Epigenetic scores for the circulating proteome as tools for disease prediction

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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

70 mortality and disease onset, providing strong evidence to suggest that methylation is an
71 important measure of disease risk (Hillary, Stevenson, et al., 2020; Lu et al., 2019; Y. Zhang
72 et al., 2017). DNAm can regulate gene transcription (Lea et al., 2018), and epigenetic
73 differences can be reflected in the variability of the proteome (Hillary et al., 2019; Hillary,
74 Trejo-Banos, et al., 2020; Zaghlool et al., 2020). Low-grade inflammation, which is thought to
75 exacerbate many age-related morbidities, is particularly well-captured through DNAm studies
76 of plasma protein levels (Zaghlool et al., 2020). Connecting the epigenome, proteome and time
77 to disease onset may help to identify predictive biological signatures.

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79 Epigenetic predictors have utilised DNAm from the blood to estimate a person's 'biological
80 age' (Lu et al., 2019), measure their exposure to lifestyle and environmental exposures
81 (McCartney, Hillary, et al., 2018; McCartney, Stevenson, Hillary, et al., 2018; Peters et al.,
82 2021) and predict circulating levels of inflammatory proteins (A. Stevenson et al., 2020; A. J.
83 Stevenson et al., 2021). A leading epigenetic predictor of biological aging, the GrimAge
84 epigenetic clock incorporates methylation scores for seven proteins along with smoking and
85 chronological age, and is associated with numerous incident disease outcomes (Hillary,
86 Stevenson, et al., 2020; Lu et al., 2019). This suggests that there is predictive value in utilising
87 DNAm relevant to protein levels for disease predictions. A portfolio of protein EpiScores
88 across the circulating proteome may aid in the prediction of disease and may offer a
89 complementary signal to that of composite scores. Generation of an extensive range of
90 proteomic scores has not been attempted to date. The capability of specific protein scores to
91 predict a range of morbidities has also not been tested. However, DNAm scores for Interleukin-
92 6 and C-Reactive protein have been found to associate with a range of phenotypes
93 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 reflecting a more consistent profile of stress in the body than protein measurements.

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. Finally, we 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 regressed out the effects of age on protein levels prior to training and testing; age was also included as a covariate in the time-to-event disease prediction models. We controlled for common risk factors for disease and assessed the capacity of EpiScores to identify previously reported protein-disease associations.

	Basic model			Fully-adjusted model		
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	1494	5341	5.2 (3.5)	1221	4475	5.3 (3.5)

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

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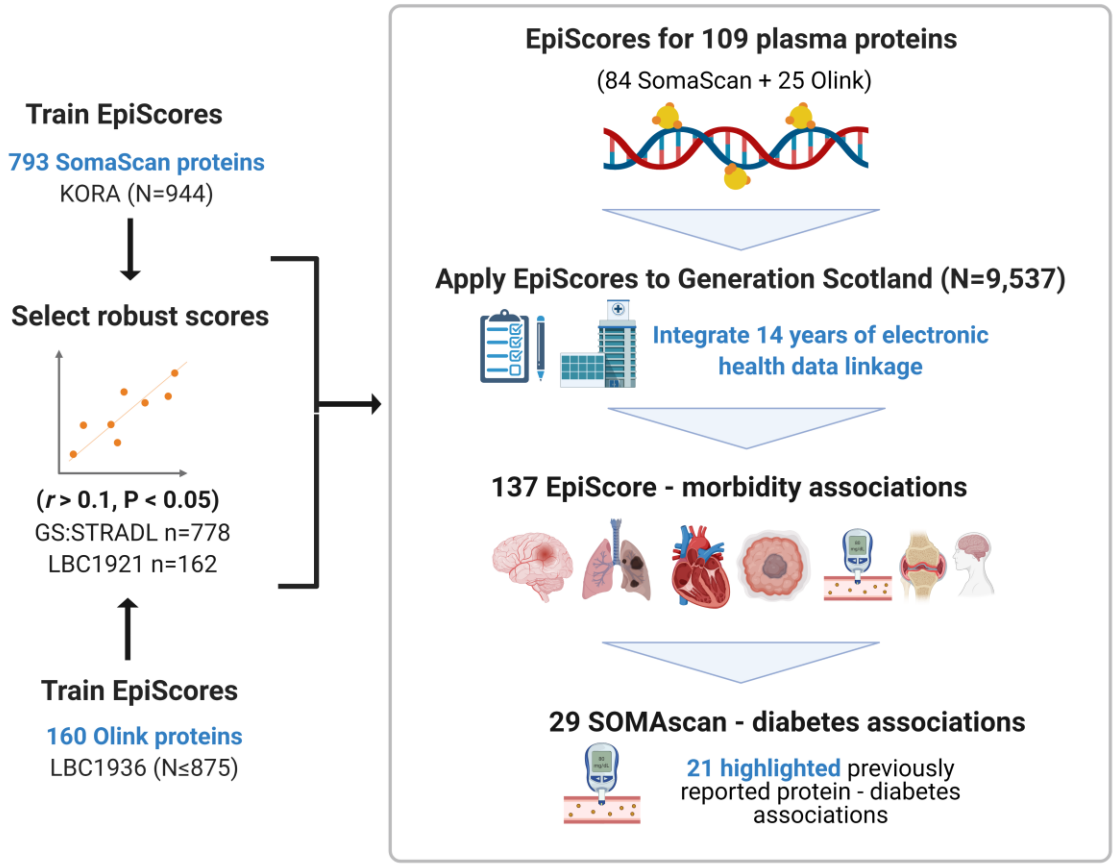

Figure 1. EpiScores for plasma proteins as tools for disease prediction study design. DNA methylation scores were trained on 953 circulating plasma protein levels in the KORA and LBC1936 cohorts. There were 109 EpiScores selected based on performance ($r > 0.1$, $P < 0.05$) in independent test sets. 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, 21 reflected highlighted previously reported protein - diabetes associations.

