrplot/ggcorrplot.pdf>. Accessed April 2021.
- Kim, S.-H., & Park, M.-J. (2017). Effects of growth hormone on glucose metabolism and insulin resistance in human. *Annals of Pediatric Endocrinology & Metabolism*, 22(3), 145–152. <https://doi.org/10.6065/apem.2017.22.3.145>
- Koenig, W., Sund, M., Fröhlich, M., Löwel, H., Hutchinson, W. L., & Pepys, M. B. (2003).

Refinement of the Association of Serum C-reactive Protein Concentration and Coronary Heart Disease Risk by Correction for Within-Subject Variation over Time The MONICA Augsburg Studies, 1984 and 1987. *American Journal of Epidemiology*, 158(4), 357–364. <https://doi.org/10.1093/AJE/KWG135>

Kolde, R. (2019). *Pheatmap : Pretty Heatmaps. R package version 1.0.12*. <https://cran.r-project.org/web/packages/pheatmap/index.html>. Accessed April 2021.

Kwak, S. H., & Park, K. S. (2016). Recent progress in genetic and epigenetic research on type 2 diabetes. *Experimental & Molecular Medicine*, 48(3), e220.

Lea, A. J., Vockley, C. M., Johnston, R. A., Del Carpio, C. A., Barreiro, L. B., Reddy, T. E., & Tung, J. (2018). Genome-wide quantification of the effects of DNA methylation on human gene regulation. *ELife*, eLife 2018;7:e37513. <https://doi.org/10.7554/eLife.37513>

Lee, J. K., Jin, H. K., Park, M. H., Kim, B. R., Lee, P. H., Nakauchi, H., Carter, J. E., He, X., Schuchman, E. H., & Bae, J. S. (2014). Acid sphingomyelinase modulates the autophagic process by controlling lysosomal biogenesis in Alzheimer's disease. *Journal of Experimental Medicine*, 211(8), 1551–1570. <https://doi.org/10.1084/jem.20132451>

Liu, Y., Buil, A., Collins, B. C., Gillet, L. C., Blum, L. C., Cheng, L., Vitek, O., Mouritsen, J., Lachance, G., Spector, T. D., Dermitzakis, E. T., & Aebersold, R. (2015). Quantitative variability of 342 plasma proteins in a human twin population. *Molecular Systems Biology*, 11(2), 786. <https://doi.org/10.15252/MSB.20145728/FORMAT/PDF>

Lord, J., & Cruchaga, C. (2014). *The epigenetic landscape of Alzheimer's disease*. 9, 1921.

Lu, A. T., Quach, A., Wilson, J. G., Reiner, A. P., Aviv, A., Raj, K., Hou, L., Baccarelli, A., Li, Y., Stewart, J. D., Whitsel, E. A., Assimes, T. L., Ferrucci, L., & Horvath, S. (2019). DNA methylation GrimAge strongly predicts lifespan and healthspan. *Aging*, 11(2), 303–327. <https://doi.org/10.18632/aging.101684>

900 Ma, Y., Liu, Y., Zhang, Z., & Yang, G. Y. (2019). Significance of complement system in
 901 ischemic stroke: A comprehensive review. *Aging and Disease*, 10(2), 429–462.
 902 <https://doi.org/10.14336/AD.2019.0119>

903 Mantovani, S., Gordon, R., Macmaw, J. K., Pfluger, C. M. M., Henderson, R. D., Noakes, P.
 904 G., McCombe, P. A., & Woodruff, T. M. (2014). Elevation of the terminal complement
 905 activation products C5a and C5b-9 in ALS patient blood. *Journal of Neuroimmunology*,
 906 276(1–2), 213–218. <https://doi.org/10.1016/j.jneuroim.2014.09.005>

907 McCartney, D. L., Hillary, R. F., Stevenson, A. J., Ritchie, S. J., Walker, R. M., Zhang, Q.,
 908 Morris, S. W., Bermingham, M. L., Campbell, A., Murray, A. D., Whalley, H. C., Gale,
 909 C. R., Porteous, D. J., Haley, C. S., McRae, A. F., Wray, N. R., Visscher, P. M.,
 910 McIntosh, A. M., Evans, K. L., ... Marioni, R. E. (2018). Epigenetic prediction of
 911 complex traits and death. *Genome Biology*, 19(1), 136.

