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ORIGINAL ARTICLE

Prediction of Risk for Myeloid Malignancy in Clonal Hematopoiesis

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Abstract

BACKGROUND Clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS) are defined by somatic mutations in genes associated with myeloid neoplasms (MN) at a variant allele fraction (VAF) of 0.02 or greater in the absence and presence of cytopenia, respectively. CHIP/CCUS is highly prevalent in adults, and defining predictors of MN risk would aid clinical management and research.

METHODS We analyzed sequenced exomes of healthy U.K. Biobank participants (N=438,890) in separate derivation and validation cohorts. Genetic mutations, laboratory values, and MN outcomes were used in conditional probability–based recursive partitioning and Cox regression to determine predictors of incident MN. Combined statistical weights were used to define a clonal hematopoiesis risk score (CHRS). Independent CHIP/CCUS patient cohorts were used to test the prognostic capability of the CHRS in the clinical setting.

RESULTS Recursive partitioning distinguished patients with CHIP/CCUS with 10-year probabilities of MN ranging from 0.0077 to 0.85. Multivariable analysis validated partitioning variables as predictors of MN. Key features, including single *DNMT3A* mutations, high-risk mutations, two or more mutations, a VAF of 0.2 or more, 65 years of age or older, having CCUS versus CHIP, and red blood cell indices, influenced MN risk in a variable direction. CHRS was used to define low-risk (n=10,018 [88.4%]), intermediate-risk (n=1196 [10.5%]), and high-risk (n=123 [1.1%]) groups. In clinical cohorts, most MN events occurred in high-risk patients with CHIP/CCUS.

CONCLUSIONS The CHRS provides a simple prognostic framework for CHIP/CCUS, distinguishing a high-risk minority from the majority of CHIP/CCUS, which has a

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minimal risk of progression to MN. (Funded by the National Institutes of Health, the Harold Amos Medical Faculty Development Program, and others.)

Introduction

A critical goal of early cancer detection is to identify individuals with premalignant states at the greatest risk for progression. Clonal hematopoiesis (CH), a premalignant expansion of a population of blood cells derived from a single hematopoietic stem cell,[1] is often caused by somatic mutations in leukemia driver genes.[2-4] Under the broader category of CH, two conditions are formally defined. CH of indeterminate potential (CHIP) is categorized by CH with somatic mutations detectable at a variant allele fraction (VAF) of 2% or greater in the absence of a diagnosed blood disorder or cytopenia. Clonal cytopenia of undetermined significance (CCUS) describes CHIP in the presence of unexplained, persistent cytopenias.[5,6]

More than 10% of individuals older than 60 years of age have CHIP or CCUS (CHIP/CCUS), and diagnosis rates are increasing, partially due to the use of next-generation sequencing (NGS) to evaluate unexplained cytopenias and "liquid biopsies" to evaluate solid malignancies.[7,8] The overall rate of transformation for CH is approximately 0.5 to 1% per year. Similar to many premalignant states, most individuals with CHIP/CCUS do not progress to myeloid neoplasms (MN), although progression risks as high as 90% have been reported in certain populations.[9,10] Risk stratification aids clinicians by identifying high-risk patients in whom early intervention may be appropriate while avoiding the toxicities[11] associated with overdiagnosis, unnecessary monitoring, and treatment in low-risk patients.

Several studies have identified features associated with evolution to MN, including mutations in certain high-risk genes, specific patterns of co-mutation, larger clone size as determined by VAF, and having CCUS instead of CHIP.[9,10,12-15] However, systematic risk prognostication tools do not exist for CHIP/CCUS. We leveraged analysis of genetic, laboratory, and MN outcomes data from 438,890 U.K. Biobank (UKB) participants to definitively identify features of CHIP/CCUS that predict risk of MN. Statistically weighted features combined to yield the CH risk score (CHRS), a simple prognostic model that distinguishes high-risk CHIP/CCUS from low-risk CHIP/CCUS in population and patient cohorts.

Methods

UKB COHORTS AND MOLECULAR ANNOTATION

UKB[16] data were extracted under application 50834 from a cohort of 502,490 participants 40 to 70 years of age recruited between 2006 and 2010. Detection of somatic variants in whole-exome sequencing was as previously described,[15] and pathogenic somatic variants in at least one gene associated with CH or myeloid malignancy were used to define CH.[2,17,18] A list of included genes and average coverage per gene has been published previously.[19] Individuals with low abundance clones (defined by a VAF <0.02), missing laboratory values, and myeloid malignancy preceding or within 6 months of study enrollment were excluded from the analysis. Of the 438,890 individuals eligible for study, 193,743 were used for model derivation and 245,147 were used for validation (Fig. S1 in the Supplementary Appendix). Returned single nucleotide polymorphism (SNP) array data ("Return 3094")[20,21] were used to independently annotate mosaic chromosomal abnormalities (mCAs) using estimated break points and relevance to hematologic malignancies according to the cBioPortal for Cancer Genomics[22] and the Atlas of Genetics and Cytogenetics in Oncology and Haematology.[23] For this study, mCA refers to myeloid mCA and "ambiguous" mCA that were common to both myeloid and lymphoid malignancy.[15] Lymphoid-specific mCAs were not analyzed. Additional details on molecular analyses are available in the Supplemental Methods.

CHIP AND CCUS DESIGNATIONS

CHIP and CCUS were defined by the presence of somatic mutations at a VAF of 0.02 or greater. CH in the absence of cytopenia was classified as CHIP, and CH in persons with at least one cytopenia was classified as CCUS. Cytopenias were defined by using World Health Organization[5,24] criteria (anemia = hemoglobin concentration <13.0 g/dl in male participants and <12.0 g/dl in female participants; thrombocytopenia = platelet counts $<150 \times 10^9$ cells/l; and neutropenia = absolute neutrophil count $<1.8 \times 10^9$ cells/l). Bone marrow analysis was unavailable for UKB participants.

VARIABLES AND OUTCOMES OF INTEREST

Participants were followed up from the time of study enrollment until death or December 31, 2021, whichever

was earliest. Extracted variables included age, sex, laboratory values, self-reported smoking history, and history of cancer (defined as solid or lymphoid malignancies occurring before initial study assessment). Table S1 shows the distribution of prior cancers.

The primary outcome of interest included incident MN, in which MN was defined by diagnosis with myelodysplastic syndrome (MDS), acute myeloid leukemia (AML), chronic myelomonocytic leukemia (CMML), or Philadelphia chromosome–negative myeloproliferative neoplasms (MPNs) any time after month 6 of study enrollment. Diagnoses were assessed by self-report and International Classification of Diseases, Tenth Revision (ICD-10), codes in linked national health record hospital data. Table S2 lists the ICD-10 codes used.

STATISTICAL ANALYSIS

Statistical analyses were performed by using R statistical software (R Foundation for Statistical Computing). Figures were made with R (version 4.2.1) and GraphPad Prism (Prism version 9.4.0). All statistical tests were two-sided with statistical significance determined by a P value <0.05. Categorical variables were compared by using Fisher's exact test, and Wilcoxon's rank-sum test was used to compare continuous variables. Cumulative incidence of MN was estimated by using a competing risks approach with P values determined by Gray's test. Cumulative incidences are reported at 5 and 10 years. Overall survival was estimated by using the Kaplan–Meier method, with P values determined by using the log-rank test.

