Plasma Proteomic Signature Predicts Myeloid Neoplasm Risk

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ABSTRACT

Purpose: Clonal hematopoiesis (CH) is thought to be the origin of myeloid neoplasms (MN). Yet, our understanding of the mechanisms driving CH progression to MN and clinical risk prediction of MN remains limited. The human proteome reflects complex interactions between genetic and epigenetic regulation of biological systems. We hypothesized that the plasma proteome might predict MN risk and inform our understanding of the mechanisms promoting MN development.

Experimental Design: We jointly characterized CH and plasma proteomic profiles of 46,237 individuals in the UK Biobank at baseline study entry. During 500,036 person-years of follow-up, 115 individuals developed MN. Cox proportional hazard regression was used to test for an association between plasma protein levels and MN risk.

Results: We identified 115 proteins associated with MN risk, of which 30% ($N = 34$) were also associated with CH. These were enriched for known regulators of the innate and adaptive immune system. Plasma proteomics improved the prediction of MN risk (AUC = 0.85; $P = 5\times10–9$) beyond clinical factors and CH (AUC = 0.80). In an independent group ($N = 381,485$), we used inherited polygenic risk scores (PRS) for plasma protein levels to validate the relevance of these proteins toMNdevelopment. PRS analyses suggest that most MN-associated proteins we identified are not directly causally linked toMN risk, but rather represent downstream markers of pathways regulating the progression of CH to MN.

Conclusions: These data highlight the role of immune cell regulation in the progression of CH to MN and the promise of leveraging multi-omic characterization of CH to improveMN risk stratification.

See related commentary by Bhalgat and Taylor, p. 3095

Introduction

Although it is known that myeloid neoplasm (MN) development is characterized by the sequential acquisition of somatic mutations, there is a poor understanding of the mechanisms underlying the early stages of carcinogenesis. Work over the past decade has revealed that clonally expanded, mutant hematopoietic stem cells, termed clonal hematopoiesis (CH), are common with human aging, but confer an increased risk of hematologic cancer (1–3). Among individuals who develop hematologic cancer, CH can often be detected years and sometimes even decades before diagnosis (4, 5). The prevalence of CH far exceeds the rate of hematologic cancer, and only a small subset of individuals with CH will ultimately progress to cancer. Although large-scale retrospective studies have characterized CH mutational and clinical features that modify risk of progression, our ability to predict which individuals with CH will progress to hematologic cancer remains poor (6, 7). This may partly be due to limitations in our knowledge of the biological pathways that regulate clonal fitness, resultant CH expansion, and secondary mutation acquisition.

The human proteome reflects the downstream impact of complex interactions between genetic and epigenetic regulation of biologic systems. Proteomic characterization of MNs has provided insights into disease biology and clinical disease heterogeneity beyond what is captured by genomic alterations alone (8–12). Proteomic tumor profiling studies have also proved useful in prioritizing therapeutic targets in cancer (13–18). We hypothesized that the plasma proteome might be associated with MN risk and if so, could inform our understanding of the mechanisms promoting the development of MN. Here, we jointly characterized CH and plasma proteomic profiles from 46,237 individuals in the UK Biobank (UKBB) and discovered 115 proteins associated with risk of incident MN, many of which were also associated with CH. We go on to validate these findings through protein quantitative trait loci (pQTL) genetic risk scores and Mendelian randomization (MR) in an independent cohort of 381,485 individuals in the UKBB.

Materials and Methods

Study population

The UKBB is a population-based, prospective study of 502,368 individuals living in the United Kingdom. Individuals were enrolled between 2006 and 2010 and were required to be between 40 and 69 years at study entry (19). At their initial visit at the time of enrollment, participants provided blood samples and answered questionnaires regarding socio-demographic, lifestyle, and health information. We ascertained clinical information from the baseline visit, including blood counts, age, gender, and smoking status. All participants provided written informed consent. The UKBB study was approved by the North West Multi-Center Research Ethics Committee (REC reference: 11/NW/0382), and the research was carried out in accordance with the Declaration of Helsinki of the World Medical Association.

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Translational Relevance

Clonal hematopoiesis (CH) is thought to be the origin of myeloid neoplasms (MN). Yet our understanding of the mechanisms driving CH progression to MN and clinical risk prediction of MN remains limited. The human proteome reflects complex interactions between genetic and epigenetic regulation of biological systems. We hypothesized that the plasma proteome might predictMN risk and inform our understanding of the mechanisms promoting MN development.We demonstrate that plasma protein levels can be used to predict the risk of MN. Many proteins associated with subsequent MN development are also associated with the presence of CH. We also show that proteomics improves MN prediction beyond clinical factors and CH. This highlights the promise of integrating protein and genetic biomarkers for early diagnosis of MN. Furthermore, our findings identify proteins and critical pathways that should be prioritized for experimental validation aimed at prevention of CH progression to MN.

Identification of MN cases

The development of MN after the baseline visit was ascertained using cancer registries from the National Health Service Information Centre of England (last updated December 31, 2020) and Wales (last updated December 31, 2016), and the National Records of Scotland Central Registrar (last updated November 30, 2021). MN were determined by both International Classification of Diseases Tenth Revision (ICD-10) codes and International Classification of Diseases for Oncology, Third Edition (ICD-O-3) histology codes (Data-Field 40006 and 40011), including: Acute myelogenous leukemia (AML) cases [ICD-10 codes C92 (except 92.3), C93, C94]; myelodysplastic syndrome (MDS) cases (ICD-10 codes D46 or ICD-O-3 9989); myeloproliferative neoplasm (MPN) cases (ICD-10 codes C96.9, D45, D47.1, D47.3 or ICD-O-3 9875, 9960, 9876); and other MN (ICD-10 codes C92.7, C92.9, C96.1, C96.2 or ICD-O-3 9741, 9750, 9860, 9945, 9975). We excluded individuals with hematologic cancers diagnosed before or within 3 months of the baseline visit ($N = 2{,}221$).