912 McCartney, D. L., Min, J. L., Richmond, R. C., Lu, A. T., Sobczyk, M. K., Davies, G., Broer,
 913 L., Guo, X., Jeong, A., Jung, J., Kasela, S., Katrinli, S., Kuo, P. L., Matias-Garcia, P. R.,
 914 Mishra, P. P., Nygaard, M., Palviainen, T., Patki, A., Raffield, L. M., ... Marioni, R. E.
 915 (2020). Genome-wide association studies identify 137 loci for DNA methylation
 916 biomarkers of ageing. *BioRxiv*. <https://doi.org/10.1101/2020.06.29.133702>

917 McCartney, D. L., Stevenson, A. J., Hillary, R. F., Walker, R. M., Bermingham, M. L.,
 918 Morris, S. W., Clarke, T. K., Campbell, A., Murray, A. D., Whalley, H. C., Porteous, D.
 919 J., Visscher, P. M., McIntosh, A. M., Evans, K. L., Deary, I. J., & Marioni, R. E. (2018).
 920 Epigenetic signatures of starting and stopping smoking. *EBioMedicine*, 37, 214–220.
 921 <https://doi.org/10.1016/j.ebiom.2018.10.051>

922 McCartney, D. L., Stevenson, A. J., Walker, R. M., Gibson, J., Morris, S. W., Campbell, A.,
 923 Murray, A. D., Whalley, H. C., Porteous, D. J., McIntosh, A. M., Evans, K. L., Deary, I.

- J., & Marioni, R. E. (2018). Investigating the relationship between DNA methylation age acceleration and risk factors for Alzheimer's disease. *Alzheimer's and Dementia: Diagnosis, Assessment and Disease Monitoring*, 10, 429–437.
<https://doi.org/10.1016/j.dadm.2018.05.006>
- Messner, C. B., Demichev, V., Wendisch, D., Michalick, L., White, M., Freiwald, A., Textoris-Taube, K., Vernardis, S. I., Egger, A. S., Kreidl, M., Ludwig, D., Kilian, C., Agostini, F., Zelezniak, A., Thibeault, C., Pfeiffer, M., Hippenstiel, S., Hocke, A., von Kalle, C., ... Ralser, M. (2020). Ultra-High-Throughput Clinical Proteomics Reveals Classifiers of COVID-19 Infection. *Cell Systems*, 11(1), 11-24.e4.
<https://doi.org/10.1016/j.cels.2020.05.012>
- Min, J. L., Hemani, G., Hannon, E., Dekkers, K. F., Castillo-Fernandez, J., Luijk, R., Carnero-Montoro, E., Lawson, D. J., Burrows, K., Suderman, M., Bretherick, A. D., Richardson, T. G., Klughammer, J., Iotchkova, V., Sharp, G., Khleifat, A. Al, Shatunov, A., Iacoangeli, A., McArdle, W. L., ... Relton, C. L. (2020). Genomic and phenomic insights from an atlas of genetic effects on DNA methylation. *MedRxiv*.
<https://doi.org/10.1101/2020.09.01.20180406>
- Moldoveanu, A. I., Shephard, R. J., & Shek, P. N. (2000). Exercise elevates plasma levels but not gene expression of IL-1 β , IL-6, and TNF- α in blood mononuclear cells. *Journal of Applied Physiology*, 89(4), 1499–1504.
<https://doi.org/10.1152/JAPPL.2000.89.4.1499>/ASSET/IMAGES/LARGE/DG1000209003.JPEG
- Morgan, B. P., & Harris, C. L. (2015). Complement, a target for therapy in inflammatory and degenerative diseases. *Nature Reviews Drug Discovery*, 14, 857–877.
<https://doi.org/10.1038/nrd4657>

948 MRC-IEU. (2021). *The MRC-IEU catalog of epigenome-wide association studies. Available*
949 *at: <http://www.ewascatalog.org>. Accessed April 2021.*

950 Navrady, L. B., Wolters, M. K., MacIntyre, D. J., Clarke, T. K., Campbell, A. I., Murray, A.
951 D., Evans, K. L., Seck, J., Haley, C., Milburn, K., Wardlaw, J. M., Porteous, D. J.,
952 Deary, I. J., & McIntosh, A. M. (2018). Cohort profile: Stratifying Resilience and
953 Depression Longitudinally (STRADL): A questionnaire follow-up of Generation
954 Scotland: Scottish Family Health Study (GS: SFHS). *International Journal of*
955 *Epidemiology*, 47(1), 13-14g. <https://doi.org/10.1093/ije/dyx115>

