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Clonal haematopoiesis of indeterminate potential and impaired kidney function—A Danish general population study with 11 years follow-up

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Abstract

The myeloproliferative neoplasms are associated with chronic kidney disease but whether clonal haematopoiesis of indeterminate potential (CHIP) is associated with impaired kidney function is unknown. In the Danish General Suburban Population Study ($N = 19\,958$) from 2010 to 2013, 645 individuals were positive for *JAK2V617F* ($N = 613$) or *CALR* ($N = 32$) mutations. Mutation-positive individuals without

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haematological malignancy were defined as having CHIP ($N = 629$). We used multiple and inverse probability weighted (IPW)-adjusted linear regression analysis to estimate adjusted mean (95% confidence interval) differences in estimated glomerular filtration rate (eGFR; ml/min/1.73 m^2) by mutation status, variant allele frequency (VAF%), blood cell counts, and neutrophil-to-lymphocyte ratio (NLR). We performed 11-year longitudinal follow-up of eGFR in all individuals. Compared to CHIP-negative individuals, the mean differences in eGFR were −5.6 (−10.3, −0.8, $p = .02$) for *CALR*, −11.9 (−21.4, −2.4, $p = 0.01$) for *CALR* type 2, and −10.1 (−18.1, −2.2, $p = .01$) for *CALR* with VAF ≥ 1%. The IPW-adjusted linear regression analyses showed similar results. NLR was negatively associated with eGFR. Individuals with *CALR* type 2 had a worse 11-year longitudinal follow-up on eGFR compared to CHIP-negative individuals ($p = .004$). In conclusion, individuals with *CALR* mutations, especially *CALR* type 2, had impaired kidney function compared to CHIP-negative individuals as measured by a lower eGFR at baseline and during 11-year follow-up.

KEYWORDS
CALR, CHIP, clonal haematopoiesis of indeterminate potential, eGFR, epidemiology, impaired kidney function, *JAK2V617F*, population studies

1 | INTRODUCTION

The Philadelphia-chromosome negative classical myeloproliferative neoplasms (MPNs) cover essential thrombocythaemia (ET), polycythaemia vera (PV) and primary myelofibrosis (PMF).[1,2] MPNs are caused by acquired somatic driver mutations in the haematopoietic stem cells, including *JAK2V617F*, *CALR* and *MPL* mutations.[3–5]

In contrast, clonal haematopoiesis of indeterminate potential (CHIP) constitutes an age-dependent acquisition of leukaemia-associated mutations in peripheral blood, typically with a variant allele frequency (VAF) >2%,[6] but without the presence of a haematological malignancy.[7–9] However, the clinical impact of clones <2% is largely unknown. Clonal haematopoiesis (CH) and CHIP are associated with an increased risk of cardiovascular disease (CVD),[10,11] chronic obstructive pulmonary disease,[12] autoimmune vasculitis,[13] haematological and non-haematological malignancies.[14,15]

MPN is associated with several chronic inflammatory-mediated diseases[16,17] and a progressive reduction in estimated glomerular filtration rate (eGFR).[18] In addition, in patients with MPN, moderate to severe chronic kidney disease (CKD) is associated with thrombosis,[19,20] disease severity[20] and reduced survival.[21] CKD in MPN may be caused by an underlying glomerulonephritis[22] such as focal segmental glomerulosclerosis, mesangial sclerosis and hypercellularity and renal interstitial extramedullary haematopoiesis.[23–25] Clonal myelopoiesis has just recently been associated with CKD in the UK Biobank using whole exome sequencing but without measurement of *CALR* mutations.[26] Although only supported by a single case-report, *CALR* may be associated with MPN nephropathy,[23] as calreticulin is involved in fibrosis.[27]

We, therefore, hypothesised that CHIP mutations, including the *JAK2V617F* or *CALR* mutations, is associated with impaired kidney function in the Danish General Suburban Population Study (GESUS).

2 | MATERIALS AND METHODS

2.1 | Study population

From 2010 to 2013, the GESUS study enrolled 19 958 individuals age ≥20 years who consented to research and return of results critical for health care.[9] GESUS was approved by the regional ethical committee (SJ-114, SJ-452), the Danish Data Protection Agency (REG-50-2015) and adheres to the Declaration of Helsinki. The health examination included anthropometric, haematological and biochemical measurements, and a detailed questionnaire as previously described.[28]

2.2 | Measurement of *JAK2V617F* and *CALR* mutation

In GESUS, molecular screening was performed using a multiplex droplet digital PCR (ddPCR) assay as previously described.[9] Briefly, DNA from four individuals were pooled and evaluated for both *JAK2V617F* and *CALR* type 1 and type 2. Both assays were multiplexed with wild-type. If mutation-positive, the four samples were re-analysed separately for either *JAK2V617F* or *CALR* to identify the positive sample(s) and quantify the mutant VAF (%). The sensitivity of the assays was 0.009% for *JAK2V617F* and 0.01% for *CALR* types 1 and 2.[9] Thus, CHIP was defined as the presence of *JAK2V617F* or *CALR*, irrespective of the VAF, and absence of haematological disease at study entry.

Among 645 mutation-positive individuals, 16 were diagnosed with MPN disease (ET = 4, PV = 10, PMF = 1, PreMF = 1) at the time of study entry. Hence, 629 individuals were eligible for inclusion and considered CHIP positive, including 599 with *JAK2V617F* and 30 with *CALR* (Figure S1). Currently, there is an ongoing clinical follow-up of CHIP-positive individuals.