Whole-exome sequencing

Sample preparation and sequencing was performed by the Regeneron Genetics Center as previously described (20) and summarized in the Supplementary Methods.

SNP array

Genotyping was performed by Affymetrix as summarized in the Supplementary Methods. We limited our analyses to variants with a minor allele frequency >0.1% and imputation score >0.7.

CH variant calling and annotation

CH calling and annotation were performed blinded to participant information as specified in the Supplementary Methods. We included 939 genes with known relevance to cancer in our annotation pipeline (Supplementary Table S1).

Mosaic chromosomal alteration calling

We used MoChA v10.2.0 (21) to identify acquired (i.e., mosaic) chromosomal alterations (mCA) in the UKBB genotyping array data as specified in the Supplementary Methods.

Proteomics assay

Proteomic profiling of blood plasma samples (obtained at the time of study enrollment) was available for 54,306 UKBB participants using the Olink Explore 1,536 platform, which quantitates 1,472 total analytes and 1,463 unique proteins (Supplementary Table S2) across four protein panels (Cardiometabolic, Inflammation, Neurology and Oncology). UKBB participants were selected for Olink proteomic profiling by randomization ($N = 46{,}673$), UK Biobank Pharma Proteomics Project consortium selection ($N = 6{,}385$), or enrollment in a COVID-19 imaging study ($N = 1{,}268$). All analyses of the Olink proteomics data in this study were limited to randomly selected UKBB samples to avoid selection bias. Detailed information on the Olink assay, data processing, and quality control are described by *Sun and colleagues* (22).

Statistical analysis

We used Cox regression to estimate the association between plasma protein levels and the subsequent risk of MN. Gene set enrichment analysis (GSEA) was performed on the proteins significantly associated with risk of MN using clusterProfiler v4.6.2 (RRID:SCR_016884) and Reactome pathways [curated from MSigDB v7.5.1 (RRID:SCR_016863), filtered to only include measured proteins]. In subgroup analyses, we divided MN into MPN and MDS/AML (MDS was combined with AML into one category to preserve statistical power). We modeled CH status driven by SNVs/indels as a continuous variable using the maximum CH VAF for each patient. mCAs were modeled as binary instead of continuous variables due to known difficulties in quantifying cell fraction for low frequency copy-number events (21). Linear regression was used to model the association between protein levels and CH. To investigate the effect of CH allelic burden on plasma protein levels, we compared the strength of the association between plasma proteins and CH separately among individuals with low VAF (<10%) and high VAF (≥10%) CH. We used a heterogeneity test among CH-positive individuals, testing for a difference between protein levels by CH VAF (comparing individuals with VAF ≥10% to VAF <10%). Unless otherwise stated, all models included potential confounders as covariates, including age, sex, smoking status, year of sample collection, blood count measurements, and the top 10 principal components of genetic ancestry (Data-Field 22009).

To evaluate whether the plasma proteome improves MN risk prediction beyond CH and clinical factors alone, we used Cox regression with least absolute shrinkage and selection operator (LASSO) penalty to identify the most predictive CH, proteomics, and clinical features for risk of MN. This analysis was performed with all UKBB participants who had both proteomics profiling and WES data available ($N = 46{,}237$). Internal validation was performed using 5-fold nested cross validation with 100 replicates. In each replicate, we randomly split the data into a training set (80% of the data) and a test set (20% of the data). We then fit the model using the training set and calculated the AUC using the test set. We calculated the mean and standard deviation of the AUCs generated using the test set data across replicates. The significance of a feature was calculated as the frequency of that feature appearing in the 100 replicates of the LASSO Cox regression.

For each UKBB participant with proteomics data, we calculated the "Clonal Hematopoiesis Risk Score" (CHRS) developed by Weeks and colleagues (7). Individuals with "medium" or "high-risk" CH were defined as having a CHRS score of 10 or greater. Using LASSO regression with internal validation as above, we calculated the average AUC of the CHRS model with and without proteomics features. We

repeated this analysis separately among individuals with (i) "low-risk" CH or no CH and (ii) "medium" or "high-risk" CH.

Polygenic risk score for plasma protein levels

Among individuals with both proteomic and SNP array data in the UKBB ($N = 42{,}129$), we identified genetic loci throughout the genome that were significantly associated with protein levels (pQTL) after multiple-testing correction ($P < 3.4 \times 10^{-11}$) using the data from Sun and colleagues (22). We then calculated polygenic risk scores (PRS) for protein levels for each individual in the UKBB using the beta coefficients generated from the SNP-protein association analysis. We refer to this as the global (e.g., genome-wide) PRS ($PRS^{global\text{-}pQTL}$). We used a Cox regression approach to test the association between the $PRS^{global\text{-}pQTL}$ and the risk of MN among the remaining UKBB cohort ($N = 381{,}485$). We limited our analysis to the proteins, where the $PRS^{global\text{-}pQTL}$ explained ≥1% of the variance in protein levels.