153 # Results

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155 **Selecting the most robust EpiScores for protein levels**

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

166 Prior to running the elastic net models, we rank-based inverse normalised protein levels and
167 adjusted for age, sex, cohort-specific variables and, where present, *cis* and *trans* pQTL effects
168 identified from previous analyses (Hillary et al., 2019; Hillary, Trejo-Banos, et al., 2020; Suhre
169 et al., 2017) (**Methods**). Of a possible 793 proteins in KORA, 84 EpiScores had Pearson $r >$
170 0.1 and $P < 0.05$ when tested in an independent subset of Generation Scotland (The Stratifying
171 Resilience and Depression Longitudinally [STRADL] study, n=778) (**Supplementary file 1B**).
172 These EpiScores were selected for EpiScore-disease analyses. Of the 160 Olink proteins trained
173 in LBC1936, there were 21 with $r > 0.1$ and $P < 0.05$ in independent test sets (STRADL,
174 n=778, Lothian Birth Cohort 1921: LBC1921, n=162) (**Supplementary file 1C**). Independent
175 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 1E**. The number of CpG features selected for EpiScores ranged from one (Lyzozyme) to 395 (Aminoacylase-1), with a mean of 96 **Supplementary file 1F**). 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 1G.** 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).

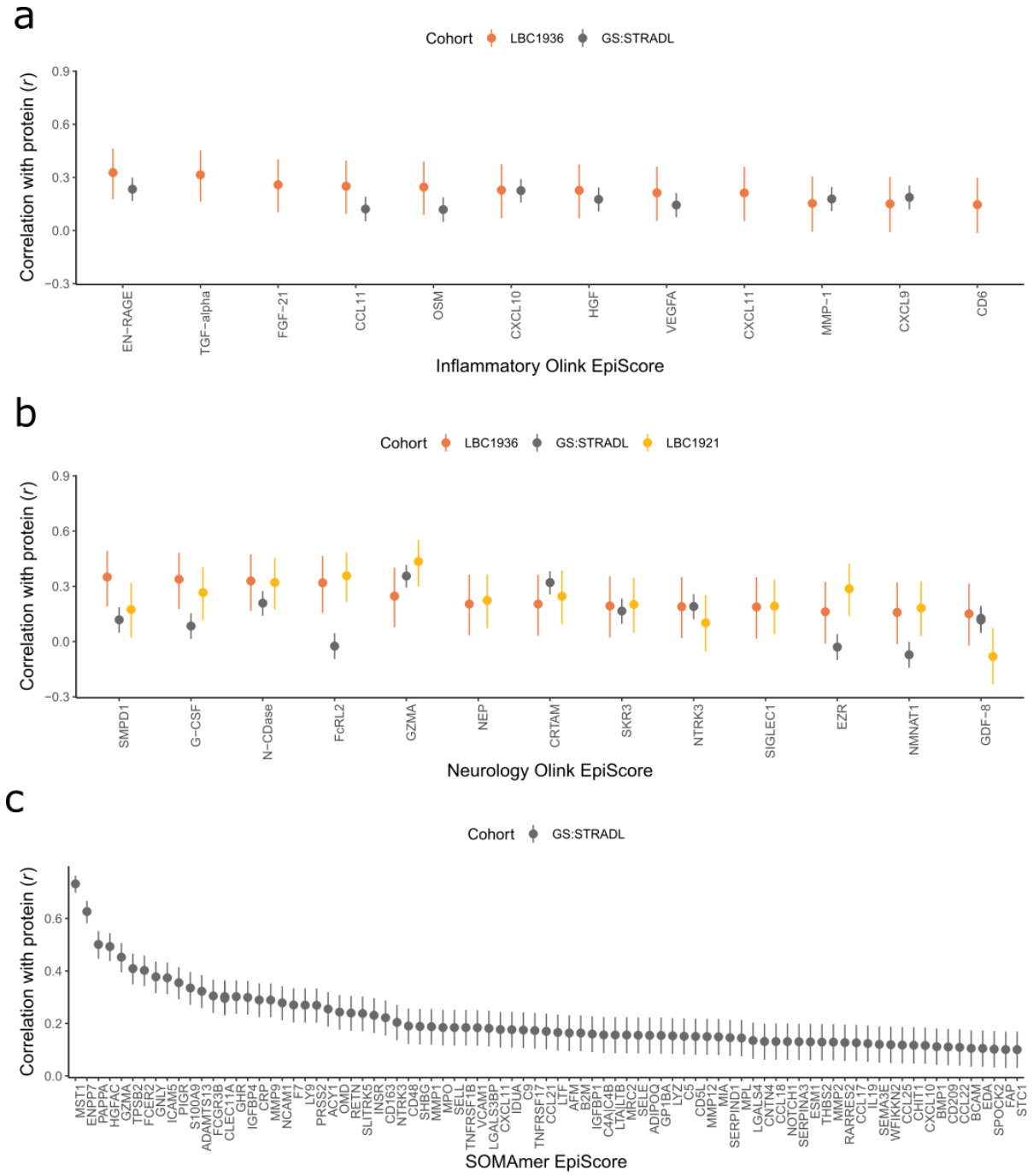

Figure 2. Test performance for the 109 selected protein EpiScores. Test set correlation coefficients for associations between protein EpiScores for (**a**) inflammatory Olink, (**b**) neurology Olink and (**c**) SOMAmer protein panel EpiScores and measured protein levels are plotted. Upper and lower 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**.

206 **EpiScore-disease associations in Generation Scotland**

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208 The Generation Scotland dataset contains extensive electronic health data from GP and hospital
209 records available as well as DNA methylation data for 9,537 individuals. This makes it
210 uniquely positioned to test whether EpiScore signals can predict disease onset. We ran nested
211 mixed effects Cox proportional hazards models (**Figure 3**) to determine whether the levels of
212 each EpiScore at baseline associated with the incidence of 12 morbidities over a maximum of
213 14 years of follow up. The correlation structures for the 109 EpiScore measures used for Cox
214 modelling are presented in **Supplementary file 2A**.