2.3 | Blood samples

Non-fasting blood samples were drawn, and pre-analytically managed according to institutional guidelines at the Department of Clinical Biochemistry, Naestved Hospital, Denmark.[28] Haematological blood cell counts were analysed from fresh EDTA whole blood using Sysmex XE-5000 (Sysmex Corporation). A composite dichotomous variable for elevated blood cell counts in at least one cell type was defined according to regional laboratory reference values and depending on sex: haemoglobin concentration >10.5 mmol/L (male) or >9.5 mmol/L (female), haematocrit >0.50 (male) or >0.46 (female), erythrocytes $>5.7 \times 10^{12}$/L (male) or $>5.2 \times 10^{12}$/L (female), thrombocytes $>390 \times 10^{9}$/L, leukocytes $>8.8 \times 10^{9}$/L, neutrophils $>7.0 \times 10^{9}$/L, monocytes $>0.7 \times 10^{9}$/L, eosinophils $\geq 0.5 \times 10^{9}$/L, basophils $\geq 0.1 \times 10^{9}$/L and lymphocytes $>3.5 \times 10^{9}$/L. Neutrophil-to-lymphocyte ratio (NLR) and thrombocyte-to-lymphocyte ratio (PLR) were calculated as proxies for chronic inflammation.[29,30]

Biochemical variables (plasma creatinine [μmol/L], high-sensitive C-reactive protein [hsCRP; mg/L], cholesterol [mmol/L]) were analysed using Cobas-600 (Roche Diagnostics). We excluded individuals with a hsCRP ≥10 mg/L ($N = 857$) in the regression analysis to exclude individuals with potential infections. Plasma creatinine was measured using a multistep enzymatic assay with sarcosine as the primary intermediate metabolite, and subsequently oxidised by sarcosine oxidase.[31,32] Renal function was assessed by eGFR according to the eGFR EPI-CKD (ml/min/1.73 m^2) formula by Levey[33] based on plasma creatinine (μmol/L), age, sex, and race.

2.4 | Comorbidities

Information on self-reported health was obtained through questionaries at baseline. Smoking status was categorised as never, previous, or current smoker. Blood pressure was assessed during the examination. Body mass index (BMI) was calculated from weight (kg)/height (cm^2) and classified as underweight (<18.5 kg/cm^2), normal weight (18.5–24.9 kg/cm^2), overweight (25.0–29.9 kg/cm^2) and obese (≥30 kg/cm^2). Hypertension and dyslipidaemia were defined as use of antihypertensive or antilipidemic medication, respectively. Ischemic heart disease (IHD), a history of acute myocardial infarction and coronary heart disease was self-reported and validated through the Danish National Patient Registry[34] using the International Classification of Diseases version 8 and 10 (ICD-8: 410-414 and ICD-10: DI20-DI25). These data were used as prevalent data to assess the validity of self-reported myocardial infarction and coronary heart disease.

2.5 | Follow-up of eGFR

Among all 19 942 individuals, a longitudinal follow-up on plasma creatinine to calculate eGFR was obtained from study entry in GESUS until December 2021 using data from the regional laboratory system. Any incident diagnosis of CKD among individuals with a *CALR* mutation (ICD-10: N00-N06, N11-N19) was obtained from study entry in GESUS until June 2021 by reviewing electronic medical records (Figure S1).

2.6 | Statistics

We used Stata SE/14 (STATA Corp.), Rstudio 4.0.3, R package (ggplot2), and GraphPad Prism version 7 (GraphPad Inc.). A two-sided $p < .05$ was considered as statistically significant. Summary statistics were presented as mean and standard deviation (SD). hsCRP was logarithmically transformed to obtain geometric mean (SD). Pearson's χ^2 or Fisher's exact tests were used for categorical variables. An unpaired Student's *t*-test was used for continuous variables with equal variance between groups, whereas unequal variance between groups qualified a Welch's correction. For continuous variables only, age and sex-adjusted means (95% confidence interval [CI]) and *p* values were obtained using regression analysis.

We investigated if CHIP mutations were associated with increased NLR and PLR by multiple linear regression analysis adjusted for age, sex, blood pressure, BMI, smoking status, IHD, cholesterol and hsCRP (<10 mg/L).

We investigated if CHIP mutations and elevated blood cell counts were associated negatively with eGFR by multiple linear regression analysis with and without inverse probability weighting (IPW). IPW-adjusted multiple regression analysis was used to obtain mean (95% CI) outcome differences by balancing confounding variables between individuals with or without CHIP, and individuals with elevated versus normal blood cell counts. The analyses were stratified by CHIP mutation type (*JAK2V617F* and *CALR*), subtype (*CALR* type 1 and type 2), and VAF (<1% and ≥1%). All regression analyses were adjusted for age, sex, blood pressure, BMI, smoking status, IHD, NLR, PLR, cholesterol and hsCRP (<10 mg/L). The regression analyses for elevated blood cell counts were not adjusted for NLR or PLR. The IPW-adjusted multiple regression analysis for *CALR* was not adjusted for PLR, since we were unable to balance PLR to CHIP-negative individuals.

We also investigated if increased NLR associated negatively with eGFR by multiple linear regression analysis adjusted for age, sex, blood pressure, BMI, smoking status, IHD cholesterol, hsCRP (<10 mg/L) and PLR. To visualise the distribution of eGFR on NLR, a two-dimensional density contour plot was applied.