To investigate whether there might be a causal relationship between protein levels and MN risk, we used a two-sample MR approach. MR is based on Mendel's laws of inheritance and instrumental variable methods, which allow estimating causal effects even when there is unobserved confounding (23). We repeated testing for SNP-protein associations, restricting our analysis to SNPs within 1 Mb of the coding regions of the proteomics markers to reduce genetic pleiotropy. We identified SNPs that were significantly associated with plasma protein levels after correcting for multiple hypothesis testing ($P < 3.4 \times 10^{-11}$). We then performed clumping using PLINK v1.9 (24) with the settings "–clump-r2 0.10–clump-p1 3.4e–11–clump-p2 1e 3–clump-kb 10,000" to identify independent pQTLs. We constructed PRS for the protein, limited to SNPs in cis ($PRS^{cis\text{-}pQTL}$). We then tested for an association between protein levels and MN in the remaining individuals in the UKBB ($N = 381{,}485$). We used the weighted median method (WMM) to calculate the causal effect. We also compared results generated using the WMM with other commonly used methods in MR, including inverse-variance weighted, robust inverse-variance weighted, MR-Egger, and robust MR-Egger using the R package MendelianRandomization v0.8.0 (RRID: SCR_025049). We limited our analysis to proteins where genetic loci explained ≥10% of the variance of the protein level to avoid weak instrument bias. We calculated the statistical power for our MR analysis using the method described by Brion and colleagues (25).

Data availabilty

All original data used in this study are publicly available from the UK Biobank through a procedure described at https://www.ukbiobank.ac.uk/enable-your-research. All the code used to generate the results presented in this article are publicly available on GitHub (https://github.com/kbolton-lab/CCR_Proteomics).

Results

Among 46,237 UKBB participants, we observed 115 cases of MN (41 cases of AML, 20 of MDS, 41 of MPN, and 13 of other subtypes) over 12 years of follow-up (**Table 1**). The median time to the development of MN was 81.6 months (range, 3–141.6 months). Overall, 8.7% of UKBB participants had detectable CH; 6.1% with SNVs/indels in genes known to predispose to hematologic cancers (CH-heme) and 3% with large scale copy-number events in autosomal chromosomes (mCAs; **Table 1**). As expected, individuals who had CH-heme and mCAs were more likely to develop MN ($P = 2.6 \times 10^{-29}$ for CH-heme and 5.5×10^{-13} for mCAs). Correspondingly, individuals who went on to develop MN were more likely to have detectable CH at baseline (49.6% vs. 6%), multiple CH mutations (14.8% vs. 0.5%), and higher VAF mutations (mean 0.26 vs. 0.15; **Table 1**). Beyond CH, male sex, older age, and former or current smoking status were associated with an increased risk of MN (**Table 1**). Select blood count parameters were also associated with MN risk (higher platelets, monocytes, and RDW; lower hemoglobin; **Table 1**).

Association between plasma protein levels and MN risk

We tested for an association between the plasma levels of 1,463 unique proteins and the subsequent risk of MN, while adjusting for age, race, sex, smoking status, blood count parameters, and year of recruitment. Levels of 115 proteins were significantly associated with risk of MN after multiple hypothesis testing correction (FDR-corrected $P < 0.05$; Supplementary Table S2). Among MNassociated proteins, many have important roles in the hematopoietic system. These include, among the top 20 associated proteins, known regulators of the adaptive immune system [AMIGO2 (26, 27), LAG3 (28), LY9 (29, 30), SIGLEC6 (31, 32)], innate immune system [GCNT1 (33, 34), ICAM2 (35), CD244 (36), CLEC6A (37), FCGR2A, VCAM1 (38, 39), SEMA7A (40, 41), SIRBP1 (42)], leukocyte trafficking [CXCL11 or I-TAC (43, 44), SDC4 or Syndecan-4 (45)], hematopoietic stem cell self-renewal/differentiation [ADGRE5 or CD97 (46), THPO (47), AXL (48, 49)], and platelet activation [GP1BA (50), PEAR1 (51, 52)]. Correspondingly, GSEA showed a significant enrichment in genes involved in immunoregulatory interactions between lymphoid and non-lymphoid cells, and the adaptive immune response (Supplementary Table S3). Most of the MN risk proteins ($N = 112$) showed directionally consistent risk for both MDS/AML and MPN (Supplementary Fig. S1; Supplementary Table S4). Many ($N = 23$, 20%) showed a significantly stronger association with MPN compared with MDS/AML (Supplementary Fig. S2; Supplementary Table S4).

Relationship between proteomic predictors of MN and CH

Given that CH can represent a genetic precursor to MN, we sought to characterize the extent to which the association between plasma proteins and MN risk might be explained by CH. Among the 115 plasma proteins that were significantly associated with MN, 34 were significantly associated (pFDR < 0.05) and 59 nominally associated ($P < 0.05$) with CH, and all but five were directionally consistent (**Fig. 1A**; Supplementary Fig. S3 and Supplementary Table S2). After adjusting for CH (by the maximum VAF), the strength of the association between plasma protein markers and MN risk was generally reduced (0.1%–47.6% HR reduction), but remained significant for many (44 proteins, **Fig. 1B**; Supplementary Table S5). This suggests that some, but not all of the impact of plasma proteomic profiles on MN risk is mediated by baseline CH.