215 The Cox proportional hazard assumption dictates that hazard ratios for EpiScore – disease
216 associations should remain constant over time. We correlated the Schoenfeld residuals from
217 the models with time to test this. Two associations in the basic model adjusting for age and sex
218 failed to satisfy the global assumption (across all covariates) and were excluded. There were
219 294 remaining EpiScore-disease associations with a False Discovery Rate (FDR)-adjusted $P <$
220 0.05 in the basic model. After further adjustment for common risk factor covariates (smoking,
221 social deprivation status, educational attainment, body mass index (BMI) and alcohol
222 consumption), 137 of the 294 EpiScore-disease associations from the basic model had $P < 0.05$
223 in the fully-adjusted model (**Supplementary files 1H-I**). Eleven of the 137 fully-adjusted
224 associations failed the Cox proportional hazards assumption for the EpiScore variable ($P < 0.05$
225 for the association between the Schoenfeld residuals and time; **Supplementary file 1J**). When
226 we restricted the time-to-event/censor period by each year of possible follow-up, there were
227 minimal differences in the EpiScore - disease hazard ratios between follow-up periods that did
228 not violate the assumption and those that did (**Supplementary file 1K**). The 137 associations
229 were therefore retained as the primary results.

230 The 137 associations found in the fully-adjusted model comprised 78 unique EpiScores that
231 were related to the incidence of 11 of the 12 morbidities studied. Diabetes and chronic
232 obstructive pulmonary disease (COPD) had the greatest number of associations, with 33 and
233 41, respectively. **Figure 4** presents the EpiScore-disease relationships for COPD and the
234 remaining nine morbidities: stroke, lung cancer, ischaemic heart disease, inflammatory bowel
235 disease, rheumatoid arthritis, depression, bowel cancer, pain and Alzheimer's dementia. There
236 were 13 EpiScores that associated with the onset of three or more morbidities. **Figure 5**
237 presents relationships for these 13 EpiScores in the fully-adjusted Cox model results. Of note
238 is the EpiScore for Complement 5 (C5), which associated with five outcomes: stroke, diabetes,
239 ischaemic heart disease, rheumatoid arthritis and COPD. Of the 29 SOMAscan-derived
240 EpiScore associations with incident diabetes, 21 replicated previously reported protein
241 associations (Elhadad et al., 2020; Gudmundsdottir et al., 2020) with incident or prevalent
242 diabetes in one or more cohorts (**Figure 6 and Supplementary file 1L**).

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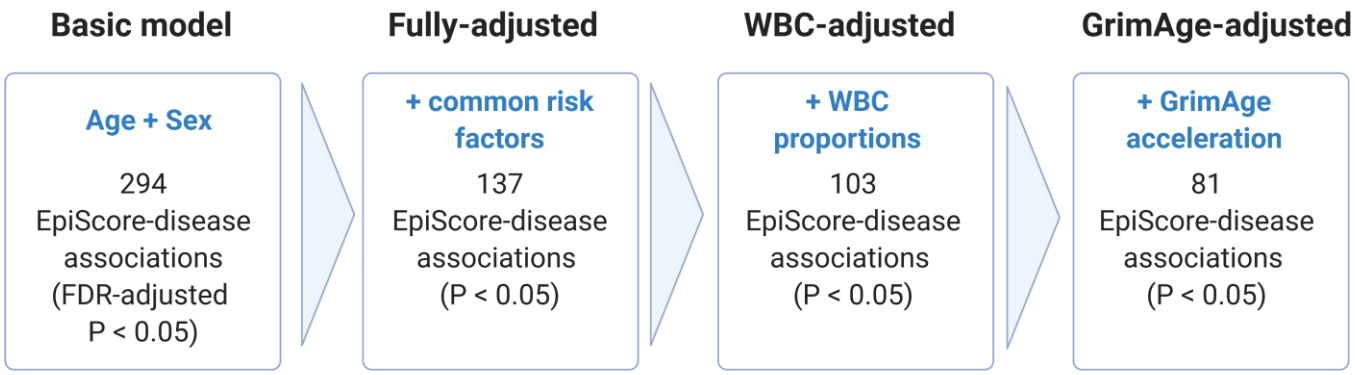

Figure 3. Nested Cox proportional hazards assessment of EpiScore-disease prediction. Mixed effects Cox proportional hazards analyses in Generation Scotland (n = 9,537) tested the relationships between each of the 109 selected EpiScores and the incidence of 12 leading causes of morbidity **(Supplementary files 1H-I)**. 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.