Finally, we compared eGFR longitudinally in all individuals. We calculated an annual mean eGFR for each year of follow-up if participants had more than one value per year. We used repeated measures analysis of variance (ANOVA) with both follow-up time and age as

TABLE 1 Baseline characteristics by mutation status in the Danish General Suburban Population Study

Characteristics	CHIP-negative		CHIP		
	N	%/Mean (SD)	*N*	%/Mean (SD)	*p* Value
Number of participants	19 313	96.8	629	3.2	*NA*
Sex					
Women	10 566	54.7	285	45.3	3.2×10^{-6}
Men	8747	45.3	344	54.7	
Age (years)	19 313	56 (13.6)	597	60 (12.9)	4.4×10^{-14}
BMI (kg/m^2)					
<18.5	193	1	5	0.8	.2
18.5–24.9	7392	38.4	230	36.7	
25–29.9	7656	39.8	274	43.7	
≥30	3990	20.8	118	18.8	
Smoking					
Never smoker	8486	43.9	260	41.3	.4
Former smoker	7384	38.2	246	39.1	
Current smoker	3 443	17.8	123	19.6	
Systolic pressure (mmHg)	19 281	141 (21.4)	629	144 (22.2)	.0005
Diastolic pressure (mmHg)	19 289	85 (11.2)	629	86 (11.4)	.3
CVD					
Ischemic heart disease	1471	7.6	61	9.7	.05
Hypertension	4340	22.5	190	30.2	5.2×10^{-6}
Hyperlipidaemia	2778	14.4	120	19.1	.001
Laboratory test					
VAF (%)	–	–	629	1.2 (5.1)	*NA*
Leukocytes ($\times 10^9$/L)	19 267	7.3 (1.9)	627	7.6 (2.0)	.00002
Neutrophils ($\times 10^9$/L)	19 067	4.1 (1.3)	621	4.4 (1.4)	8.9×10^{-6}*
Eosinophils ($\times 10^9$/L)	19 075	0.19 (0.1)	622	0.21 (0.1)	.002
Basophils ($\times 10^9$/L)	19 075	0.04 (0.4)	622	0.04 (0.03)	.3*
Lymphocytes ($\times 10^9$/L)	19 080	2.3 (0.7)	622	2.3 (0.8)	.7*
Monocytes ($\times 10^9$/L)	19 078	0.56 (0.2)	622	0.59 (0.2)	.0001*
Thrombocytes ($\times 10^9$/L)	19 258	250 (57.3)	627	281 (99.5)	4.4×10^{-14}*
Erythrocytes ($\times 10^{12}$/L)	19 267	4.6 (0.7)	627	4.7 (0.4)	.0002*
Haemoglobin (mmol/L)	19 266	8.7 (0.8)	627	8.8 (0.8)	.0001
Haematocrit (ratio)	19 267	0.43 (0.03)	627	0.43 (0.04)	.00002*
hsCRP (mg/L)	19 275	1.43 (3.0)	628	1.30 (2.8)	.02*
Creatinine (μmol/L)	19 283	76.4 (20.0)	628	78.0 (17.0)	.02*
Total cholesterol (mmol/L)	19 283	5.5 (1.0)	628	5.4 (1.1)	.3
NLR	19 066	1.9 (0.9)	621	2.1 (1.0)	.0008*
PLR	19 072	116 (42.5)	622	131 (68.0)	1.2×10^{-7}*

Note: *p* Values obtained using χ^2 test for categorical data and independent *t*-test for continous data or independent *t*-test with Welch's correction *.
Abbreviations: BMI, body mass index; CHIP, clonal haematopoiesis of indeterminate potential; NA, not applicable; NLR, neutrophil/lymphocyte ratio; PLR, thrombocyte/lymphocyte ratio; VAF, variant allele frequency.

underlying timescale with the addition of a random subject effect to account for repeated measures for each individual. In addition, we analysed interactions between CHIP mutation type and follow-up time or age on reduction in eGFR.

3 | RESULTS

Individuals with CHIP versus CHIP-negative were older ($p = 4.4 \times 10^{-14}$) and had a higher systolic blood pressure ($p = .0005$). The proportion of