Prognostic model derivation was performed by using a two-stage approach. For patients with CHIP/CCUS in the UKB with at least 10 years of follow-up (n=10,559), conditional probability–based recursive partitioning (RP) analysis was performed by using the rpart package (https://cran.r-project.org/web/packages/rpart/index.html) and minimal complexity pruning with incident MN within 10 years as the single binary outcome. Additional information on the RP method, including all variables used, is available in the Supplemental Methods. Variables identified by RP analysis were then used in multivariable Cox models to generate statistical weights. Regression models were adjusted for smoking status and cancer history, both independent risk factors for MN.[25,26] Summed variable weights determined the CHRS values, which were used to define low-, intermediate-, and high-risk groups. Model performance was evaluated by using receiver-operating-characteristic (ROC) analysis and model concordance (c-index).

HEMATOLOGY PATIENT COHORTS

The Dana-Farber Cancer Institute/Brigham and Women's Hospital (DFCI/BWH) CHIP/CCUS cohort included all patients diagnosed in hematology clinics with CHIP/CCUS between 2014 and 2019, with follow-up until December 1, 2021. The Pavia CCUS cohort is an independent group of 99 patients with bone marrow biopsy–confirmed CCUS with follow-up between 2003 and 2019 at the Department of Hematology, Policlinico San Matteo at the University of Pavia. Patients with an MN history and those missing more than one CHRS variable were excluded. The remaining missing values were handled by stochastic regression imputation. Information on racial/ethnic distribution of UKB and DFCI/BWH cohorts and what is known of the distribution of CHIP and CCUS is included in Table S13 in the supplemental appendix. This information is unavailable for the Pavia cohort.

Results

BASELINE CHARACTERISTICS OF THE UKB DERIVATION COHORT

We analyzed whole-exome sequencing data from 193,743 study-eligible UKB participants and identified 11,337 individuals who met the criteria for having CHIP or CCUS.[5] Median follow-up time was 11.7 years (interquartile range, 10.9 to 12.6 years). Compared with CHIP (n=10,479), a greater proportion of CCUS (n=858) was male (51.4 vs. 44.5%, $P=9.87\times10^{-5}$), and CCUS was more commonly associated with a cancer history (10.5 vs. 7.8%, P=0.0072). No difference in age or smoking history was noted between CHIP and CCUS (Table 1 and Table S3). Anemia, thrombocytopenia, and neutropenia were mostly mild and detected in 58.3%, 34.6%, and 14.7% of CCUS, respectively (Table S4). Red cell distribution width (RDW) and mean platelet volume were higher in CCUS compared with CHIP.

Consistent with prior reports,[15,27] *DNMT3A*, *TET2*, and *ASXL1* mutations were the most commonly mutated genes in CHIP/CCUS (Fig. S2A). Individuals with CCUS had a higher VAF compared with CHIP (0.128 [interquartile range, 0.076 to 0.237] vs. 0.111 [interquartile range, 0.071 to 0.189], $P=1.28\times10^{-6}$; Fig. S2B). Greater clonal complexity, defined by the presence of more than one mutation, was higher in CCUS versus CHIP (15.0 vs. 8.42%, $P=1.56\times10^{-9}$), and CHIP/CCUS with a single mutation

Table 1. Characteristics of U.K. Biobank Derivation Cohort.*

Characteristic	No CHIP/CCUS (n=182,406)	All CHIP/CCUS (n=11,337)	CHIP (n=10,479)	CCUS† (n=858)
Sex — no. (%)				
Female	100,200 (54.9)	6,235 (55.0)	5,818 (55.5)	417 (48.6)
Male	82,206 (45.1)	5,102 (45.0)	4,661 (44.5)	441 (51.4)
Age — median (IQR), yr	57.0 (50–63)	62.0 (57–66)	62.0 (57–66)	62.0 (56–66)
Follow-up — median (IQR), yr‡	11.7 (10.8–12.5)	11.6 (10.8–2.4)	11.6 (10.8–12.4)	11.2 (10.6–12.1)
Any smoking history — no. (%)				
Yes	102,085 (56.0)	5,703 (50.3)	5,272 (50.3)	431 (50.2)
No	80,322 (44.0)	5,634 (49.7)	5,207 (49.7)	427 (49.8)
Cancer history — no. (%)§				
Prior malignancy	9,844 (5.4)	908 (8.0)	818 (7.8)	90 (10.5)
No prior malignancy	172,562 (94.6)	10,429 (92.0)	9,804 (92.2)	768 (89.5)
Laboratory values and cytopenias¶				
White blood cell count ($\times 10^9$ cells/l) — median (IQR)	6.68 (5.69–7.85)	6.84 (5.77–8.10)	6.90 (5.82–8.10)	6.28 (4.87–7.64)
Hemoglobin — median (IQR), g/dl	14.1 (13.3–15.0)	14.1 (13.4–15.0)	14.2 (13.5–15.1)	12.6 (11.7–14.0)
Platelets ($\times 10^9$ cells/l) — median (IQR)	247 (213–286)	249 (213–289)	250 (216–290)	216 (142–284)
Neutrophil count ($\times 10^9$ cells/l) — median (IQR)	4.03 (3.30–4.95)	4.16 (3.36–5.13)	4.20 (3.40–5.15)	3.67 (2.59–4.83)
No. of cytopenias — no. (%)				
0	169,801 (93.1)	10,479 (92.4)	10,479 (100)	0 (0)
1	12,106 (6.6)	797 (7.0)	0 (0)	797 (92.9)
2	464 (0.3)	57 (0.5)	0 (0)	57 (6.6)
3	35 (0.0)	4 (0.04)	0 (0)	4 (0.5)
Anemia — no. (%)				
No	174,878 (95.9)	10,837 (95.6)	10,479 (100)	358 (41.7)
Yes	7,528 (4.1)	500 (4.4)	0 (0)	500 (58.3)
Thrombocytopenia — no. (%)				
No	178,330 (97.8)	11,040 (97.4)	10,479 (100)	561 (65.4)
Yes	4,076 (2.2)	297 (2.6)	0 (0)	297 (34.6)
Neutropenia — no. (%)				
No	18,0871 (99.2)	11,211 (98.9)	10,479 (100)	732 (85.3)
Yes	1,535 (0.8)	126 (1.1)	0 (0)	126 (14.7)
Mean corpuscular volume — median (IQR), fl	91.3 (88.6–93.9)	91.4 (88.8–94.1)	91.4 (88.9–94.1)	90.6 (86.7–94.1)
Mean platelet volume — median (IQR), fl	9.20 (8.58–9.94)	9.18 (8.51–9.92)	9.15 (8.50–9.90)	9.47 (8.70–10.60)
Red cell distribution width — median (IQR), %	13.3 (12.9–13.9)	13.4 (13.0–14.0)	13.4 (12.9–13.9)	14.0 (13.3–15.1)

* A derivation cohort of 193,744 U.K. Biobank participants was evaluated by whole-exome sequencing for the presence or absence of clonal hematopoiesis and further classified as having clonal hematopoiesis of indeterminate potential (CHIP) or clonal cytopenia of undetermined significance (CCUS) on the basis of the absence or presence of cytopenia. Categorical variables are summarized by the number of events (n) and proportion (%). Continuous variables are summarized by using median and interquartile range (IQR).