956 Ngo, D., Benson, M. D., Long, J. Z., Chen, Z. Z., Wang, R., Nath, A. K., Keyes, M. J., Shen,
957 D., Sinha, S., Kuhn, E., Morningstar, J. E., Shi, X., Peterson, B. D., Chan, C., Katz, D.
958 H., Tahir, U. A., Farrell, L. A., Melander, O., Mosley, J. D., ... Gerszten, R. E. (2021).
959 Proteomic profiling reveals biomarkers and pathways in type 2 diabetes risk. *JCI*
960 *Insight*, 6(5), e144392. <https://doi.org/10.1172/JCI.INSIGHT.144392>

961 NHS England. (2016). *Improving Outcomes Through Personalised Medicine.*
962 [https://www.england.nhs.uk/wp-content/uploads/2016/09/improving-outcomes-](https://www.england.nhs.uk/wp-content/uploads/2016/09/improving-outcomes-personalised-medicine.pdf)
963 [personalised-medicine.pdf](https://www.england.nhs.uk/wp-content/uploads/2016/09/improving-outcomes-personalised-medicine.pdf). Accessed April 2021.

964 Ort, M., Dingemanse, J., van den Anker, J., & Kaufmann, P. (2020). Treatment of Rare
965 Inflammatory Kidney Diseases: Drugs Targeting the Terminal Complement Pathway. In
966 *Frontiers in Immunology* (Vol. 11). Frontiers Media S.A.
967 <https://doi.org/10.3389/fimmu.2020.599417>

968 Park, M. H., Jin, H. K., & Bae, J. sung. (2020). Potential therapeutic target for aging and age-
969 related neurodegenerative diseases: the role of acid sphingomyelinase. *Experimental and*
970 *Molecular Medicine*, 52, 380–389. <https://doi.org/10.1038/s12276-020-0399-8>

971 Pedersen, T. L. (2021). *ggraph: An Implementation of Grammar of Graphics for Graphs and*

972 *Networks. R package version 2.0.5. <https://CRAN.R-project.org/package=ggraph>.*

973 *Accessed April 2021.*

974 Pérez-Pérez, R., García-Santos, E., Ortega-Delgado, F. J., López, J. A., Camafeita, E., Ricart,

975 W., Fernández-Real, J. M., & Peral, B. (2012). Attenuated metabolism is a hallmark of

976 obesity as revealed by comparative proteomic analysis of human omental adipose tissue.

977 *Journal of Proteomics*, 75(3), 783–795. <https://doi.org/10.1016/j.jprot.2011.09.016>

978 Peters, A., Nawrot, T. S., & Baccarelli, A. A. (2021). Hallmarks of environmental insults.

979 *Cell*, 184(6), 1455–1468. <https://doi.org/10.1016/j.cell.2021.01.043>

980 Petersen, A. K., Zeilinger, S., Kastenmüller, G., Werner, R. M., Brugger, M., Peters, A.,

981 Meisinger, C., Strauch, K., Hengstenberg, C., Pagel, P., Huber, F., Mohny, R. P.,

982 Grallert, H., Illig, T., Adamski, J., Waldenberger, M., Gieger, C., & Suhre, K. (2014).

983 Epigenetics meets metabolomics: An epigenome-wide association study with blood

984 serum metabolic traits. *Human Molecular Genetics*, 23(2), 534–545.

985 <https://doi.org/10.1093/hmg/ddt430>

986 Pietzner, M., Wheeler, E., Carrasco-Zanini, J., Raffler, J., Kerrison, N. D., Oerton, E.,

987 Auyeung, V. P. W., Luan, J., Finan, C., Casas, J. P., Ostroff, R., Williams, S. A.,

988 Kastenmüller, G., Ralser, M., Gamazon, E. R., Wareham, N. J., Hingorani, A. D., &

989 Langenberg, C. (2020). Genetic architecture of host proteins involved in SARS-CoV-2

990 infection. *Nature Communications*, 11, 6397. [https://doi.org/10.1038/s41467-020-](https://doi.org/10.1038/s41467-020-19996-z)

991 19996-z

992 R. (2020). *R Core Team. R: A language and environment for statistical computing. R*

993 *Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.*

994 *Accessed April 2021.*

995 Revelle, W. (2020). *psych: Procedures for Personality and Psychological Research*,

996 Northwestern University, Evanston, Illinois, USA. (Version 2.0.9). [https://CRAN.R-](https://CRAN.R-project.org/package=psych)
997 [project.org/package=psych](https://CRAN.R-project.org/package=psych). Accessed April 2021.