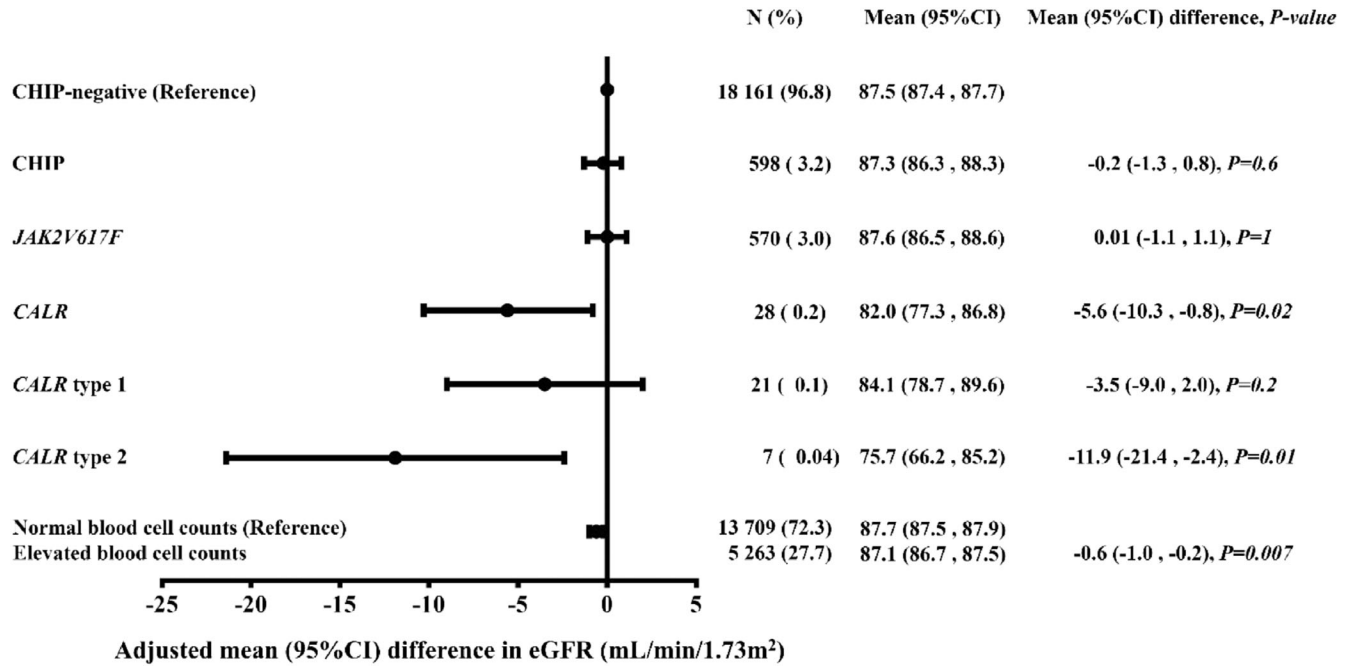


FIGURE 1 Adjusted mean (95% CI) difference in eGFR using linear regression analysis. CHIP includes *JAK2V617F* and *CALR*, *CALR* includes *CALR* type 1 and *CALR* type 2. *CALR*, calreticulin mutation includes type 1 and type 2 mutations; CHIP, clonal haematopoiesis of indeterminate potential; eGFR, estimated glomerular filtration rate; *JAK2V617F*, Janus kinase 2 (JAK2) valine-to-phenylalanine substitution at codon 617

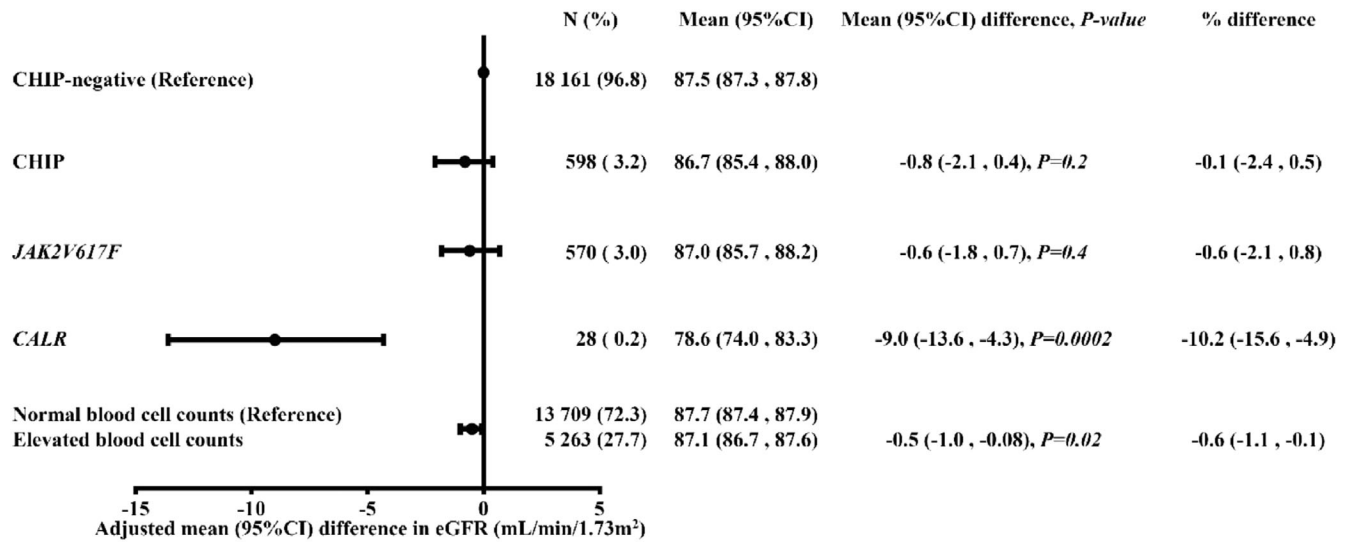


FIGURE 2 Adjusted mean (95% CI) difference in eGFR using inverse probability weighted regression analysis. CHIP includes *JAK2V617F* and *CALR*, *CALR* includes *CALR* type 1 and *CALR* type 2. The estimate for *CALR* is without thrombocyte-to-lymphocyte adjustment with a mean (95% CI) of 87.6 (87.4, 87.9) for the mutation-negative participants. *CALR*, calreticulin mutation; CHIP, clonal haematopoiesis of indeterminate potential; eGFR, estimated glomerular filtration rate; *JAK2V617F*, Janus kinase 2 (JAK2) valine-to-phenylalanine substitution at codon 617

CVD were also higher among individuals with CHIP (Table 1). In addition, those with CHIP versus CHIP-negative had higher myeloid blood cell counts (Tables 1 and S1–S3), NLR (Figure S2) and PLR (Figure S3).