† Cytopenias were defined such that individuals classified as CCUS had one or more of the following: anemia (hemoglobin level <13 g/dl for male participants and <12 g/dl for female participants), thrombocytopenia (platelet count <150 x 10^9 cells/l), or neutropenia (absolute neutrophil count <1.8 10^9 cells/l).

‡ Follow-up time is the number of years from sequencing to death or last follow-up (January 2021), whichever is earliest.

§ Cancer history is defined as a history of solid or lymphoid malignancy in the years before enrollment in the study on the basis of aggregated self-report and hospital records by International Classification of Disease, Tenth Revision, code (codes are listed in Table S1). Individuals with histories of myeloid malignancy were excluded. Cancer types contributing to cancer history in this population are indicated in Table S3.

¶ Hematologic parameters were obtained from complete blood count and differential obtained at the time of sequencing.

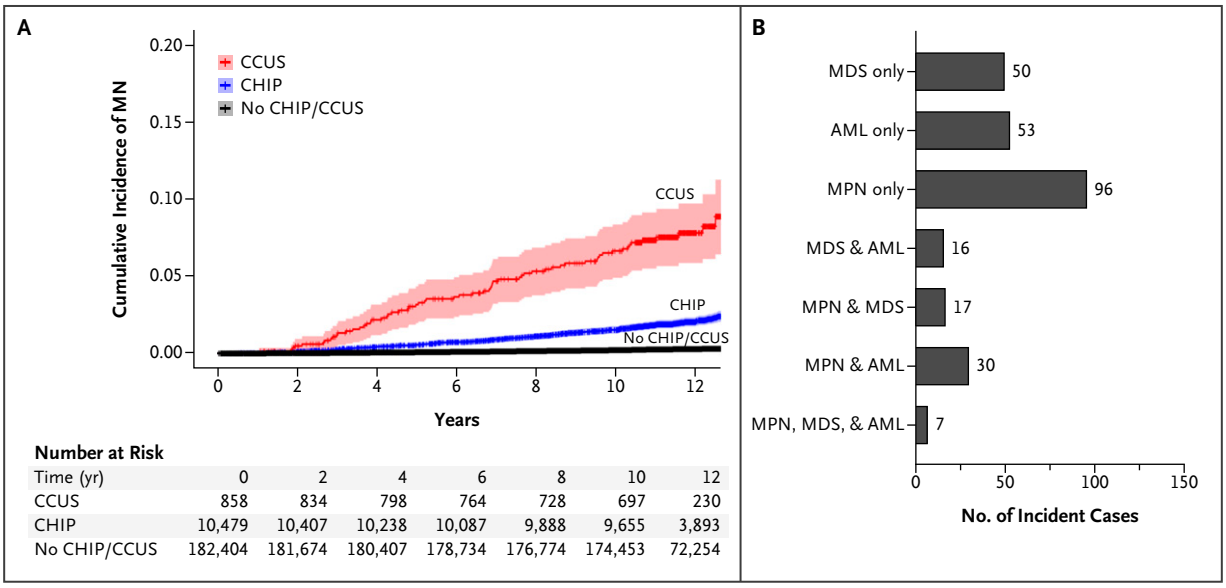


Figure 1. Features Influencing the Risk of MN in U.K. Biobank Participants with CHIP/CCUS.

Panel A shows the cumulative incidence of myeloid neoplasms (MN) in individuals with clonal hematopoiesis of indeterminate potential (CHIP)/clonal cytopenia of undetermined significance (CCUS) compared with those without CHIP/CCUS. (Panel B) Subtypes of MN among patients with CHIP/CCUS in whom MN develops. Univariate Cox proportional-hazards regression analysis for the 14 most commonly mutated genes in CH (Panel C) and for groups of mutations, including single mutations in *DNMT3A*, *TET2*, or *ASXL1* (single D-T-A), splicing factor mutations (*SRSF2*, *SF3B1*, and *ZRSR2*) and acute myeloid leukemia (AML)-like mutations (*IDH1*, *IDH2*, *FLT3*, and *RUNX1*) (Panel D). (Panel E) Univariate analysis of single *DNMT3A*, *TET2*, and *ASXL1*. (Panel F) Cumulative incidence of MN for CHIP/CCUS possessing a single *DNMT3A* mutation (green) compared with the cumulative incidence for all other CHIP/CCUS genotypes (red) and individuals without CHIP/CCUS (black). (Panel G) For U.K. Biobank participants with at least 10 years of follow-up (n=10,559), recursive partitioning analysis was performed on the basis of conditional probability of incident MN within 10 years. Of these, 207 incident MN events were recorded. Each node is annotated with the number of individuals and probability of incident MN. Nodes are color coded as follows: probability of 0.02 or lower is green, 0.02 to 0.4 is yellow, and more than 0.4 is red with white typeface. Partitioning variables are all binary (presence vs. absence of feature) and include high-risk mutation (mutations in *SRSF2*, *SF3B1*, *ZRSR2*, *IDH1*, *IDH2*, *FLT3*, *RUNX1*, and *JAK2*), single *DNMT3A*, having two or more mutations, a variant allele fraction (VAF) of 0.2 or more, having CCUS instead of CHIP, red cell distribution width (RDW) of 15% or more, mean corpuscular volume (MCV) of 100 fL or more, and being 65 years of age or older. (Panel H) Multivariable Cox regression adjusted for assigned sex at birth, history of cancer, and any history of smoking as confounders was performed on the entire cohort (N=11,337) using features selected in recursive partitioning analysis. For all Cox regression models, hazard ratios are shown with error bars representing 95% confidence intervals and numerical values for hazard ratios (95% confidence intervals) and P values for each feature analyzed. MDS denotes myelodysplastic syndrome; and MPN, myeloproliferative neoplasm.

in *DNMT3A* (single *DNMT3A*) was the most common genotype (Fig. S2C to S2E).

GENOTYPE-SPECIFIC RISK OF INCIDENT MN IN THE UKB DERIVATION COHORT

Among the 11,337 individuals with CHIP/CCUS, there were 269 (2.37%) incident MN events (Fig. 1A and 1B). The cumulative incidence of MN was higher in CCUS compared with CHIP (Fig. 1A and Table S5). Cox proportional-hazards models performed with time-to-incident MN were conducted for each gene and adjusted for sex, cancer history, and smoking history. Hazard ratios of more than 5 were observed for mutations in *SRSF2*, *SF3B1*, *ZRSR2*, *JAK2*, *RUNX1*, and *IDH2* (Fig. 1C), and we classified these mutations as high risk. Mutations in splicing factor genes (*SRSF2*, *SF3B1*, and *ZRSR2*), AML-like genes (*IDH1*, *IDH2*, *FLT3*, and *RUNX1*), and *TP53*-related genes (*TP53* and *PPM1D*) were also evaluated as grouped variables in regression models (Fig. 1D). Mutations in splicing factors and AML-like genes were associated with a 9.26-fold (interquartile range, 5.29 to 16.2) and 13.8-fold (interquartile range, 10.3 to 18.4) increased risk of incident MN relative to other genotypes of CHIP/CCUS, respectively. We also classified genes in these variable groups as high risk. Although no association was observed between