Given the overlap between markers associated with both MN and CH, we sought to identify unique factors that might influence CH, but not MN risk. We used logistic regression to test for an association between CH and plasma protein level (including CH driven by both SNVs/indels and mCAs) adjusted for age, race, sex, smoking status, blood counts, and year of recruitment. We identified 79 proteins significantly associated with CH; 34 of which were significantly associated and 53 nominally associated with MN risk (Supplementary Tables S2 and S6). CH is present on a continuum with the VAF reflecting the proportion of blood cells bearing the CH mutation. The adverse effects of CH generally increase with higher VAF, which led us to investigate whether proteomic markers might show a stronger association with higher VAF CH. For the majority of CH-associated proteins ($N = 74$), we saw a stronger

Table 1. Characteristics of UKBB participants with plasma proteomic profiling ($N = 46{,}237$).

Characteristic	Myeloid neoplasm development: Yes ($N = 115$)	Myeloid neoplasm development: No ($N = 46{,}122$)	Overall ($N = 46{,}237$)	*P*
Age, mean (SD; y)	60.79 (7.5)	57.31 (8.2)	57.32 (8.2)	2.1E−06
Sex, female	48 (41.7%)	24,770 (53.7%)	24,818 (53.7%)	0.01
Race				0.82
White	110 (95.7%)	42,973 (93.2%)	43,083 (93.2%)	
Black	2 (1.7%)	1,068 (2.3%)	1,070 (2.3%)	
Asian	2 (1.7%)	1,016 (2.2%)	1,018 (2.2%)	
Other	1 (0.9%)	836 (1.8%)	837 (1.8%)	
Missing	0 (0%)	229 (0.5%)	229 (0.5%)	
Smoking status				4.7E−03
Never	47 (40.9%)	24,968 (54.1%)	25,015 (54.1%)	
Current or former	68 (59.1%)	20,936 (45.4%)	21,004 (45.4%)	
Missing	0 (0%)	218 (0.5%)	218 (0.5%)	
Blood counts, mean (SD)				
Neutrophil count, $\times 10^9$ cells/L	4.42 (2.1)	4.24 (1.4)	4.24 (1.4)	0.38
Lymphocyte count, $\times 10^9$ cells/L	1.90 (0.7)	1.95 (0.7)	1.95 (0.7)	0.44
Monocyte count, $\times 10^9$ cells/L	0.59 (0.6)	0.48 (0.2)	0.48 (0.2)	0.04
Eosinophil count, $\times 10^9$ cells/L	0.17 (0.1)	0.17 (0.1)	0.17 (0.1)	0.75
Basophil count, $\times 10^9$ cells/L	0.04 (0.1)	0.03 (0.0)	0.03 (0.0)	0.65
Hemoglobin concentration, g/dL	13.9 (1.4)	14.2 (1.3)	14.2 (1.3)	0.05
Mean corpuscular volume, fL	92.4 (7.4)	91.1 (4.6)	91.1 (4.6)	0.06
RBC distribution width (%)	14.3 (1.8)	13.5 (1.0)	13.5 (1.0)	5.0E−06
Platelet count, $\times 10^9$ cells/L	299 (126)	252 (59)	252 (59)	1.7E−04
Clonal hematopoiesis (CH)				
Detectable	49.6%	8.60%	8.7%	
CH-heme (SNV/indel)	49 (42.6%)	2,757 (6.0%)	2,806 (6.1%)	2.6E−29
0 variants	66 (57.4%)	43,365 (94.0%)	43,431 (93.9%)	1.6E−126
1 variant	32 (27.8%)	2,538 (5.5%)	2,570 (5.6%)	
≥2 variants	17 (14.8%)	219 (0.5%)	236 (0.5%)	
Mean VAF, mean (SD)	0.26 (0.1)	0.15 (0.1)	0.15 (0.1)	6.0E−08
CH-mCA	23 (20.0%)	1,369 (3.0%)	1,392 (3.0%)	5.5E−13

Note: Unless noted otherwise, data are *n* (%).
Abbreviations: SD, standard deviation; SNV, single-nucleotide variant; VAF, variant allele frequency; mCA, mosaic chromosomal alteration.

association with higher VAF (≥10%) CH compared with lower VAF (<10%) CH (Supplementary Table S6). In analyses restricted to CHpositive individuals, levels of 29 proteins were associated with CH VAF (Supplementary Table S6). In summary, CH-associated plasma proteins are associated with clonal expansion and risk of MN.

The risk factors determining CH expansion and progression to MN are known to vary by gene, reflecting gene-specific fitness effects and molecular dependencies. Among individuals with detectable CH, we tested whether the strength of the association between plasma proteins and CH varied by CH gene (Supplementary Fig. S4; Supplementary Table S7). Across many proteins, we found clear heterogeneity between the strength of the association with specific CH genes (Supplementary Fig. S4). Overall, 36 proteins had a stronger association for specific CH genes compared with other CH genes. These included decreased levels of AMIGO2, GALNT3, KAZALD1, PCDH17, and SLITRK2 that had a stronger association with *JAK2* CH; increased CD1C, CD200R1, CD28, CD300E, CLEC4C, CLEC6A, FLT3, GCNT1, ICAM3, IFNLR1, LILRA5, LILRB1, LRR25C, OSCAR, SEMA7A, SIGLEC7, TNFSF13B, and decreased FLT3LG, NID1, RETN with *TET2* CH; increased DDAH1, DSC1, MANSC1, SLAMF8 with *ASXL1* CH; increased AXL, CD160, FASLG, LAG3, and decreased ANGPT1, PDGFA, RSPO1 with *DNMT3A* CH (Supplementary Fig. S4; Supplementary Table S7). Similar to previous work by *Bick and colleagues* (53) analyzing cytokine levels by ELISA, we observed a significant association between IL18 and *JAK2* CH and directionally consistent correlations of IL18 with SF3B1 and IL1B with *TET2* (Supplementary Fig. S5). Unlike *Bick and colleagues*, we did not observe a positive association between IL6 and *TET2* CH. However, we did observe a positive association between *TET2* CH and the IL6 receptor (IL6R). Differences between the extent to which Olink versus ELISA assays preferentially measure free or bound forms of cytokine levels could, at least in part, drive these discrepancies.