In the multiple linear regression analysis, eGFR was not different in individuals with CHIP ($p = .6$) or *JAK2V617F* ($p = 1.0$) compared to CHIP-negative individuals (Figure 1). However, eGFR in individuals with *CALR* was reduced compared to CHIP-negative individuals with a mean (95% CI) difference of −5.6 (−10.3, −0.8) ml/min/1.73 m^2, $p = .02$ (Figure 1). When stratifying by *CALR* mutation subtype, only *CALR* type 2 had reduced eGFR with a mean (95% CI) difference of −11.9 (−21.4, −2.4) ml/min/1.73 m^2, $p = .01$ (Figure 1). In the IPW-adjusted linear regression analysis (Figure 2), results were similar to the multiple linear regression analysis; however, due to the low

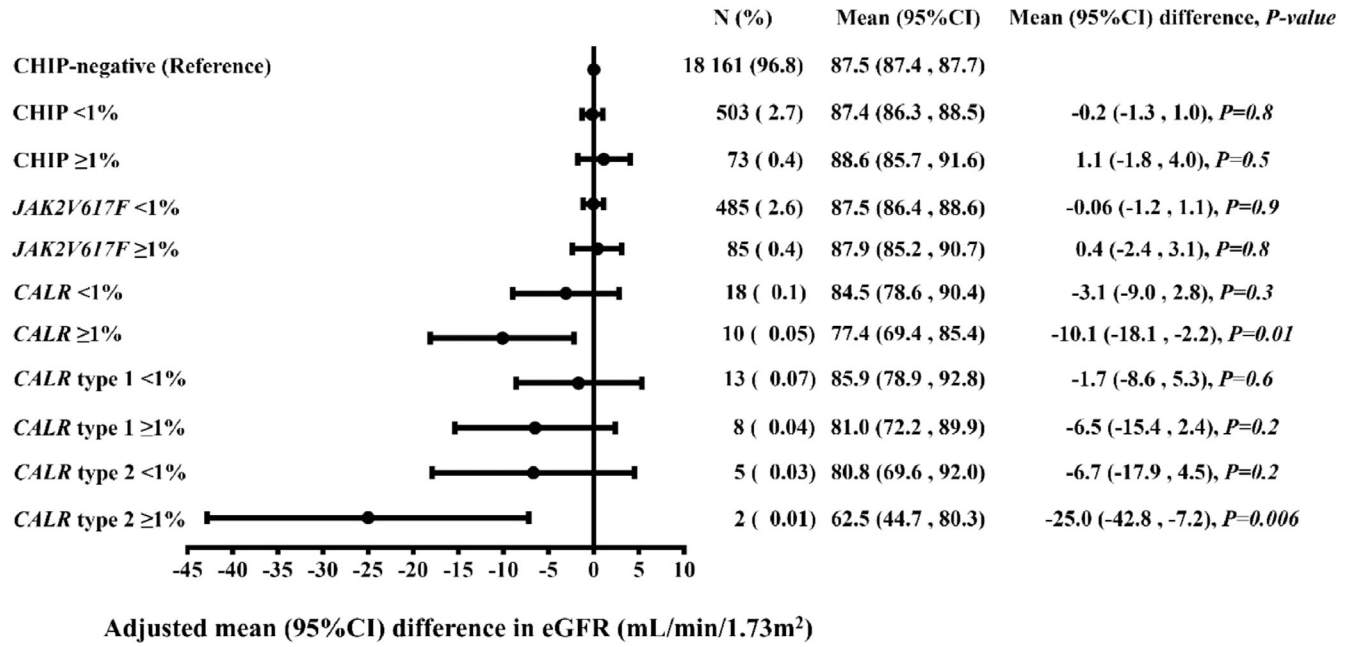


FIGURE 3 Adjusted mean (95% CI) difference in eGFR stratified by mutation type, subtype, and VAF% using linear regression analysis. CHIP includes *JAK2V617F* and *CALR* stratified by variant allele frequency (VAF%) if <1% or ≥1%. *CALR*, calreticulin mutation includes type 1 and type 2 mutations; CHIP, clonal haematopoiesis of indeterminate potential; eGFR, estimated glomerular filtration rate; *JAK2V617F*, Janus kinase 2 (JAK2) valine-to-phenylalanine substitution at codon 617