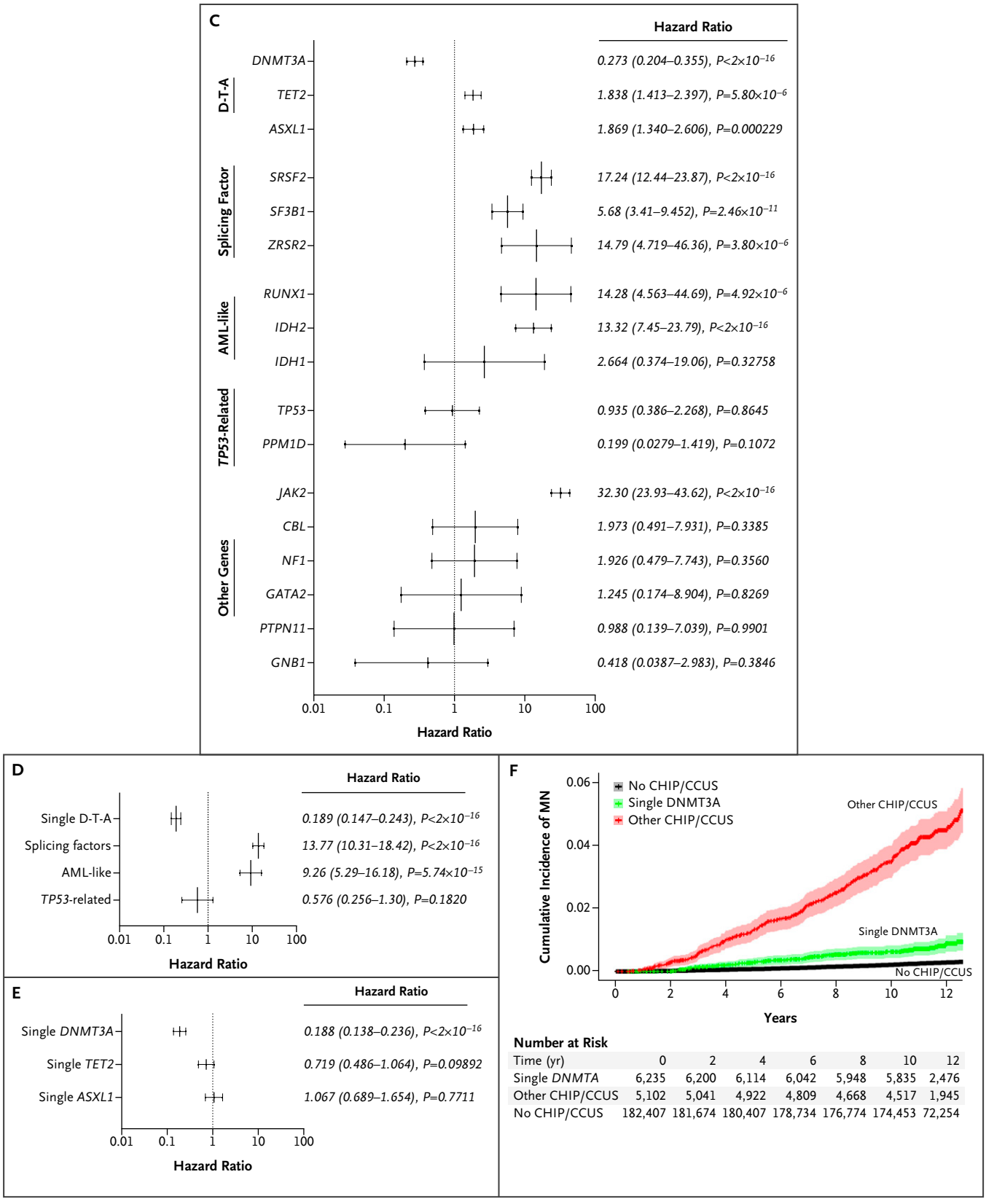


Figure 1. Continued.

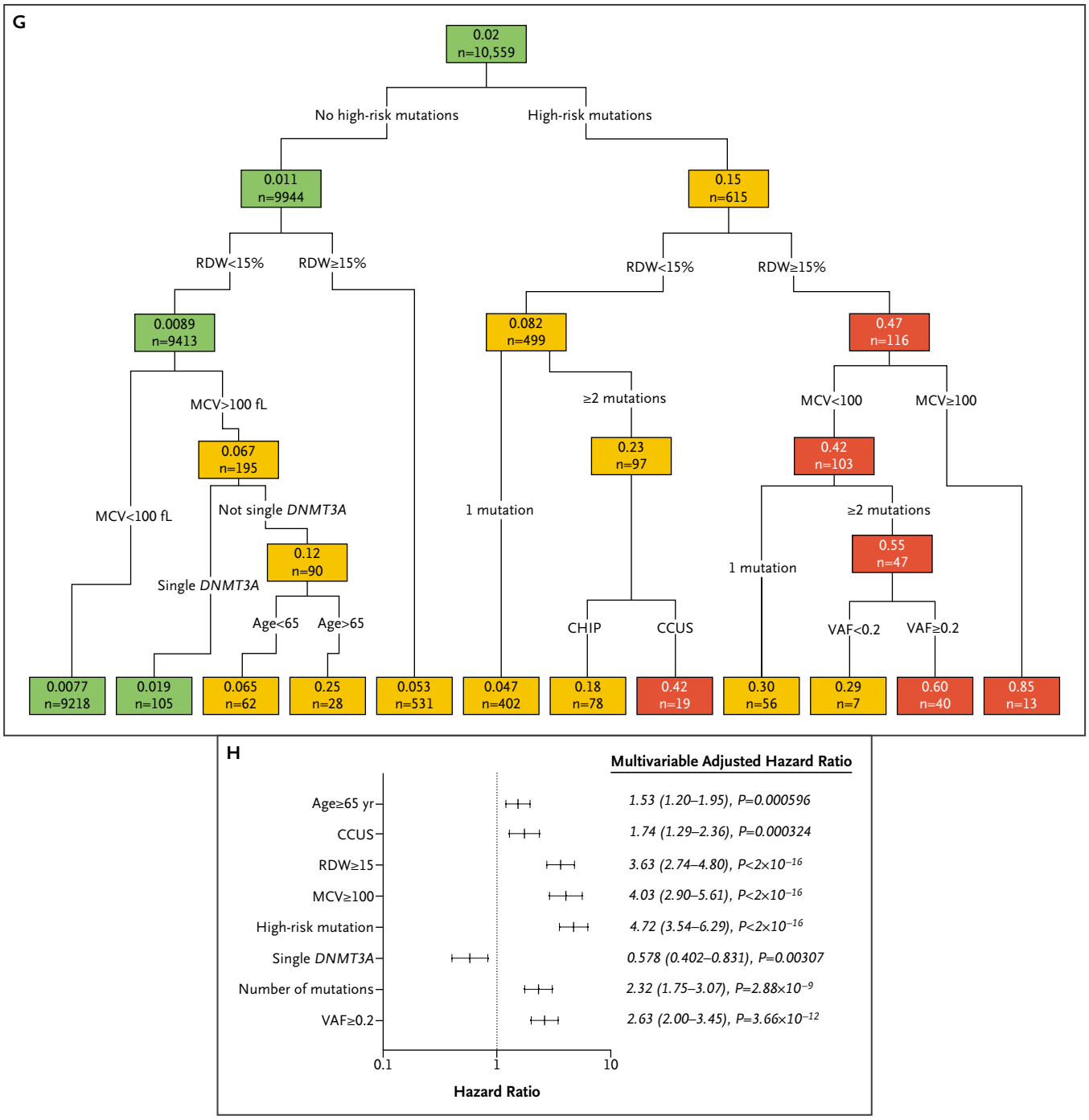


Figure 1. Continued.