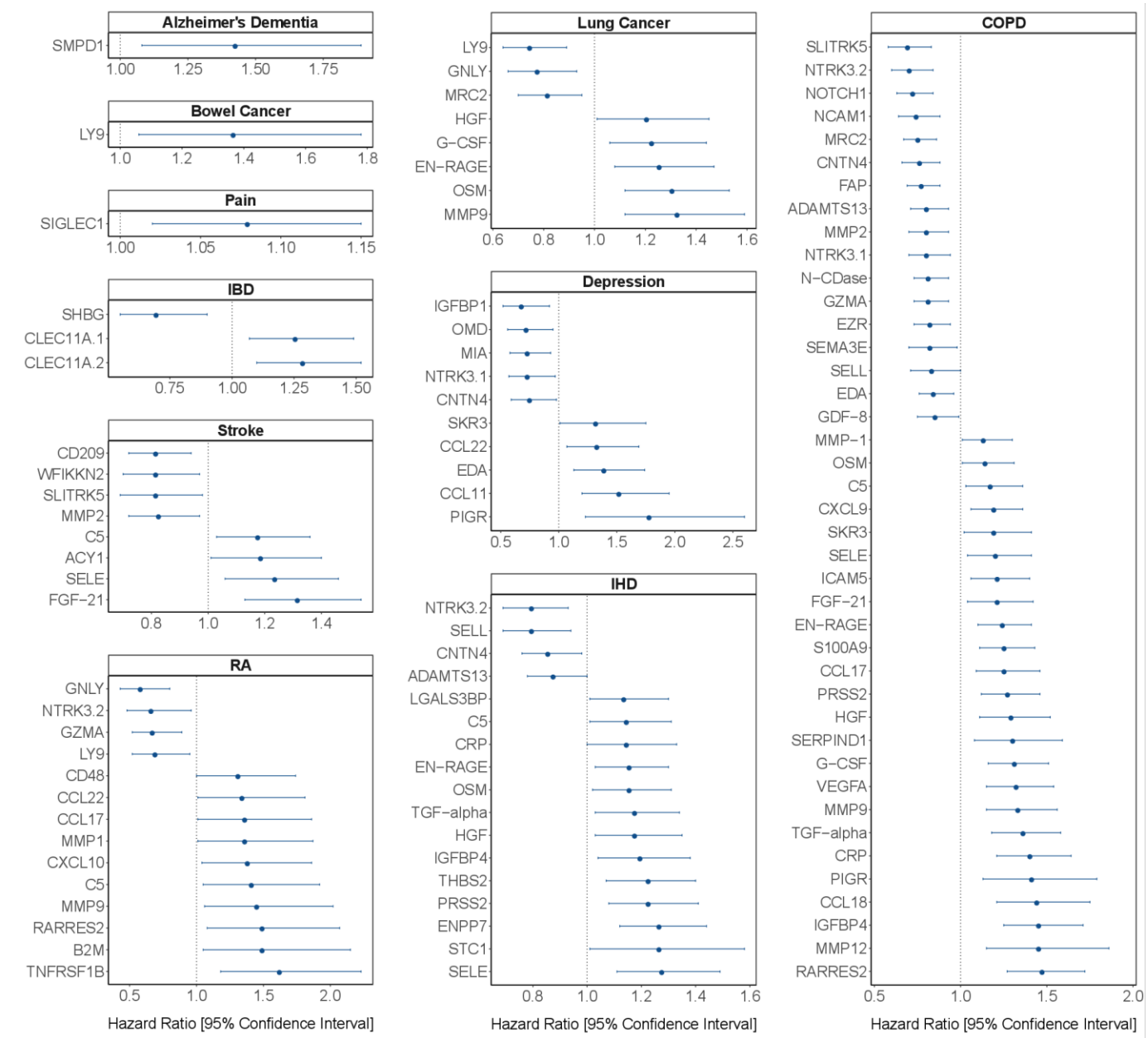

Figure 4. EpiScore associations with incident disease. EpiScore-disease associations for ten of the eleven morbidities with associations where $P < 0.05$ in the fully-adjusted mixed effects Cox proportional hazards models in Generation Scotland (n=9,537). Hazard ratios are presented with confidence intervals 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 1I**.

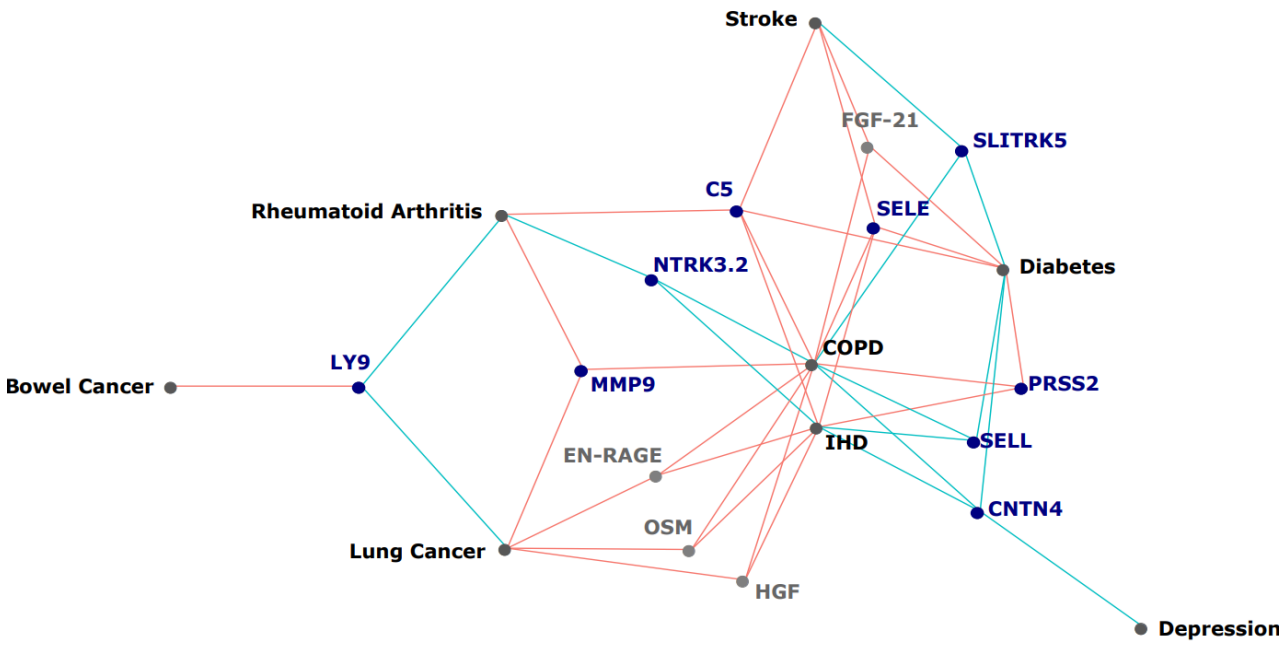

Figure 5. **EpiScores that associated with the greatest number of morbidities.** EpiScores with a minimum of three relationships with incident morbidities in the fully-adjusted Cox models. The network includes 13 EpiScores as dark blue (SOMAscan) and grey (Olink) nodes, with disease outcomes in black. 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 1I**.