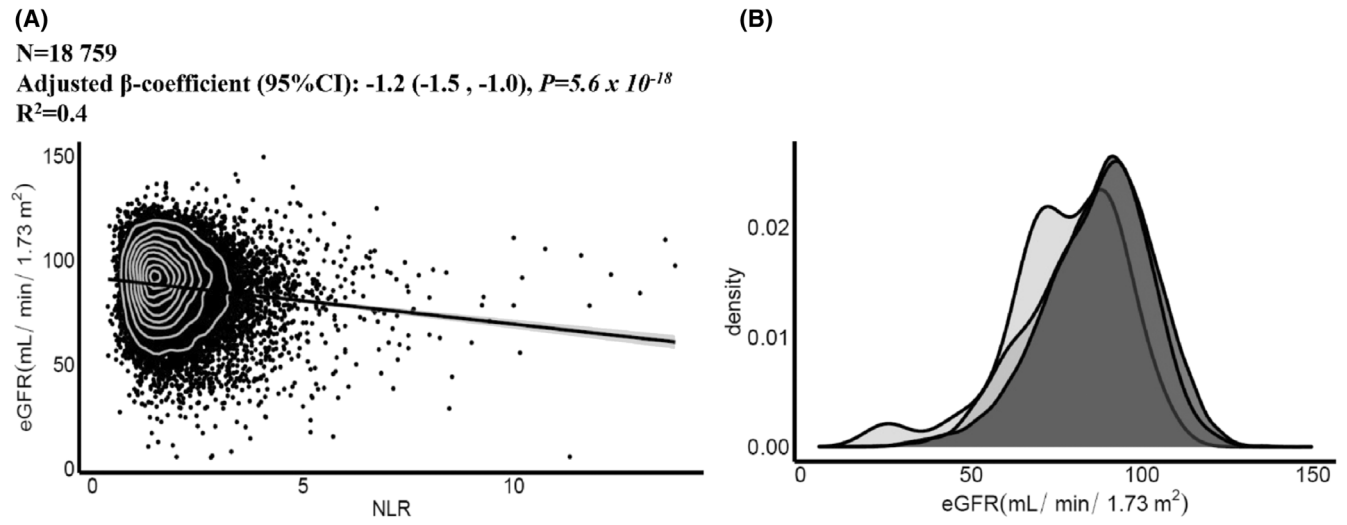
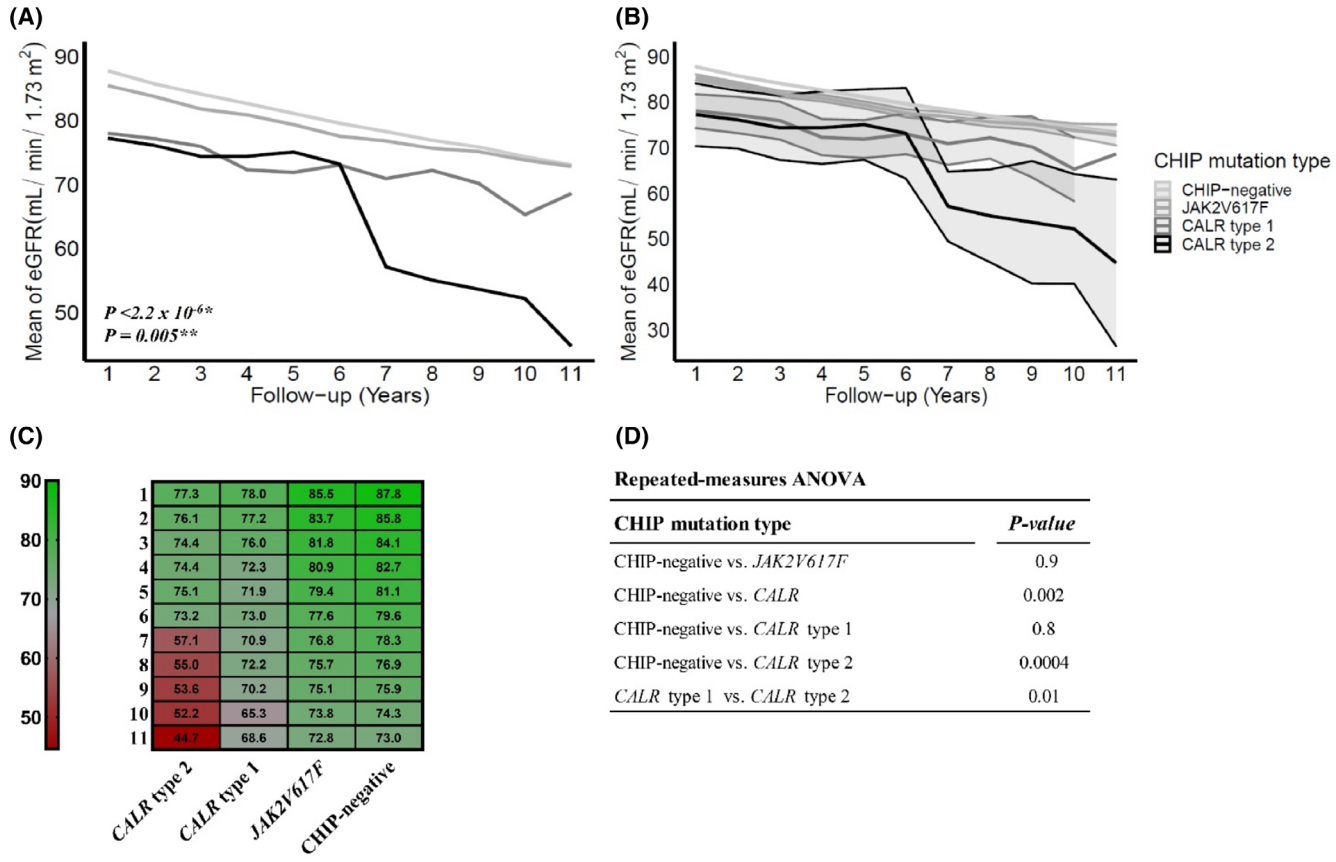


FIGURE 4 Scatterplot with linear regression line of eGFR on NLR. (A) The dark solid line represents the regression line (unadjusted) whereas the grey shading represents the 95% CI of the regression line. The 2D density contour plot was used to visualise the distribution of eGFR on NLR due to large dataset. (B) Density plot of eGFR was used to visualise the distribution for individuals without CHIP (black), *JAK2V617F* positive individuals (dark grey), and *CALR* positive individuals (light grey). The β coefficients are adjusted for age, sex, blood pressure, BMI, smoking status, IHD, total cholesterol, hsCRP (<10 mg/L) and PLR. eGFR, estimated glomerular filtration rate; NLR, neutrophils-to-lymphocyte ratio

prevalence of the *CALR* mutations we could not stratify by *CALR* type 1 and *CALR* type 2.