TP53-related mutations and incident MN, we empirically added *TP53* mutations to our final list of high-risk mutations given prior data showing high penetrance for AML evolution,[12,13] poor outcomes in *TP53* mutant MDS/AML,[28-31] and the potential hazards of underestimating risk in *TP53* mutant CHIP/CCUS. Compared with individuals without CHIP/CCUS, those with *DNMT3A* mutant CHIP/CCUS had a 4.25-fold increased risk of MN (Fig. S3). However, MN risk was markedly lower in *DNMT3A* mutant CHIP/CCUS compared with other genotypes

(co-mutated *DNMT3A*: hazard ratio, 0.273 [95% confidence interval (CI), 0.209 to 0.355; $P<2\times10^{-16}$]; single *DNMT3A*: hazard ratio, 0.188 [95% CI, 0.138 to 0.236; $P<2\times10^{-16}$]; Fig. 1C to 1F).

CLONAL HEMATOPOIESIS RISK SCORE

We used conditional probability–based RP analysis to identify critical predictors of the 10-year probability of incident MN. A list of candidate variables used in the analysis is provided in the Supplemental Methods. High-risk mutations and single *DNMT3A* mutations were genotypes of the greatest importance for classification. Other partitioning variables included age 65 years or older, CCUS versus CHIP, two or more mutations, a maximum VAF of 0.2 or greater (for any CH variant), mean corpuscular volume of 100 fl or greater, and RDW of 15% or greater (Fig. 1G). Within sample size limitations, severity of cytopenia was not identified as a key partitioning variable. With the exception of single *DNMT3A* which had a lower risk of MN compared to other genotypes, cumulative incidence of MN was higher for patients with CHIP/CCUS possessing each feature compared with those lacking that feature (Fig. S4 and Table S6).

RP analysis distinguished groups of patients with CHIP/CCUS with a probability of incident MN by year 10, ranging from 0.0077 to 0.85 (Fig. 1G), highlighting marked variability in risk of MN. Features identified in RP retained statistical significance in multivariable Cox regression analysis (Fig. 1H). Regression coefficients (Table S6) for each variable were rounded to the nearest 0.5 and increased by 1, providing weighted scores for each prognostic variable (Table 2). CHRS values were calculated as the sum of scores for each prognostic variable. Most patients with CHIP/CCUS had low CHRS values (Fig. 2A). Score boundaries for risk groups were selected to prioritize creation of a low-risk group with 10-year probability of incident MN less than 2%. Three risk groups were defined (Fig. 2B and Table S7): high risk (CHRS ≥12.5, n=123 [1.1%]), intermediate risk (CHRS 10 to 12, n=1196 [10.5%]), and low risk (CHRS ≤9.5, n=10,018 [88.4%]). Sample CHRS calculations and a provisional calculator are provided in the Supplemental Methods. The 10-year cumulative incidence of MN was 52.2±4.96%, 7.83±0.807%, and 0.669±0.0827% in the high-, intermediate-, and low-risk CHIP/CCUS groups, respectively (Fig. 2B and 2C).

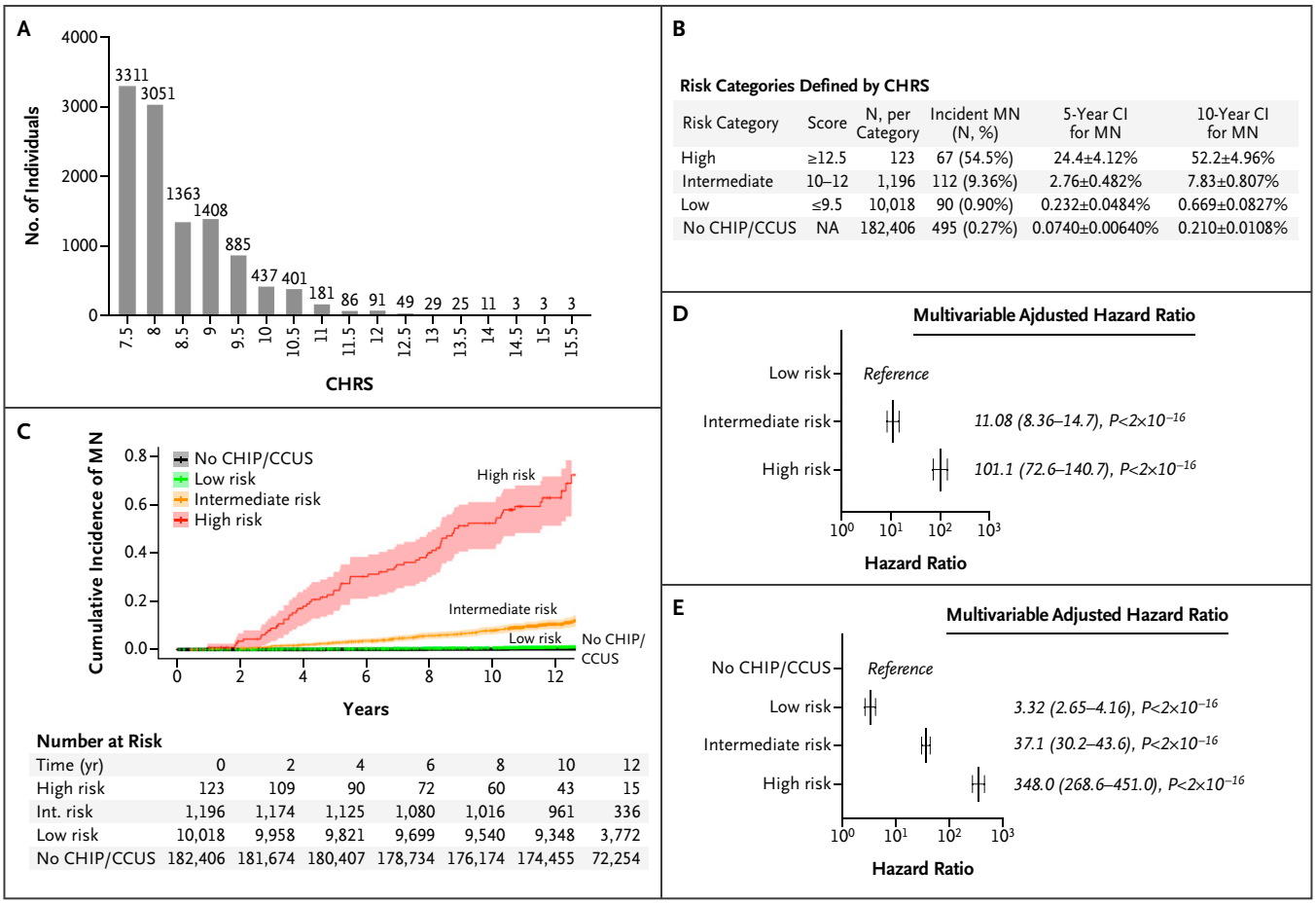
ROC analysis for the CHRS at each year of observation indicated an overall model c-index of 0.807±0.016 in the UKB derivation cohort. Notably, empiric addition of *TP53* mutations as high risk did not significantly affect the model c-index (Fig. S5). Relative to low-risk CHIP/CCUS, risk of incident MN was 11.8- and 101.1-fold higher in intermediate- and high-risk CHIP/CCUS ($P<2\times10^{-16}$; Fig. 2D). When we compared risk of incident MN in CHRS risk groups with individuals without CHIP/CCUS, risk increases of 3.32-, 37.1-, and 348-fold were observed in low-, intermediate-, and high-risk CHIP/CCUS, respectively ($P<2\times10^{-16}$; Fig. 2E).