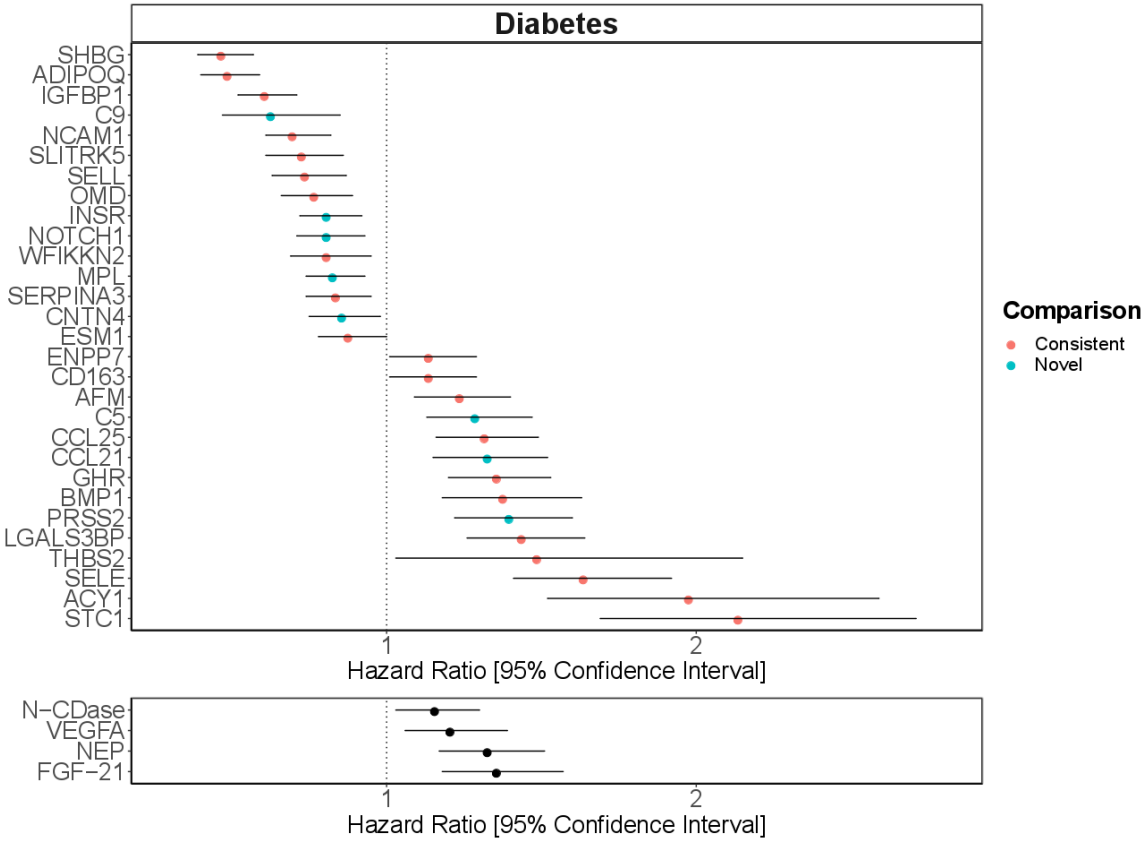

Figure 6. Replication of known protein-diabetes associations with EpiScores. EpiScore – incident diabetes associations in Generation Scotland (n=9,537). The 29 SOMAscan (top panel) and four Olink (bottom panel) associations shown with $P < 0.05$ in fully-adjusted mixed effects Cox proportional hazards models. Of the 29 SOMAscan-derived EpiScores, 21 associations were consistent with protein – diabetes associations (pink) in one or more of the four comparison cohorts that used SOMAscan protein levels. Eight associations were novel (blue). Data shown corresponds to the results included in **Supplementary files 1I and 1L** .

306 **Immune cell and GrimAge sensitivity analyses**

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308 Correlations of the 109 EpiScores with covariates suggested interlinked relationships with both
309 immune cells and GrimAge acceleration (**Supplementary file 2B)**. These covariates were
310 therefore added incrementally to the fully-adjusted Cox models (**Figure 3**). There were 103
311 associations that remained statistically significant (FDR $P < 0.05$ in the basic model and $P <$
312 0.05 in the fully-adjusted model) after adjustment for immune cell proportions, of which 81
313 remained significant when GrimAge acceleration scores were added to this model
314 **(Supplementary file 1I)**. In a further sensitivity analysis, relationships between both estimated
315 White Blood Cell (WBC) proportions and GrimAge acceleration scores with incident diseases
316 were assessed in the Cox model structure independently of EpiScores. Of the 60 possible
317 relationships between WBC measures and the morbidities assessed, four were statistically
318 significant (FDR-adjusted $P < 0.05$) in the basic model and remained significant with $P < 0.05$
319 in the fully-adjusted model **(Supplementary file 1M**). A higher proportion of Natural Killer
320 cells was linked to decreased risk of incident COPD, rheumatoid arthritis, diabetes and pain.
321 The GrimAge acceleration composite score was associated with COPD, IHD, Diabetes and
322 Pain in the fully-adjusted models ($P < 0.05$) (**Supplementary file 1N**). The magnitude of the
323 GrimAge effect sizes were comparable to the EpiScore findings.

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328 ## Discussion

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330 Here, we report a comprehensive DNA methylation scoring study of 953 circulating proteins.
331 We define 109 robust EpiScores for plasma protein levels that are independent of known pQTL
332 effects. By projecting these EpiScores into a large cohort with extant data linkage, we show
333 that 78 EpiScores associate with the incidence of 11 leading causes of morbidity (137 EpiScore
334 – disease associations in total). Finally, we show that EpiScore - disease associations highlight
335 previously measured protein - disease relationships. The bulk of EpiScore-disease associations
336 are independent of common lifestyle and health factors, differences in immune cell
337 composition and GrimAge acceleration. EpiScores therefore provide methylation-proteomic
338 signatures for disease prediction and risk stratification.