Individuals with *CALR* mutations and a VAF ≥1% had a reduced eGFR compared to CHIP-negative individuals with a mean (95% CI) difference of −10.1 (−18.1, −2.2) ml/min/1.73m^2, $p = .01$ (Figure 3). However, when stratifying by *CALR* mutation subtype and VAF only those with a *CALR* type 2 and a VAF ≥1% had a reduced eGFR compared to CHIP-negative individuals with a mean (95% CI) difference of −25.0 (−42.8, −7.2), $p = .0006$ (Figure 3).

Individuals with elevated versus normal blood cell counts had a reduced eGFR with a mean (95% CI) difference of −0.6 (−1.0, −0.2) ml/min/1.73m^2, $p = .007$ (Figure 1). Also, we observed that NLR was negatively associated with eGFR in the general population with an



CHIP mutation type	*P-value*
CHIP-negative vs. *JAK2V617F*	0.9
CHIP-negative vs. *CALR*	0.002
CHIP-negative vs. *CALR* type 1	0.8
CHIP-negative vs. *CALR* type 2	0.0004
CALR type 1 vs. *CALR* type 2	0.01

FIGURE 5 Longitudinal follow-up on eGFR. (A and B) Mean eGFR (ml/min/1.73 m^2) by follow-up in years with and without standard error. Longitudinal data was grouped by CHIP mutation type using grey-colour scale gradient. (C) Change in eGFR by each follow-up year grouped by CHIP mutation type and illustrated as a heatmap with double gradient from green to red. (D) Repeated-measures ANOVA with interaction between CHIP mutation types and follow-up time on reduction in eGFR. *ANOVA with random subject effect adjusted for age, sex, and follow-up time (years). **ANOVA with random subject effect adjusted for age and sex with interaction between *CALR* type and follow-up time (years) on eGFR. *CALR*, calreticulin; eGFR, estimated glomerular filtration rate; *JAK2V617F*, Janus kinase 2 (JAK2) valine-to-phenylalanine substitution at codon 617

adjusted β coefficient (95% CI) of −1.2 (−1.5, −1.0), $p = 5.6 \times 10^{-18}$ (Figure 4).

During 11 years of follow-up, the combined repeated measures ANOVA for the different CHIP mutation types had a more rapid decline in eGFR compared to CHIP-negative individuals (Figure 5), $p < 2.2 \times 10^{-6}$. Both follow-up time and age showed a significant interaction with CHIP mutation types on eGFR reduction, $p = .005$ and $p = 8.3 \times 10^{-5}$, respectively (Figures 5 and S4). In the stratified analysis, only *CALR* mutations and *CALR* type 2 showed significant interaction with follow-up time on eGFR reduction compared to CHIP-negative individuals. Also, individuals with *CALR* type 2 showed significant interaction with follow-up time and had a more rapid decline in eGFR compared to individuals with *CALR* type 1 (Figure 5). The annual mean eGFR in individuals with *CALR* type 2 reached CKD cut-off of 60 ml/min/1.73 m^2 only after 7 years of follow-up, corresponding to an average annual decline of 2 ml/min/1.73 m^2 per year. Only one individual, with *CALR* type 1, was diagnosed with CKD during this follow-up period.

4 | DISCUSSION

In the GESUS study, individuals with *CALR* mutations, particularly *CALR* type 2, had lower eGFR than CHIP-negative individuals. Furthermore, those individuals with *CALR* mutations and VAF ≥1% had lower eGFR than CHIP-negative individuals. However, in individuals with CHIP overall, *JAK2V617F* and *JAK2V617F* stratified by VAF, eGFR was not reduced. Increased NLR was negatively associated with eGFR. During 11 years of follow-up individuals with *CALR* mutations and *CALR* type 2 had a more rapid decline in eGFR compared to CHIP-negative individuals and individuals with *CALR* type 1, respectively. The findings for *CALR* are novel findings.

Although the mean eGFR at baseline in individuals with *CALR* type 2 was reduced more than 10 ml/min/1.73 m^2 compared to CHIP-negative individuals, it did not reach the CKD cut-off of less than 60 ml/min/1.73 m^2 at baseline.[35] However, the annual mean eGFR in individuals with *CALR* type 2 reached CKD cut-off only after 7 years of follow-up, corresponding to an average annual decline of

2 ml/min/1.73 m^2 per year. The reduction of eGFR in individuals with *CALR* mutations cannot only be explained by an age-related decline, which on average is 1 ml/min/1.73 m^2 per year by the CKD-EPI formula[36] since we observed no significant difference in age between *CALR* type 1 and type 2.