Given evidence of heterogeneity in protein-CH associations by gene, we tested for associations between CH mutation classes and plasma proteins. We divided CH genes into epigenetic modifiers, DNA damage response, splicing factors and growth factor signaling (Supplementary Table S8). Pathway analysis based on these results identified additional pathways that were enriched in specific CH mutation classes but not with CH overall (Supplementary Table S3). This included IL2, IL10, and TNF signaling with DDR CH, ERBB4, EGFR, PI3K and estrogen signaling for splicing factor CH and response to elevated platelet cytosolic $Ca2^+$ enriched with growth factor CH and epigenetic modifier CH.

CH is known to influence hematopoietic architecture as reflected by alterations in blood count parameters among CH carriers. Among CHassociated plasma proteins, we observed several hematopoietic growth factors, including FGF2, EGF, THPO, PDGFB, and PDGFA among others. We sought to characterize whether proteins associated with CH

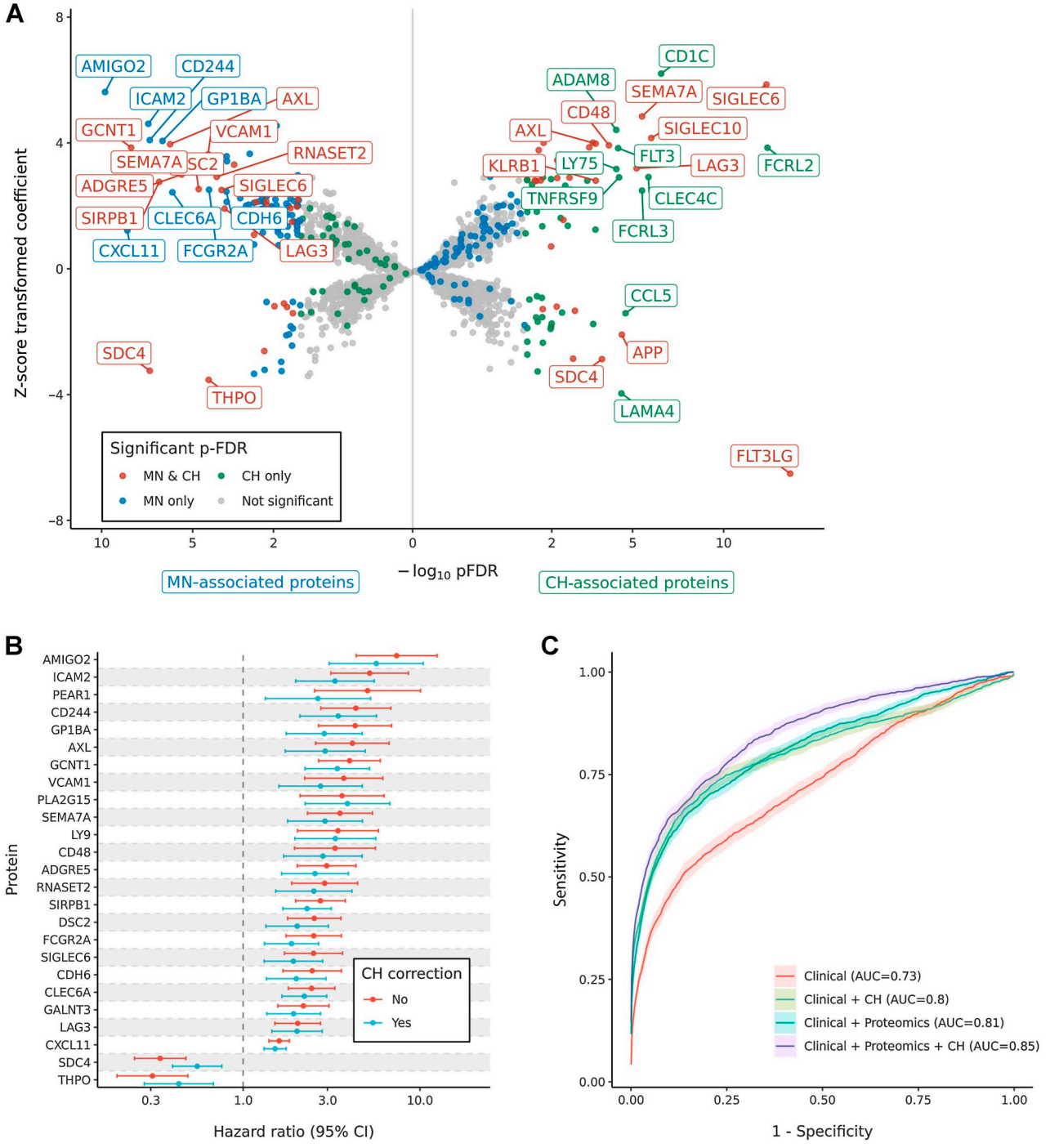

Figure 1.