CHRS groups displayed clear survival differences. In high-risk CHIP/CCUS, 10-year survival was 51.2±4.51% compared with 84.0±1.06% in intermediate-risk CHIP/CCUS, 93.7±0.243% in low-risk CHIP/CCUS, and 95.8±0.0471% in individuals without CHIP/CCUS (Fig. 2F). Higher risks for CH-related comorbidities, including ischemic cardiovascular disease, arterial and venous thromboembolic disease, chronic kidney disease, and chronic obstructive pulmonary disease, were observed in high-risk CHIP/CCUS compared with intermediate- and low-risk CHIP/CCUS (Fig. 2G and Table S8).

Table 2. CHRS Values.*

Prognostic Variable	0.5	1	1.5	2	2.5
Single *DNMT3A*	Present	Absent			
High-risk mutation		Absent			Present
Mutation number		1		≥2	
Variant allele fraction		<0.2		≥0.2	
Red cell distribution width		<15			≥15
Mean corpuscular volume		<100			≥100
Cytopenia		CHIP	CCUS		
Age (yr)		<65	≥65		

* CCUS denotes clonal cytopenia of undetermined significance; CHIP, clonal hematopoiesis of indeterminate potential; and CHRS, clonal hematopoiesis risk score.



Risk Category	Score	N, per Category	Incident MN (N, %)	5-Year CI for MN	10-Year CI for MN
High	≥12.5	123	67 (54.5%)	24.4±4.12%	52.2±4.96%
Intermediate	10–12	1,196	112 (9.36%)	2.76±0.482%	7.83±0.807%
Low	≤9.5	10,018	90 (0.90%)	0.232±0.0484%	0.669±0.0827%
No CHIP/CCUS	NA	182,406	495 (0.27%)	0.0740±0.00640%	0.210±0.0108%

Time (yr)	0	2	4	6	8	10	12
High risk	123	109	90	72	60	43	15
Int. risk	1,196	1,174	1,125	1,080	1,016	961	336
Low risk	10,018	9,958	9,821	9,699	9,540	9,348	3,772
No CHIP/CCUS	182,406	181,674	180,407	178,734	176,174	174,455	72,254

Figure 2. CHRS Distribution and Risk Stratification in the UKB Derivation Cohort.

(Panel A) Number of individuals with each possible clonal hematopoiesis risk score (CHRS) value (number of individuals with each score is indicated above the bar). (Panel B) Risk categories were defined by CHRS value, with cutoffs chosen to minimize risk in low-risk strata. For each category, the number of individuals in the risk group, number of myeloid neoplasms (MN) events, and crude event rate (N, %), as well as the 5- and 10-year cumulative incidences (±standard deviation), are shown. Cumulative incidence of MN for individuals without clonal hematopoiesis of indeterminate potential (CHIP) or clonal cytopenia of undetermined significance (CCUS) (No CHIP/CCUS) in the derivation cohort is included for reference. (Panel C) Cumulative incidence curves of MN according to CHRS risk category. Curves correspond to the cumulative incidence analysis used to derive figures in Panel B. Hazard ratios for incident MN were determined for CHRS risk strata using Cox proportional-hazards models adjusted for sex, smoking history, and history of cancer. Hazard ratios were calculated in models with low-risk strata as the reference population (Panel D) and using the population of 182,406 U.K. Biobank (UKB) participants in the No CHIP/CCUS group as the reference population (Panel E). (Panel F) Survival according to CHRS risk category is shown, with 10-year survival annotated to the right of the graph for each category. For both cumulative incidence and survival curves, black = No CHIP/CCUS, green = Low risk, orange = Intermediate risk, and red = High risk. The ribbon about each curve indicates the 95% confidence interval (CI). The table shows the number at risk. (Panel G) Results of Cox regression analyses for nonmalignant outcomes according to CHRS risk group. For all outcomes, No CHIP/CCUS is the reference population. Outcomes shown include ischemic cardiovascular disease (CVD), which is a composite of atherosclerosis, ischemic heart failure, myocardial infarction, and stroke; arterial thromboembolic events (ATE); venous thromboembolic events (VTE); chronic kidney disease (CKD); and chronic obstructive pulmonary disease (COPD). For Panels D, E, and G, forest plots indicate hazard ratios (95% CIs) and P values for main effects. Int. denotes intermediate; and NA, not applicable.

VALIDATION OF CHRS MODEL

We used a distinct subset of 245,147 UKB participants as a validation cohort. Validation and derivation cohort characteristics were similar (Table S9). Of the 16,274 individuals with CHIP/CCUS, 14,755 (90.6%) were at low risk for incident MN (Fig. S6 and Table S10), similar to the rate of low-risk CHIP/CCUS in the derivation cohort. The c-index for the CHRS in the validation cohort was 0.799±0.015,