The clinical impact of *CALR* type 1 and 2 is different between ET[37] and PMF,[38] with *CALR* type 2 exhibiting the most unfavourable phenotype in PMF patients.[38] Prior evidence suggests that *CALR* may be associated with fibrotic diseases through the TGF-β signalling pathway[27,39,40] and that the extent of circulating calreticulin (CALR) in myelofibrosis patients seem to correlate with disease severity.[41] Although only supported by a single case-report, *CALR* may be associated with MPN nephropathy,[23] as calreticulin is involved in fibrosis.[27]

We observed that NLR was negatively associated with eGFR, and that individuals with the *CALR* mutation have an increased NLR compared to CHIP-negative individuals. This may indicate that a hyperproliferative and chronic inflammatory drive of the myeloid compartment may contribute to a reduced eGFR. This finding is supported by previous evidence that: patients with *CALR* positive MPN have aberrant IL-6 signalling[42] and that IL-6 is correlated with NLR.[29] Although individuals with elevated versus normal blood cell counts had lower eGFR, the magnitude of the difference was negligible and not of clinical importance. Thus, elevated blood cell counts are not a good indicator for impaired kidney function.

Despite the impaired kidney function among individuals with *CALR* type 2, no individuals with *CALR* type 2 and only one individual with type 1 was diagnosed with CKD during follow-up. This may indicate that impaired kidney function among *CALR* positive individuals is potentially underdiagnosed. Also, it may indicate that not only do aberrant blood cell counts precede the MPN-diagnosis[43] but also that the development of MPN-related nephropathy could be facilitated through a chronic inflammatory state as reflected by an increased NLR, which is negatively associated with eGFR, in our analysis. This is in part supported by the proportion of moderate to severe CKD among MPN patients at the time of diagnosis,[16] but also by the observation that neutrophilia associate with kidney dysfunction.[20]

Individuals with CHIP and the *JAK2V617F* mutation did not associate with reduced eGFR when compared to CHIP-negative individuals. This observation may be explained by the lower allele burden at study entry for the *JAK2V617F* positive individuals than the *CALR* positive individuals. Furthermore, it may suggest that a threshold of the *JAK2V617F* malignant clone is required to initiate, sustain and propagate a reduction in eGFR as observed in some MPN patients.[18,20] Interestingly, we also observed a lower eGFR in individuals with the *CALR* mutations and a VAF ≥1% indicating that both the mutation type and VAF is of importance for the impaired kidney function.

Most recently, clonal haematopoiesis with and without prevalent myeloid malignancy was associated with CKD.[26] Interestingly, CHIP *JAK2* and CHIP *TET2* were only associated with a reduced cystatin-C calculated eGFR, but not with creatinine calculated eGFR.[26] Similarly, in our study we did not observe an association between *JAK2V617F* and creatinine calculated eGFR, but we did not measure cystatin-C, as this kidney biomarker is not routine in Denmark. Thus, we cannot exclude that a cystatin-C based eGFR would have been a more sensitive biomarker for impaired kidney function in *JAK2V617F* in our study.

Several strengths and limitations are relevant to emphasise. The strengths were that despite the very low prevalence of *CALR* mutations in the general population, we were able to detect lower eGFR in both the traditional multiple linear regression and IPW-adjusted analysis. Furthermore, the findings were consistent in both the cross-sectional and longitudinal study with *CALR* mutations and *CALR* type 2 individuals having a lower eGFR than CHIP-negative individuals and individuals with *CALR* type 1, respectively. Finally, the longitudinal eGFR study were real-life heterogeneous data with more measurements in hospitalised patients than in non-hospitalised patients and with different indications of eGFR measurements during follow-up. Renal biopsies were not available to investigate the prevalence of histopathological characteristics associated with MPN-related glomerulopathy.[23–25] Although, the CHIP definition in some studies is defined as the absence of haematological malignancy with a VAF larger than 2%,[6] we defined CHIP as the absence of haematological malignancy with the presence of the somatic mutations *JAK2V617F* and *CALR*, irrespective of the VAF for two main reasons: first, the VAF cut-off of 2% is arbitrary reflecting analytical capabilities across different platforms. Second, little is known about the clinical impact of clones <2%. Further studies are required to validate if our findings are generalizable to other well-defined CHIP-cohorts derived from a large general population study, with CHIP defined as the presence of leukaemia-associated mutations and the absence of a haematological malignancy.

In conclusion, the *CALR* mutation, particularly *CALR* type 2, was associated with impaired kidney function at baseline. In individuals with *CALR* mutations, a VAF ≥1% also associated with impaired kidney function. Individuals with both *CALR* mutations and *CALR* type 2 had a faster decline in eGFR during 11 years of follow-up compared to CHIP-negative individuals and individuals with *CALR* type 1. Furthermore, we observed that individuals with CHIP had increased NLR. NLR was negatively associated with eGFR.

AUTHOR CONTRIBUTIONS

Christina Ellervik and Morten K. Larsen collected GESUS baseline data. Hans C. Hasselbalch, Christina Ellervik, Thomas Stiehl, Vibe Skov, Lasse Kjær and Morten K. Larsen designed the study. Morten Dahl extracted laboratory values for follow-up. Morten K. Larsen performed statistics with supervision by Christina Ellervik. Morten K. Larsen made tables and figures. Hans C. Hasselbalch, Christina Ellervik, Thomas Stiehl, Vibe Skov, Lasse Kjær and Morten K. Larsen interpreted the results. Morten K. Larsen wrote the paper. All authors contributed substantially to revision and interpretation. All authors approved the final version.

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CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

Due to the General Data Protection Regulation in the European Union, data from GESUS cannot be shared publicly. For queries about data access, contact Dr Christina Ellervik.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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