339

340 The consistency between our EpiScore – diabetes associations and previously identified protein
341 – diabetes relationships (Elhadad et al., 2020; Gudmundsdottir et al., 2020) suggests that
342 epigenetic scores may identify candidate disease-protein pathways. In addition to the
343 comprehensive lookup of SOMAscan proteins with diabetes, several of the markers we
344 identified for COPD and IHD also reflect previous associations with measured proteins (Ganz
345 et al., 2016; Serban et al., 2021). The two studies used for the diabetes comparison represent
346 the largest candidate protein characterisations of diabetes to date and the top markers identified
347 included aminoacylase-1 (ACY-1), sex hormone binding globulin (SHBG), growth hormone
348 receptor (GHR) and Insulin-like growth factor-binding protein 2 (IGFBP-2) (Elhadad et al.,
349 2020; Gudmundsdottir et al., 2020). Our EpiScores for these top markers are also associated
350 with diabetes, in addition to EpiScores for several other protein markers reported in these

351 studies. A growing body of evidence suggests that type 2 diabetes is mediated by genetic and
352 epigenetic regulators (Kwak & Park, 2016) and proteins such as ACY-1 and GHR are thought
353 to influence a range of diabetes-associated metabolic mechanisms (Kim & Park, 2017; Pérez-
354 Pérez et al., 2012). In the case of diabetes, EpiScores may therefore be used as disease-relevant
355 risk biomarkers, many years prior to onset. Validation should be tested when sufficient data
356 become available for the remaining morbidities.

357

358 With modest test set performances (for example, SHBG r = 0.18 and ACY-1 r = 0.25), it is
359 perhaps surprising that such strong synergy is observed between EpiScores for proteins that
360 associated with diabetes and the trends seen with measured proteins. Nonetheless, DNA
361 methylation scores for CRP and IL6 have been shown to perform modestly in test sets ($r \sim 0.2$,
362 equivalent to ~ 4% explained variance in protein level), but augment and often outperform the
363 measured protein related to a range of phenotypes (A. Stevenson et al., 2020; A. J. Stevenson
364 et al., 2021). Upper bounds for DNAm prediction of complex traits, such as proteins, can be
365 estimated by variance components analyses (Hillary, Trejo-Banos, et al., 2020; Trejo Banos et
366 al., 2020; F. Zhang et al., 2019).

367

368 Compared to epigenetic clocks like GrimAge, EpiScores enable the granular study of
369 individual protein predictor signatures with disease outcomes. For example, levels of the acid
370 sphingomyelinase (ASM) EpiScore predicted onset of Alzheimer's dementia, several years
371 prior to diagnosis. ASM (encoded by *SMPD1*) has been discussed as a therapeutic candidate
372 for Alzheimer's disease (Cataldo et al., 2004; Kamil et al., 2016; Lee et al., 2014; Park et al.,
373 2020) and has been shown to disrupt autophagic protein degradation and associate with

374 accumulation of amyloid-beta in murine models of Alzheimer's pathology (Lee et al., 2014;
375 Park et al., 2020). The EpiScore for Complement Component 5 (C5) was associated with the
376 onset of five morbidities, the highest number for any EpiScore. Elevated levels of C5 peptides
377 have been associated with severe inflammatory, autoimmune and neurodegenerative states (Ma
378 et al., 2019; Mantovani et al., 2014; Morgan & Harris, 2015) and a range of C5-targetting
379 therapeutic approaches are in development (Alawieh et al., 2018; Brandolini et al., 2019;
380 Hawksworth et al., 2017; Hernandez et al., 2017; Morgan & Harris, 2015; Ort et al., 2020).
381 EpiScores that occupy central hubs in the disease-prediction framework may therefore provide
382 evidence of early methylation signatures common to the onset of multiple diseases. Our large-
383 scale assessment of EpiScores provides a platform for future studies, as composite predictors
384 may be created using our EpiScore database. These should be tested in incident disease
385 predictions when sufficient case data are available.

386

387 This study has several limitations. First, like with protein – disease association studies, we
388 cannot infer causality from our EpiScore – disease models. However, both protein levels and
389 EpiScores may have utility in risk prediction – future studies where both modalities are
390 available should assess paired protein and CpG contributions to traits. This should entail the
391 direct measurement of proteins, as inference from EpiScores alone, while useful for disease
392 risk stratification, is not sufficient to determine mechanisms. Second, the epitope nature of the
393 protein measurement in the SOMAscan panel may incur probe cross-reactivity and non-
394 specific binding; there may also be differences in how certain proteins are measured across
395 panels (Pietzner et al., 2020; Sun et al., 2018). Comparisons of both protein measurement
396 technologies on the same samples would help to explore this in more detail. Third, there may
397 also be pQTLs with small effect sizes that were not regressed from the proteins prior to

398 generating the EpiScores. Finally, associations present between EpiScore measures and disease
399 incidence may have been influenced by external factors such as prescription medications for
400 comorbid conditions and comorbid disease prevalence.

401

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

413

414 Materials and Methods

415

416 The KORA sample population

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418 The KORA F4 study includes 3,080 participants who reside in Southern Germany. Individuals
419 were between 32 and 81 years of age when recruited to the study from 2006 and 2008. In the

420 current study, there were 944 individuals with methylation, proteomics and genetic data
421 available. The Infinium HumanMethylation450 BeadChip platform was used to generate
422 DNA methylation data for these individuals. The Affymetrix Axiom array was used to
423 generate genotyping data and the SOMAscan platform was used to generate proteomic
424 measurements in the sample.

425

426 **DNA methylation in KORA**

427

428 Methylation data were generated for 1,814 individuals(Petersen et al., 2014); 944 also had
429 protein and genotype measurements available. During preprocessing, 65 SNP probes were
430 excluded and background correction was performed in minfi (Aryee et al., 2014). Samples
431 with a detection rate of less than 95% were excluded. Next, the minfi R package was used to
432 perform normalization on the intensity methylation measures (Aryee et al., 2014), with a
433 method consistent with the Lumi:QN +BMIQ pipeline. After excluding non-cg sites and CpGs
434 on sex chromosomes or with fewer than 100 measures available, 470,837 CpGs were available
435 for analyses.