998 Saffari, A., Silver, M. J., Zavattari, P., Moi, L., Columbano, A., Meaburn, E. L., &
999 Dudbridge, F. (2017). *Estimation of a significance threshold for epigenome-wide*
1000 *association studies*. 42(1), 22–23.

1001 Safiri, S., Kolahi, A. A., Hoy, D., Smith, E., Bettampadi, D., Mansournia, M. A., Almasi-
1002 Hashiani, A., Ashrafi-Asgarabad, A., Moradi-Lakeh, M., Qorbani, M., Collins, G.,
1003 Woolf, A. D., March, L., & Cross, M. (2019). Global, regional and national burden of
1004 rheumatoid arthritis 1990-2017: a systematic analysis of the Global Burden of Disease
1005 study 2017. *Annals of the Rheumatic Diseases*, 78(11), 1463–1471.
1006 <https://doi.org/10.1136/annrheumdis-2019-215920>

1007 Seeboth, A., McCartney, D. L., Wang, Y., Hillary, R. F., Stevenson, A. J., Walker, R. M.,
1008 Campbell, A., Evans, K. L., McIntosh, A. M., Hägg, S., Deary, I. J., & Marioni, R. E.
1009 (2020). DNA methylation outlier burden, health, and ageing in Generation Scotland and
1010 the Lothian Birth Cohorts of 1921 and 1936. *Clinical Epigenetics*, 12, 49.
1011 <https://doi.org/10.1186/s13148-020-00838-0>

1012 Serban, K. A., Pratte, K. A., & Bowler, R. P. (2021). Protein biomarkers for COPD
1013 outcomes. *Chest*, ISSN 0012-3692. <https://doi.org/10.1016/j.chest.2021.01.004>

1014 Shah, S., McRae, A. F., Marioni, R. E., Harris, S. E., Gibson, J., Henders, A. K., Redmond,
1015 P., Cox, S. R., Pattie, A., Corley, J., Murphy, L., Martin, N. G., Montgomery, G. W.,
1016 Starr, J. M., Wray, N. R., Deary, I. J., & Visscher, P. M. (2014). Genetic and
1017 environmental exposures constrain epigenetic drift over the human life course. *Genome*
1018 *Research*, 24(11), 1725–1733.

1019 Smith, B. H., Campbell, A., Linksted, P., Fitzpatrick, B., Jackson, C., Kerr, S. M., Deary, I.

1020 J., MacIntyre, D. J., Campbell, H., McGilchrist, M., Hocking, L. J., Wisely, L., Ford, I.,
 1021 Lindsay, R. S., Morton, R., Palmer, C. N. A., Dominiczak, A. F., Porteous, D. J., &
 1022 Morris, A. D. (2013). Cohort profile: Generation scotland: Scottish family health study
 1023 (GS: SFHS). The study, its participants and their potential for genetic research on health
 1024 and illness. *International Journal of Epidemiology*, 42(3), 689–700.
 1025 Stevenson, A. J., Gadd, D. A., Hillary, R. F., McCartney, D. L., Campbell, A., Walker, R. M.,
 1026 Evans, K. L., Harris, S. E., Spires-Jones, T. L., McRae, A. F., Visscher, P. M.,
 1027 McIntosh, A. M., Deary, I. J., & Marioni, R. E. (2021). Creating and validating a DNA
 1028 methylation-based proxy for interleukin-6. *Gerontol A Biol Sci Med Sci.*, 17:glab046.
 1029 <https://doi.org/10.1093/gerona/glab046>.
 1030 Stevenson, A., McCartney, D., Hillary, R., Campbell, A., Morris, S., Bermingham, M.,
 1031 Walker, R., Evans, K., Boutin, T., Hayward, C., McRae, A., McColl, B., Spires-Jones,
 1032 T., McIntosh, A., Deary, I., & Marioni, R. (2020). Characterisation of an inflammation-
 1033 related epigenetic score and its association with cognitive ability. *Clinical Epigenetics*,
 1034 12, 113. <https://doi.org/10.1101/802009>
 1035 Suhre, K., Arnold, M., Bhagwat, A. M., Cotton, R. J., Engelke, R., Raffler, J., Sarwath, H.,
 1036 Thareja, G., Wahl, A., Delisle, R. K., Gold, L., Pezer, M., Lauc, G., Selim, M. A. E. D.,
 1037 Mook-Kanamori, D. O., Al-Dous, E. K., Mohamoud, Y. A., Malek, J., Strauch, K., ...
 1038 Graumann, J. (2017). Connecting genetic risk to disease end points through the human
 1039 blood plasma proteome. *Nature Communications*, 8, 14357.
 1040 <https://doi.org/10.1038/ncomms14357>
 1041 Sun, B. B., Maranville, J. C., Peters, J. E., Stacey, D., Staley, J. R., Blackshaw, J., Burgess,
 1042 S., Jiang, T., Paige, E., Surendran, P., Oliver-Williams, C., Kamat, M. A., Prins, B. P.,
 1043 Wilcox, S. K., Zimmerman, E. S., Chi, A., Bansal, N., Spain, S. L., Wood, A. M., ...