Association between plasma protein levels, incident myeloid neoplasm (MN), and clonal hematopoiesis (CH). **A,** Multivariable Cox regression adjusted for age, sex, smoking status, year of sample collection, blood count measurements, and the top 10 principal components of genetic ancestry was used to test for an association between plasma protein expression and MN (left). Multivariable logistic regression adjusted for the same covariates was used to test for an association with CH (right). The *y*-axis displays the z-score transformed beta coefficients from the regressions. Each circle represents one protein colored by statistical significance (FDR-corrected *P* value < 0.05) for MN (blue), CH (green), MN and CH (red), or none (gray). The top 20 results in each plot are labeled by protein name. **B,** Multivariable Cox for the top 25 proteins associated with MN risk with (teal) or without (red) correction for CH (by maximum VAF) regression for MN risk. Shown are the hazard ratios and their 95% confidence intervals. **C,** Discrimination of MN risk incorporating basic clinical features alone or alongside CH and/or proteomics. The clinical model incorporates age, sex, ancestry, smoking status, blood counts, and sample age. Receiver operating (ROC) curves include the mean AUC and 95% confidence interval (lighter shading) generated from 100-fold cross-validation.

and/or MN ($N = 160$) might be associated with blood counts after accounting for the burden of CH as reflected by maximum VAF. In multivariable analysis, we observed that the majority (99%) of CHassociated plasma proteins showed at least one association with blood counts. Out of 160, 139 were associated with platelet counts, 142 with lymphocyte counts, and 133 with hemoglobin concentration (Supplementary Fig. S6; Supplementary Table S9).

Risk prediction of MN incorporating CH and plasma proteins

We sought to assess whether proteomic markers could improve prediction of MN risk beyond CH and clinical factors. Cox proportional hazards regression was used to test for an association between demographic factors, blood counts, CH, and proteomic markers with MN risk. In multivariable analyses, the most significant features for CH were SNV/indel max VAF, *SRSF2* SNV, *SF3B1* SNV, *TET2* SNV, and mCA 9p; the top features for demographic factors and blood counts were sex, age, smoking status, platelet count, and hemoglobin concentration. Plasma levels of five proteins remained significant after adjustment for CH and clinical characteristics and were retained in the multivariable model. This included BIN2 (54) that plays a critical role in cell adhesion and calcium-mediated platelet activation, FGF2 (55) a hematopoietic growth factor, LY9 (29, 30) a self-ligand receptor important in modulating the activation and differentiation of a wide variety of hematopoietic cells, and CXCL11 (44) and SORT1 (56) known regulators of immune cell activation and cytokine secretion (Supplementary Fig. S7). We compared models with and without the addition of proteomic features through internal validation (100-fold cross validation). Inclusion of proteomics features improved prediction of incident MN (AUC, 0.85; 95% CI, 0.84–0.86) beyond clinical factors and CH (AUC, 0.80; 95% CI, 0.79–0.82; **Fig. 1C**). Limiting proteomics features to the 25 or 10 most significant proteins (versus all 115 MN risk proteins) had no significant impact on model discrimination (AUC, 0.85–0.86), suggesting that most the MN risk signal could be captured with a smaller set of proteins (Supplementary Fig. S8). We sought to characterize whether plasma proteomics might improve the CHRS developed by *Weeks and colleagues* (7). The addition of proteomics features to the CHRS improved discrimination of MN risk MN (AUC 0.79 vs. 0.86). The improvement in AUC appeared driven by improved risk stratification among individuals with "low-risk" CH or no detectable CH (Supplementary Fig. S9).

Inherited PRSs for protein levels to further characterize the mechanisms of protein–MN associations

The observed association between plasma proteins and MN risk could be explained by three main possibilities. First, the protein could be causally linked to the development of MN. Second, the protein level could reflect other upstream events that are causally linked to MN. Third, these associations could reflect false-positive events driven by statistical type I error. We constructed PRSs for plasma protein levels using inherited pQTLs. We leveraged PRSs for plasma protein levels to both validate our findings and explore potential causality between protein levels and MN risk. We first calculated the PRS for each of the 115 MN proteins using 876 independent genetic regions across the genome that were associated with protein levels through the UK Biobank Pharma Proteomics Project ($PRS^{global\text{-}pQTL}$). The $PRS^{global\text{-}pQTL}$ explained ≥1% of the variance ($R^2 > 0.01$) of plasma protein levels for 110 out of 115 proteins (R^2 range, 0.1–64.3%; median, 6.7%). Of note, the majority of these genetic loci (92%) had a *trans* association with the protein of interest (i.e., the locus was located >1 Mb away from the gene encoding the associated protein). Many pQTLs showed evidence of pleiotropy ($N = 125$) being associated with the levels of multiple proteins. This suggests that many of these pQTLs may exert their effect through influencing genes that are upstream regulators within the same pathway.