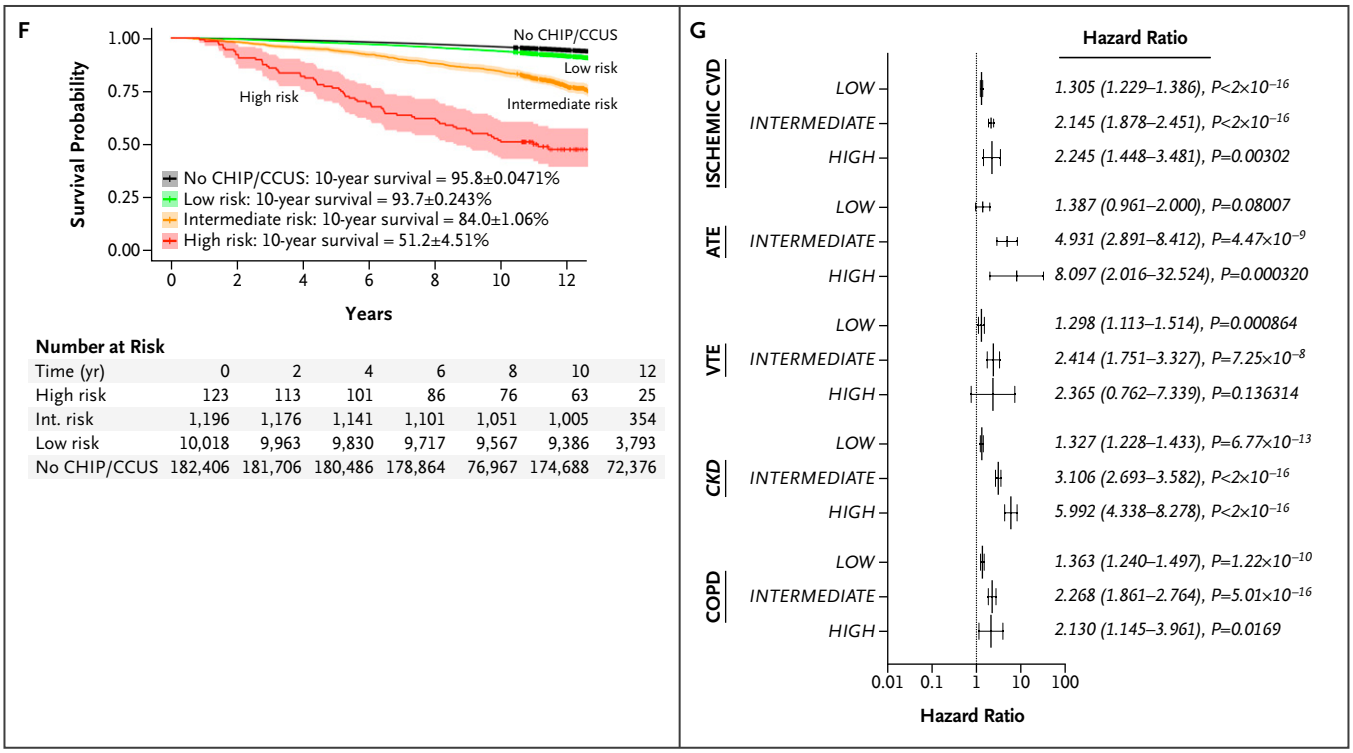


Figure 2. Continued.

indicating that the model performed equally well in both validation and derivation data sets.

Patients with CHIP/CCUS referred to hematology clinics within tertiary referral centers have a higher risk of developing MN relative to individuals with incidentally detected CH in population cohorts such as UKB. We applied the CHRS model to two independent patient cohorts for validation in the clinical setting. CH variants for the 646 patients in the DFCI/BWH CHIP/CCUS cohort were detected by clinical NGS[32] on peripheral blood or bone marrow (30.2%) in patients evaluated for unexplained cytopenia or for suspected CH identified on solid tumor or hereditary cancer sequencing panels. The Pavia CCUS cohort includes 99 patients with bone marrow biopsy–proven CCUS diagnosed in hematology clinics at the University of Pavia. Cohort characteristics are summarized in Table S11. Rates of mutations in *DNMT3A*, *TET2*, *ASXL1*, and high-risk genes are shown in Figure 3A and 3C.

Both hematology patient cohorts had higher CHRS values relative to UKB cohorts (Fig. 3B and 3D); however, the CHRS predicted MN outcomes well. Crude event rates for MN were 4.8% (n=31) and 31.3% (n=31) in the DFCI/BWH and Pavia cohorts, respectively. Most MN events occurred in high-risk patients, and the hazard ratio for MN was 40.3 (95% CI, 5.26 to 308) in the DFCI/BWH cohort and 8.80 (95% CI, 1.93 to 40.1) in the Pavia cohort (Fig. 3E). Despite brief follow-up, ROC analysis indicated a c-index of 0.788±0.040 for the DFCI/BWH cohort and 0.727±0.039 for the Pavia cohort (Fig. S7).

INFLUENCE OF mCA ON RISK OF MN

Chromosomal mosaicism is not yet routinely assessed in patients with cytopenia or CHIP/CCUS; therefore, mCAs were not used to derive the CHRS. However, 11.1% of low-risk CHIP/CCUS that progressed to MN had co-occurring mCA (Table S12). We analyzed the association of myeloid mCA with risk of incident MN. Cumulative incidence of MN was substantially higher in CHIP/CCUS, with one or more co-occurring with mCA (Fig. S8), consistent with prior reports of mCA as an independent risk factor for MN.[15,20,21,33-36] Among CHRS risk groups, MN risk was higher when mCA were present. In high-risk CHIP/CCUS, the 10-year cumulative incidence of MN was 83.3±8.20% with mCA and 43.1±5.61% without mCA. When mCA were treated as "high-risk" somatic alterations in the CHRS, 1.49% of

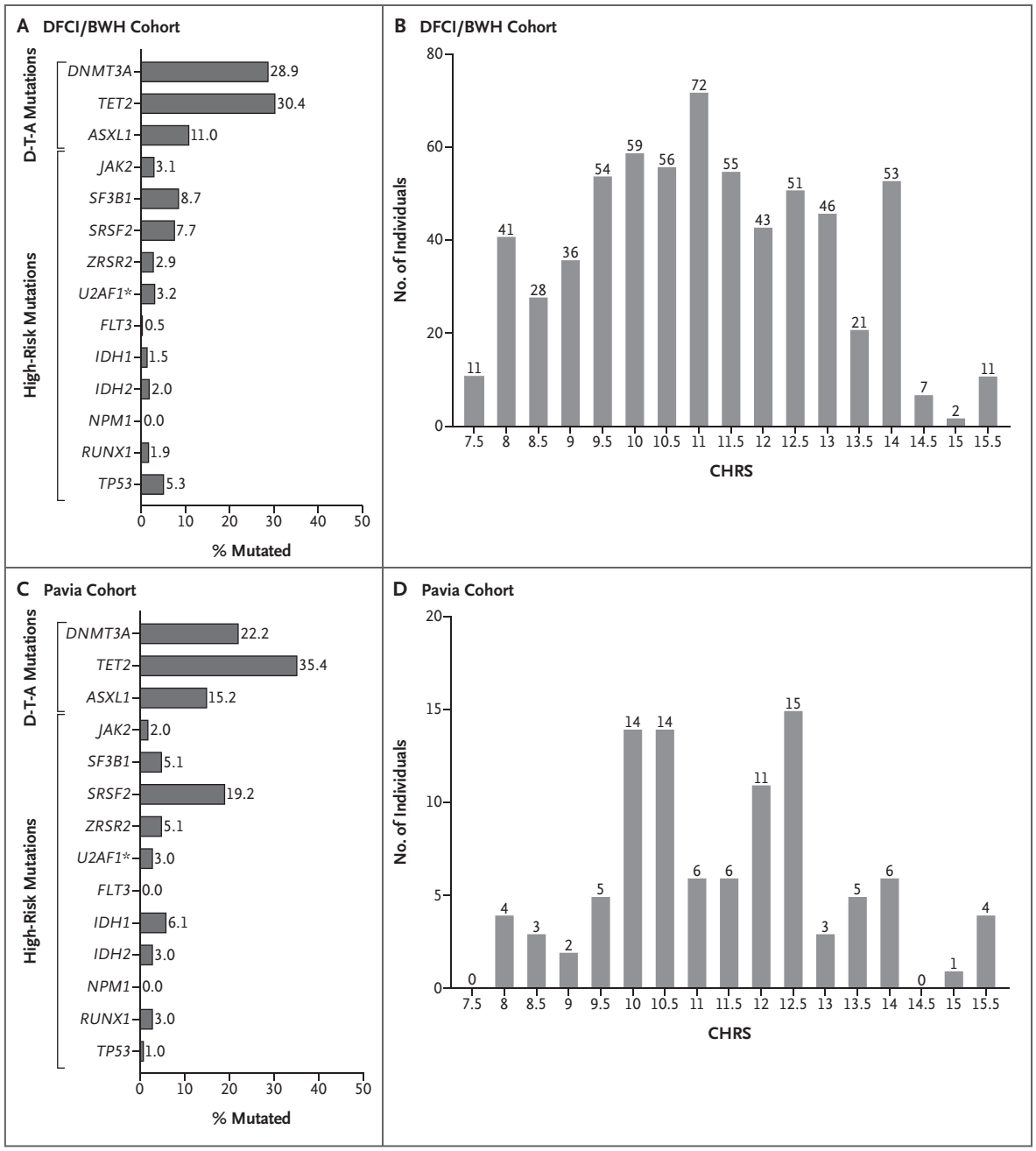


Figure 3. External Validation of CHRS in Hematology Patient Cohorts.