1044 Butterworth, A. S. (2018). Genomic atlas of the human plasma proteome. *Nature*, 558,
1045 73–79. <https://doi.org/10.1038/s41586-018-0175-2>

1046 Taylor, A. M., Pattie, A., & Deary, I. J. (2018). Cohort profile update: The Lothian birth
1047 cohorts of 1921 and 1936. *International Journal of Epidemiology*, 47(4), 1042r.

1048 Therneau, T. M. (2020a). *A Package for Survival Analysis in R. R package version 3.2-7*,
1049 <https://CRAN.R-project.org/package=survival>. Accessed April 2021.

1050 Therneau, T. M. (2020b). *coxme: Mixed Effects Cox Models. R package version 2.2-16*.
1051 <https://CRAN.R-project.org/package=coxme>. Accessed April 2021.

1052 Trejo Banos, D., McCartney, D. L., Patxot, M., Anchieri, L., Battram, T., Christiansen, C.,
1053 Costeira, R., Walker, R. M., Morris, S. W., Campbell, A., Zhang, Q., Porteous, D. J.,
1054 McRae, A. F., Wray, N. R., Visscher, P. M., Haley, C. S., Evans, K. L., Deary, I. J.,
1055 McIntosh, A. M., ... Robinson, M. R. (2020). Bayesian reassessment of the epigenetic
1056 architecture of complex traits. *Nature Communications*, 11.

1057 Watanabe, K., Taskesen, E., Van Bochoven, A., & Posthuma, D. (2017). Functional mapping
1058 and annotation of genetic associations with FUMA. *Nature Communications* 2017 8:1,
1059 8(1), 1–11. <https://doi.org/10.1038/s41467-017-01261-5>

1060 Williamson, E. J., Walker, A. J., Bhaskaran, K., Bacon, S., Bates, C., Morton, C. E., Curtis,
1061 H. J., Mehrkar, A., Evans, D., Inglesby, P., Cockburn, J., McDonald, H. I., MacKenna,
1062 B., Tomlinson, L., Douglas, I. J., Rentsch, C. T., Mathur, R., Wong, A. Y. S., Grieve,
1063 R., ... Goldacre, B. (2020). Factors associated with COVID-19-related death using
1064 OpenSAFELY. *Nature* 2020 584:7821, 584(7821), 430–436.
1065 <https://doi.org/10.1038/s41586-020-2521-4>

1066 World Health Organization. (2018). *Global Health Estimates 2016: Deaths by Cause, Age,*
1067 *Sex, by Country and by Region, 2000-2016*.

Yao, C., Chen, G., Song, C., Keefe, J., Mendelson, M., Huan, T., Sun, B. B., Laser, A.,
 Maranville, J. C., Wu, H., Ho, J. E., Courchesne, P., Lyass, A., Larson, M. G., Gieger,
 C., Graumann, J., Johnson, A. D., Danesh, J., Runz, H., ... Levy, D. (2018). Genome-
 wide mapping of plasma protein QTLs identifies putatively causal genes and pathways
 for cardiovascular disease. *Nature Communications*, 9, 3268.