436

437 **Proteomics in KORA**

438

439 The SOMAscan platform (V3.2) (Gold et al., 2010) was used to quantify protein levels in
440 undepleted plasma for 1129 SOMAmer probes (Suhre et al., 2017). Of the 1,000 samples
441 provided for analysis, two samples were excluded due to errors in bio-bank sampling and one
442 based on quality control measures. Of the 997 samples available, there were 944 individuals

443 with methylation and genotypic data. Of the 1,129 probes available, five failed the QC, leaving
444 a total of 1,124 probes for the subsequent analysis. Protein measurements were transformed
445 by rank-based inverse normalisation and regressed onto age, sex, known pQTLs and 20
446 genetic principal components of ancestry derived from the Affymetrix Axiom Array to control
447 for population structure. pQTLs for each protein were taken from a previous GWAS in the
448 sample (Suhre et al., 2017).

449

450 **The LBC1936 and LBC1921 sample populations**

451

452 The Lothian Birth Cohorts of 1921 (LBC1921; N = 550) and 1936 (LBC1936; N = 1091) are
453 longitudinal studies of aging in individuals who reside in Scotland (Deary et al., 2012; Taylor
454 et al., 2018). Participants completed an intelligence test at age 11 years and were recruited
455 for these cohorts at mean ages of 79 (LBC1921) and 70 (LBC1936). They have been
456 followed up triennially for a series of cognitive, clinical, physical and social data, along with
457 blood donations that have been used for genetic, epigenetic, and proteomic measurement.
458 DNAm, proteomic (Olink® platform) and genetic data for up to 875 individuals from Waves
459 1 and 2 of the LBC1936 (at mean ages 70 and 73 years) and 162 individuals at Wave 3 of the
460 LBC1921 (at mean age 87 years).

461

462 **DNAm in LBC1936 and LBC1921**

463

464 DNA from whole blood was assessed using the Illumina 450 K methylation array. Details of
465 quality control have been described elsewhere (Shah et al., 2014; Q. Zhang et al., 2018). Raw

466 intensity data were background-corrected and normalised using internal controls. Manual
467 inspection resulted in the removal of low quality samples that presented issues related to
468 bisulphite conversion, staining signal, inadequate hybridisation or nucleotide extension. Probes
469 with low detection rate <95% at $P < 0.01$ and samples with low call rates (<450,000 probes
470 detected at $P < 0.01$) were removed. Samples were also removed if they had a poor match
471 between genotype and SNP control probes, or incorrect DNA methylation-predicted sex.

472

473 **Proteomics in LBC1936 and LBC1921**

474

475 Plasma samples were analysed using either the Olink® neurology 92-plex or the Olink®
476 inflammation 92-plex proximity extension assays (Olink® Bioscience, Uppsala Sweden). One
477 inflammatory panel protein (BDNF) failed quality control and was removed. A further 21
478 proteins were removed, as over 40% of samples fell below the lowest limit of detection. Two
479 neurology proteins, MAPT and HAGH, were excluded due to >40% of observations being
480 below the lower limit of detection. This resulted in 90 neurology (LBC1936 Wave 2) and 70
481 inflammatory (LBC1936 Wave 1) proteins in LBC1936 and 92 neurology proteins available in
482 LBC1921. Protein levels were rank-based inverse normalised and regressed onto age, sex, four
483 genetic components of ancestry derived from multidimensional scaling of the Illumina 610-
484 Quadv1 genotype array and Olink® array plate. In LBC1936, pQTLs were adjusted for,
485 through reference to GWAS in the samples (Hillary et al., 2019; Hillary, Trejo-Banos, et al.,
486 2020).

487

488 **Generation Scotland and STRADL sample populations**

489

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

498

499 **DNA methylation in Generation Scotland and STRADL**

500

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

510

511 **Proteomics in STRADL**

512

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

520

521 **Electronic health data linkage in Generation Scotland**

522

523 Over 98% of GS participants consented to allow access to electronic health records via data
524 linkage to GP records (Read 2 codes) and hospital records (ICD codes). Data are available
525 prospectively from the time of blood draw, yielding up to 14 years of linkage. We considered
526 incident data for 12 morbidities (**Supplementary file 3A**). Prevalent cases (ascertained via
527 retrospective ICD and Read 2 codes or self-report from a baseline questionnaire) were
528 excluded. For inflammatory bowel disease (IBD) prevalent cases were excluded based on data
529 linkage alone. Included and excluded terms can be found in **Supplementary files 4A-L**.
530 Alzheimer's dementia was limited to cases/controls with age of event/censoring ≥ 65 years.
531 Breast cancer was restricted to females only. Recurrent, major and moderate episodes of
532 depression were included in depression. Diabetes was comprised of type 2 diabetes and more

533 general diabetes codes such as diabetic retinopathy and diabetes mellitus with renal
534 manifestation. Type 1 and juvenile diabetes cases were excluded.

535

536 **Elastic net protein EpiScores**

537

538 Penalised regression models were generated for 160 proteins in LBC1936 and 793 proteins in
539 KORA using Glmnet (Version 4.0-2) (J et al., 2010) in R (Version 4.0) (R, 2020). Protein levels
540 were the outcome and there were 428,489 CpG features per model in the LBC1936 training
541 and 397,630 in the KORA training. An elastic net penalty was specified (alpha=0.5) and cross
542 validation was applied. DNAm and protein measurements were scaled to have a mean of zero
543 and variance of one.

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

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

557

558 **Associations with health linkage phenotypes in Generation Scotland**

559

560 Mixed effects Cox proportional hazards regression models adjusting for age, sex, and
561 methylation set were used to assess the relationship between 109 EpiScores and 12 morbidities
562 in Generation Scotland. Models were run using coxme (Therneau, 2020b) (Version 2.2-16)
563 with a kinship matrix accounting for relatedness in Set 1. Cases included those diagnosed after
564 baseline who had died, in addition to those who received a diagnosis and remained alive.
565 Controls were censored if disease free at time of death, or at the end of the follow-up period.
566 EpiScore levels were rank-base inverse normalised. Fully-adjusted models included: the
567 following additional covariates measured at baseline: alcohol consumption (units consumed in
568 the previous week); deprivation (assessed by the Scottish Index of Multiple Deprivation
569 (GovScot, 2016)); body mass index (kg/m^2); educational attainment (an 11-category ordinal
570 variable) and a DNAm-based score for smoking status (Bollepalli et al., 2019). A false
571 discovery rate multiple testing correction $P < 0.05$ was applied to the 1306 EpiScore-disease
572 associations (109 EpiScores by 12 incident disease traits, with 2 associations excluded for
573 failing the global proportional hazards assumption). Proportional hazards assumptions were
574 checked through Schoenfeld residuals (global test and a test for the protein-EpiScore variable)
575 using the coxph and cox.zph functions from the survival package (Therneau, 2020a) (Version
576 3.2-7). For each association failing to meet the assumption (Schoenfeld residuals $P < 0.05$), a
577 sensitivity analysis was run across yearly follow-up intervals.