In an independent cohort of 381,485 UKBB participants without proteomic profiling data (985 of whom developed MN), we found that of the 110 plasma protein markers where PRS explained at least 1% of variance of the protein level, genetically predicted levels of 10 proteins were significantly associated ($P < 0.05$) with MN risk (**Fig. 2A**; Supplementary Table S10). This included FLT3LG, IL17RA, CLEC11A, SIGLEC7, AMIGO2, ADGRE5, PGLYRP1, SDC4, SPARC, and CRTAM. Of the remaining, 49 were directionally consistent (Supplementary Table S10). Similar to the overlap we saw between plasma protein levels and both risk of MN and CH, many were also associated with CH; 18 were significantly associated and 71 were directionally consistent (Supplementary Table S10). Thus, use of inherited $PRS^{global\text{-}pQTL}$ as proxies of lifetime average plasma protein levels supported the association between several plasma protein levels and MN risk in an independent subset of the UKBB.

To assess whether the association between a plasma protein and MN risk is causal, we performed an MR analysis. Using data from approximately 22.6 million imputed autosomal variants from UKBB, we identified 4,230 independent genetic loci associated with plasma protein levels at genome-wide significance ($P < 3.4 \times 10^{-11}$) that were located within 1 Mb of the gene-coding region (i.e., $PRS^{cis\text{-}pQTL}$). We limited our analysis to SNPs located nearby the corresponding gene to limit genetic pleiotropy and enrich SNPs directly regulating expression of proteins of interest. Out of 115 MN-associated proteins, *cis* genetic loci explained at least 10% of the variance for 31 plasma proteins levels. For the majority (25/31), we had at least 80% power to detect a significant association using our two-stage MR approach. Genetically proxied levels of two proteins, coagulation factor VII (F7) and IL17 receptor (IL17RA), were significantly ($P < 0.05$) associated with MN risk (**Fig. 2B**; Supplementary Fig. S10) and an additional 8 were directionally consistent (Supplementary Table S11). These associations were robust across most, but not all, MR methods (**Fig. 2B**). Overall, these analyses suggest that most MN-associated proteins do not have a direct causal role in MN development. Rather they may represent downstream markers of pathways involved in MN pathogenesis.

Discussion

Here, we demonstrate that plasma proteomic signature predicts the development of MN in healthy adults. Some, but not all of the effect of protein levels on MN risk was driven by an increased burden of CH at baseline. We observed clear heterogeneity by CH gene in regard to the strength and sometimes even the directionality of plasma protein associations, further highlighting differences in the biologic drivers of CH. Interestingly, several MN-associated proteins associated most strongly with *JAK2* CH. Correspondingly, the strongest associations we observed between plasma proteins and MN were with MPN, known to be most commonly driven by gain of function mutations in *JAK2*. Among MN-associated proteins, we observed an enrichment of known regulators of immune cell activation and signaling. Inflammation is known to play a pivotal role in MPNs, which are maintained by a continuous release of proinflammatory cytokines and chemokines (57, 58). The strong association we observed between plasma proteins and MPN could reflect this genetic dependency on an inflammatory milieu. Beyond inflammation, we also increasingly understand that dysregulation of the immune system is critical to

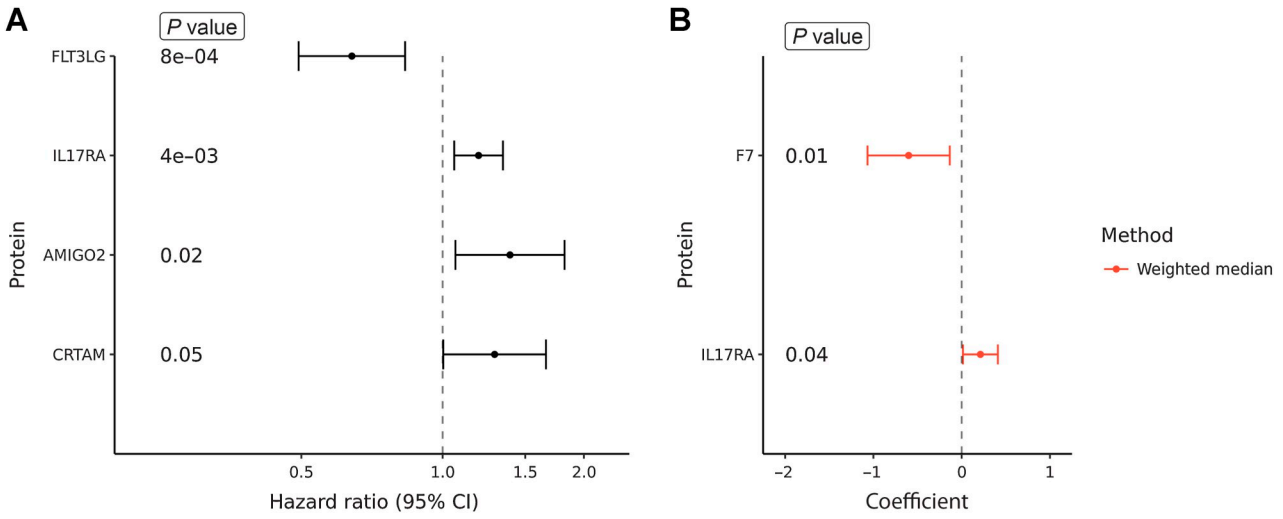

Figure 2.