Zaghlool, S. B., Kühnel, B., Elhadad, M. A., Kader, S., Halama, A., Thareja, G., Engelke, R.,
 Sarwath, H., Al-Dous, E. K., Mohamoud, Y. A., Meitinger, T., Wilson, R., Strauch, K.,
 Peters, A., Mook-Kanamori, D. O., Graumann, J., Malek, J. A., Gieger, C.,
 Waldenberger, M., & Suhre, K. (2020). Epigenetics meets proteomics in an epigenome-
 wide association study with circulating blood plasma protein traits. *Nature*
Communications, 11(1), 15. <https://doi.org/10.1038/s41467-019-13831-w>

Zhang, F., Chen, W., Zhu, Z., Zhang, Q., Nabais, M. F., Qi, T., Deary, I. J., Wray, N. R.,
 Visscher, P. M., McRae, A. F., & Yang, J. (2019). OSCA: A tool for omic-data-based
 complex trait analysis. *Genome Biology*, 20(107). <https://doi.org/10.1186/s13059-019-1718-z>

Zhang, Q., Marioni, R. E., Robinson, M. R., Higham, J., Sproul, D., Wray, N. R., Deary, I. J.,
 McRae, A. F., & Visscher, P. M. (2018). Genotype effects contribute to variation in
 longitudinal methylome patterns in older people. *Genome Medicine*, 10, 75.

Zhang, Y., Wilson, R., Heiss, J., Breitling, L. P., Saum, K. U., Schöttker, B., Holleczeck, B.,
 Waldenberger, M., Peters, A., & Brenner, H. (2017). DNA methylation signatures in
 peripheral blood strongly predict all-cause mortality. *Nature Communications*, 6, 10192.

Additional files

Supplementary file 1

- 1092 (A) Demographic and array information for the cohorts and samples used in the study.
- 1093 (B) SomaScan panel performance in the STRADL test set.
- 1094 (C) Performance of Olink protein EpiScores in holdout, STRADL and LBC1921 test sets.
- 1095 (D) Annotations for the proteins corresponding to the 109 selected EpiScores.
- 1096 (E) 1E. FUMA canonical pathway GeneSet enrichment results.
- 1097 (F) Predictor weights for the 109 selected EpiScores.
- 1098 (G) CpG feature counts for the 109 selected EpiScores.
- 1099 (H) Frequency of CpG sites selected for EpiScores with EWAS catalogue annotations to
- 1100 phenotypic traits.
- 1101 (I) Basic Cox model results in Generation Scotland.
- 1102 (J) Fully-adjusted and sensitivity analyses results for Cox models in Generation Scotland.
- 1103 (K) Schoenfeld residual Cox sensitivity analyses.
- 1104 (L) Schoenfeld residual Cox sensitivity analyses split by year of follow-up.
- 1105 (M) SOMAScan – EpiScore diabetes association lookup against two large-scale plasma
- 1106 protein – diabetes studies.
- 1107 (N) White blood cell sensitivity analyses.
- 1108 (O) GrimAge sensitivity analyses.
- 1109 (P) COVID-19 analyses. (Q-1B1 Primary and secondary health codes for each of the 12
- 1110 morbidities in this study that were used to assign case/control status of participants.

1111

1112 **Figure supplements**

1113 **Figure 2 – figure supplement 1. Correlation heatmap for protein EpiScore measures in**
 1114 **Generation Scotland.** Correlation heatmap for EpiScore measures projected into Generation
 1115 Scotland (N=9,537) for the 109 protein EpiScores selected in the test sample ($r > 0.1$, $P <$

0.05). At the top of the heatmap, an annotation bar is displayed. Olink® proteins are shown in pale green and Somalogic proteins are shown in purple.

Figure 2 – figure supplement 2. Phenotypic trait and estimated white blood cell proportion correlations with EpiScores. Heatmap of correlations between the 78 protein EpiScore measures for Olink® proteins which were associated with incident disease at $P < 0.05$ in the fully-adjusted Cox mixed effects proportional hazards models and continuous phenotypic/lifestyle trait variables and Houseman-estimated white blood cell proportions in Generation Scotland (total $N=9,357$). Protein measurements used to train the predictors were adjusted for age and sex. The maximum sample size available was used for each correlation. GrimAge: GrimAge acceleration. Units: weekly units of alcohol. EpiSmoker: DNAm-derived score for smoking. SIMD: Scottish Index of Multiple Deprivation. EA: educational attainment. Mono: Monocytes. Gran: Granulocytes. NK: Natural Killer cells.

Figure 2 – figure supplement 3. GeneSet enrichment of canonical pathways common to the genes encoding proteins used to train the 109 robust EpiScores. Genes selected for pathway enrichment (FDR adjusted $P < 0.05$) are summarised, with the proportion of overlapping genes enriched in the gene set also shown. The corresponding data for this figure can be accessed in full in **Supplementary file 1E**.