578 Fully-adjusted Cox proportional hazards models were run with Houseman-estimated White
579 Blood Cell (WBC) proportions as covariates (Houseman et al., 2012). A further sensitivity
580 analyses added GrimAge acceleration (Lu et al., 2019) as an additional covariate. Basic and
581 fully-adjusted Cox models were also run with estimated Monocyte, Bcell, CD4T, CD8T and
582 Natural Killer cell proportions as predictors.

583 Correlation structures for EpiScores, DNAm-based white cell proportions and phenotypic
584 information were assessed using Pearson correlations and pheatmap (Kolde, 2019) (Version
585 1.0.12) and ggcorrplot packages (Version 0.1.3) (Kassambara, 2019). The psych package
586 (Version 2.0.9) (Revelle, 2020) was used to perform principal components analysis on
587 EpiScores. A network visualisation was produced using the ggraph package (Version 2.0.5)
588 (Pedersen, 2021). Figures 1 and 2 were created with BioRender.com.

589

590 **Consistency of disease associations between EpiScores and measured proteins**

591

592 Comparisons were conducted between EpiScore – diabetes associations and diabetes
593 associations with measured proteins using two previous large-scale proteomic studies (Elhadad
594 et al., 2020; Gudmundsdottir et al., 2020). In both studies, two cohorts were included (Study 1:
595 KORA n= 993, HUNT n= 940 (Elhadad et al., 2020), Study 2: AGES-Reykjavik n=5,438 and
596 QMDiab n=356 (Gudmundsdottir et al., 2020)). Study 1 included the KORA dataset, which we
597 use in this study to generate SOMAscan EpiScores. We characterised which SOMAscan-based
598 EpiScore – diabetes associations from our fully-adjusted results reflected those observed with
599 measured protein levels. We included basic (nominal $P < 0.05$) and fully adjusted results (with

600 either FDR or Bonferroni-corrected P < 0.05), wherever available, across the four cohorts
601 (**Supplementary file 1L**).
602
603

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665

666 Author contributions

667

668 R.E.M., D.A.G., R.F.H., D.L.Mc.C., S. B. Z., and K. S. were responsible for the conception
669 and design of the study. D.A.G., R.F.H., D.L.Mc.C., and S. B. Z. carried out the data analyses.
670 R.E.M. and D.A.G. drafted the article. C.N., and A.C., facilitated data linkage. R.M.W., S.E.H.,
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672 and A.M.M., contributed to data collection and preparation. All authors read and approved the
673 final manuscript.

674

675 **Ethics**

676

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

679 All components of GS received ethical approval from the NHS Tayside Committee on Medical
680 Research Ethics (REC Reference Number: 05/S1401/89). GS has also been granted Research
681 Tissue Bank status by the East of Scotland Research Ethics Service (REC Reference Number:
682 20/ES/0021), providing generic ethical approval for a wide range of uses within medical
683 research.

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

689

690 ## Availability of data and materials

691

692 The datasets generated and/or analysed during the current study are not publicly available.

693 The informed consent given by the KORA study participants does not cover posting of
694 participant level phenotype and genotype data in public databases. However, data are
695 available upon request from KORA-gen (http://epi.helmholtz-muenchen.de/kora-gen).
696 Requests are submitted online and are subject to approval by the KORA board.

697 Lothian Birth Cohort data are available on request from the Lothian Birth Cohort Study,
698 University of Edinburgh (simon.cox@ed.ac.uk). Lothian Birth Cohort data are not publicly
699 available due to them containing information that could compromise participant consent and
700 confidentiality.

701 According to the terms of consent for GS and GS:STRADL participants, access to data must
702 be reviewed by the GS Access Committee. Applications should be made to
703 access@generationscotland.org.

704 Code is available with open access at the following Gitlab repository:
705 https://gitlab.com/dannigadd/episcores-for-protein-levels.

706

707 ## Competing interests

708

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

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712

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979 ## Additional files

980 **Supplementary file 1**

981 (A) Demographic and array information for the cohorts and samples used in the study.
982 (B) SomaScan panel performance in the STRADL test set.
983 (C) Performance of Olink protein EpiScores in holdout, STRADL and LBC1921 test sets.
984 (D) Annotations for the proteins corresponding to the 109 selected EpiScores.
985 (E) Predictor weights for the 109 selected EpiScores.
986 (F) CpG feature counts for the 109 selected EpiScores.
987 (G) Frequency of CpG sites selected for EpiScores with EWAS catalogue annotations to
988 phenotypic traits.
989 (H) Basic Cox model results in Generation Scotland.
990 (I) Fully-adjusted and sensitivity analyses results for Cox models in Generation Scotland.
991 (J) Schoenfeld residual Cox sensitivity analyses.
992 (K) Schoenfeld residual Cox sensitivity analyses split by year of follow-up.
993 (L) SOMAscan – EpiScore diabetes association lookup against two large-scale plasma
994 protein – diabetes studies.
995 (M) White blood cell sensitivity analyses.
996 (N) GrimAge sensitivity analyses.

997 **Supplementary file 2**

998 (A) Correlation structures for the 109 selected EpiScores.

999 (B) Correlation structures for the 109 selected EpiScores in relation to common covariates.

1000 **Supplementary file 3**

1001 (A) Summary of the rationale for including each of the 12 morbidities in this study.

1002 **Supplementary file 4**

1003 (A-L) Primary and secondary health codes for each of the 12 morbidities in this study that

1004 were used to assign case/control status of participants.

1005