Association between polygenic risk score (PRS) for plasma protein levels and MN in an independent subset of the UKBB (**A**). Association between global (i.e., genome-wide) PRS ($PRS^{global\text{-}pQTL}$) for plasma protein levels and risk of MN. For each protein, PRSs for each individual were constructed by summing the product of the beta coefficients for the association between and the number of alternate alleles for each protein quantitative loci detected through a genome-wide interrogation. The beta coefficients were obtained from SNP-protein association analysis. Displayed are the hazard ratio and 95% confidence intervals for proteins in which genetically predicted levels were significantly associated with MN risk. **B,** Two-sample Mendelian randomization (MR) analyses of the causal impact of plasma protein levels on MN risk. Each dot represents the estimated MR effect for the corresponding protein with the corresponding 95% confidence interval.

MN. To expand and progress, leukemic cells must leverage immune evasion and suppression mechanisms to avoid recognition and elimination by the immune system (59). Experimental models suggest that alteration of the inflammatory milieu influences CH fitness (60–62). Our data provide further support that alterations in the inflammasome and immune surveillance may not only influence MN maintenance and progression but also the transition from CH to MN. In addition, we identified a number of MN-associated proteins with important roles in adhesion, which is intriguing in light of the increasingly recognized role of bone marrow microenvironment stromal cells in the pathogenesis of hematological malignancies (63).

We leveraged inherited PRSs for plasma protein levels (PRS^{pQTL}) to validate the relevance of plasma proteins in predicting MN risk and to further characterize the mechanisms driving these associations. We observed several MN-associated proteins where genome-wide genetically predicted levels ($PRS^{global\text{-}pQTL}$) were also associated with risk of MN in an independent population. Many of these, including FLT3LG, SDC4, SPARC, and CRTAM, also showed evidence of association with CH. Given that $PRS^{global\text{-}pQTL}$ reflects average lifetime protein levels in an individual (64), this suggests that levels of these proteins result in an increased risk of CH and subsequent MN rather than CH influencing levels of these proteins. Given the strong association we observed between plasma protein levels and CH VAF, modification of CH fitness may represent one mechanism by which protein levels influence MN risk. Longitudinal studies, including repeat assessments of both CH and plasma protein levels, would be needed to clarify the extent to which plasma protein levels influence CH temporal dynamics and progression to MN. Conversely, we observed several CH and MNassociated proteins where the $PRS^{global\text{-}pQTL}$ was not significantly associated with CH or MN, despite having adequate power (>80%) to detect these associations. Included in this were the known platelet growth factors THPO, PDGFA, and PDGFD, where plasma levels were positively associated with platelet levels and inversely associated with CH, particularly *JAK2* CH. The inverse relationship could reflect lower activation of normal physiologic pathways for platelet growth among *JAK2* CH carriers due to hyperactivation of the JAK–STAT pathway. Thus, in summary the associations we observed between plasma proteins and MN likely reflect a combination of circulating plasma proteins influencing expansion of high-risk CH and expanded highrisk CH influencing plasma protein levels.

Restriction of PRS to genetic loci located in cis ($PRS^{cis\text{-}pQTL}$) suggests that the MN-associated proteins we identified in this study, in general, are not causally linked to MN risk. Rather, these proteins may represent downstream markers of pathways directly regulating the progression of CH to MN. Our study was limited to plasma measurements of 1,463 proteins, thus representing a small subset of the total human proteome. Broader proteomic characterization may identify additional proteins associated with MN risk, including those causally related to MN development. Two proteins, F7 and IL17-RA did show some evidence of a causal relationship with MN development based on MR analysis. IL17 has been implicated in many inflammatory conditions (65) and increased levels have been reported in hematologic cancers (66–68). F7 to our knowledge has not been previously linked to MN pathogenesis but the tissue factor–F7 complex is thought to play important roles in immune cell regulation (69, 70) and perhaps carcinogenesis (71). A potential causal role between F7 and IL17 levels and MN development would require experimental validation particularly given that our findings were not robust across all MR methods.

Validation of these findings in additional cohorts will be important to assess generalizability across diverse populations. Application of joint proteomic and CH profiling in unique patient populations (e.g., individuals at high inherited susceptibility to hematologic cancer) could highlight additional avenues for clinical utility and offer additional biologic insights into the determinates of CH progression. In summary, we show that plasma proteins influence both CH and MN development and can be used to improve MN risk prediction. This highlights the promise of integrating protein and genetic biomarkers for early diagnosis of MN. Furthermore, our findings

identify proteins and critical immune regulatory pathways that should be prioritized for experimental validation aimed at prevention of CH progression to MN.

Authors' Disclosures

S. Chlamydas reports employment with Olink. B. Busby reports employment with DNAnexus. K.L. Bolton reports grants and personal fees from Servier outside the submitted work. No disclosures were reported by the other authors.

Authors' Contributions

D. Tran: Formal analysis, writing–original draft, writing–review and editing. **J.S. Beeler:** Formal analysis, writing–original draft, writing–review and editing. **J. Liu:** Data curation. **B. Wiley:** Data curation. **I.C.C. Chan:** Data curation. **Z. Xin:** Data curation. **M.H. Kramer:** Writing–review and editing. **A.L. Batchi-Bouyou:** Data curation. **X. Zong:** Data curation. **M.J. Walter:** Writing–review and editing. **G.E.M. Petrone:** Writing–review and editing. **S. Chlamydas:** Writing–review and editing. **F. Ferraro:** Writing–review and editing. **S.T. Oh:** Writing–review and editing. **D.C. Link:** Writing–review and editing. **B. Busby:** Conceptualization, supervision. **Y. Cao:** Conceptualization, supervision. **K.L. Bolton:** Conceptualization, supervision, funding acquisition, writing–original draft, writing–review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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