Panels A and C represent the distribution of mutations and Panels B and D represent the clonal hematopoiesis risk score (CHRS) values in the Dana-Farber/Brigham and Women's Hospital (DFCI/BWH) clonal hematopoiesis cohort and Pavia clonal cytopenia of undetermined significance (CCUS) cohort. (Panel E) The CHRS risk categories and outcomes (incident myeloid neoplasms [MN]) in the DFCI/BWH cohort (left) and Pavia cohort (right) are shown. The number of patients within each category is shown with percentage. The number of patients with incident MN events and the event rate (MN events relative to number of individuals in that category, expressed as a percentage) are shown. Cox proportional-hazards models were used to obtain hazard ratios (95% confidence intervals [CIs]) for each CHRS risk strata, and performance of the CHRS model is estimated by the concordance statistic (c-index ± standard error [s.e.]) when applied to each cohort. D-T-A denotes mutations in *DNMT3A*, *TET2*, or *ASXL1*. Ref. denotes reference.

E

CHRS Risk Categories and Clinical Outcomes in Hematology Patient Cohorts

	DFCI/BWH Cohort				Pavia Cohort			
	Total	Low	Intermediate	High	Total	Low	Intermediate	High
No. of patients	646 (100%)	170 (26.3%)	285 (64.1%)	191 (29.6%)	99 (100%)	14 (14.1%)	52 (52.5%)	34 (34.3%)
MN events	31	1	7	23	34	2	12	20
Event rate	4.8%	0.588%	2.46%	15.2%	34.3%	14.3%	23.1%	58.8%
Hazard Ratio (95% CI)	—	Ref.	4.53 (0.556–36.9)	40.3 (5.26–308)	—	Ref.	2.87 (0.631–13.1)	8.803 (1.93–40.1)
	Concordance (s.e.) = 0.788 (0.040)				*Concordance (s.e.) = 0.727 (0.039)*			

Figure 3. Continued.

patients with CHIP/CCUS were reclassified, resulting in a modest increase in the c-index to 0.826±0.15 (Fig. S9).

Discussion

Here, we report genetic and laboratory features required to predict incident MN in CHIP/CCUS and incorporate them into the CHRS, a novel prognostic model that predicts myeloid malignancy outcomes. The CHRS robustly defines three distinct CHIP/CCUS risk groups and shows the low absolute risk of progression to overt MN in the vast majority of CHIP and CCUS.

This large cohort analysis enabled estimates of absolute risk of incident MN, unlike prior case-control studies in CH.[9,10,14] Among UKB participants, individuals with CHRS-defined low-risk CHIP/CCUS (~90% of patients with CHIP/CCUS), similar to those without CHIP/CCUS, have less than a 1% 10-year cumulative incidence of MN, whereas individuals with high-risk CHIP/CCUS (1.1% of patients with CHIP/CCUS) have more than a 50% 10-year cumulative incidence of MN.

CHRS risk groups correlated well with survival and select CH-related nonmalignant comorbidities such as ischemic cardiovascular disease.[37,38] Given the prevalence of CHIP/CCUS, this finding has broad implications. Shared predictors allude to common pathophysiology and the potential for early intervention strategies that improve overall survival via prevention of both malignant and nonmalignant outcomes in CHIP/CCUS. Dedicated risk models for nonmalignant outcomes would clarify this possibility.

We definitively show marked genotype specificity for risk of progression in CHIP/CCUS, as observed in MDS.[39-42] Specifically, our data highlight a more benign risk profile for patients with *DNMT3A* mutant CHIP/CCUS, particularly for those with single *DNMT3A*, who have an 80% lower risk of MN relative to other genotypes. These findings are consistent with lower growth rates for *DNMT3A* mutant hematopoietic stem cell clones compared with other CH mutations[43] and with low rates of progression to donor-derived MDS/AML in stem cell transplant recipients with *DNMT3A* mutant donor–derived CH.[44] We also established a category of high-risk mutations that includes mutations in splicing factor genes (*SRSF2*, *SF3B1*, and *ZRSR2*), AML-like genes (*IDH1*, *IDH2*, *FLT3*, and *RUNX1*), *JAK2*, and *TP53*. Notably, the splicing factor *U2AF1* is absent from our analysis because of an erroneous duplication on chromosome 21 in the hg38 reference genome,[45] which precluded reliable calls of *U2AF1* variants. Recent data have shown the rapid clonal expansion and high rate of transformation to myeloid malignancy[46] for *U2AF1* mutant CH. Data sets with reliable *U2AF1* variant calls are necessary to confirm that mutations in *U2AF1*, similar to other splicing factor genes, are high risk.

CHRS performance was not significantly altered by the empiric inclusion of *TP53* as a high-risk mutation. Heterogeneity of MN risk among *TP53* mutant CHIP/CCUS is probable, with outcomes influenced by clonal selection pressures such as chemotherapy,[47] clone size,[48] allelic state,[49] and clonal complexity.[50] Although assessment of the aforementioned factors and variant-specific risk may enhance the precision of CH risk algorithms, we were constrained by the lack of serial samples, limited information about timing and duration of selection pressures, and small sample sizes for specific variants. The importance of clonal complexity and allelic state enhances the case for assessment of mCA in patients with CHIP/CCUS, and our analysis indicates these are best incorporated into the CHRS as one would incorporate high-risk mutations.

Compared with prior published models predicting risk of MN in adults,[51,52] the CHRS permits risk assessment using

objective data obtained at a single time point from two peripheral blood tests: an NGS panel and a complete blood count with differential. Despite limited duration of follow-up, in typical patients with CHIP/CCUS, the CHRS distinguishes individuals who are high and low risk for progression to MN. A c-index of more than 0.7 in two independent cohorts validates the CHRS in clinical settings. To facilitate clinical uptake, the CHRS calculator derived in this article is available in the Supplementary Appendix at https://evidence.nejm.org/ and at www.chrsapp.com.

CHIP/CCUS provides a substrate for developing early cancer detection programs that center the identification of somatic mutations in cancer driver genes. However, because somatic mosaicism is ubiquitous, and CHIP/CCUS is highly prevalent[53] with substantial heterogeneity in outcomes, health systems would become overwhelmed if all individuals with CHIP/CCUS were referred for extensive hematologic evaluation. The CHRS provides an intuitive and adoptable framework for prognostication in CHIP/CCUS. In so doing, the CHRS aids clinical decision-making and research, allowing prioritization of intensive surveillance and therapeutic intervention in the minority of patients with CHIP/CCUS who are most likely to progress to overt MN.

Disclosures

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