EClinicalMedicine 21 (2020) 100280

Contents lists available at ScienceDirect

EClinicalMedicine

journal homepage: https://www.journals.elsevier.com/eclinicalmedicine

Research Paper

Loss-of-function polymorphism in *IL6R* reduces risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm: A Mendelian randomization study

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ARTICLE INFO

Article History:
Received 22 October 2019
Revised 17 January 2020
Accepted 24 January 2020
Available online 19 February 2020

Keywords:
Mendelian randomization
Myeloproliferative neoplasm
Essential thrombocythemia
Polycythemia vera
Myelofibrosis
Drug target

ABSTRACT

Background: Whether inflammation is independently associated with development of *JAK2*V617F mutation and myeloproliferative neoplasm is not clear. We tested the hypothesis that a loss-of-function polymorphism in *IL6R* (marked by rs4537545) reduces risk of *JAK2*V617F mutation and myeloproliferative neoplasm in a Mendelian randomization study.

Methods: We genotyped 107,969 Danes from the Copenhagen General Population Study for the *IL6R* rs4537545 genotype, where the T-allele is associated with impaired interleukin-6 receptor signaling and reduced inflammation. *JAK2*V617F was examined in a subset of 49,143 individuals. We investigated the association between *IL6R* rs4537545 and risk of *JAK2*V617F using logistic regression and myeloproliferative neoplasm using Cox regression.

Findings: 36,871 were non-carriers, 52,500 heterozygotes, and 18,598 homozygotes for the T-allele of the *IL6R* rs4537545 genotype. Among 107,969 individuals, 352 were diagnosed with myeloproliferative neoplasm, and among 49,143 individuals, 62 were *JAK2*V617F-positive (of these 62 individuals, 46 had myeloproliferative neoplasm diagnosed). Compared to non-carriers, age- and sex-adjusted odds ratios for risk of *JAK2*V617F were 0·55 (95%CI:0·32–0·94) in heterozygotes, 0·51(0·24–1·12) in homozygotes, 0·54(0·33–0·89) in carriers, and 0·66 (0·45–0·96) per T-allele. Compared to non-carriers, age- and sex-adjusted hazard ratios for risk of myeloproliferative neoplasm were 0·82(95% CI: 0·65–1·02) in heterozygotes, 0·65(0·47–0·91) in homozygotes, 0·77 (0·63–0·96) in carriers, and 0·81(0·70–0·94) per T-allele. Associations were primarily observed for polycythaemia vera and myelofibrosis, and for *JAK2*V617F-positive myeloproliferative neoplasm.

Interpretation: A loss-of-function polymorphism in *IL6R* reduces risk of *JAK2*V617F mutation and myeloproliferative neoplasm. This finding supports inflammation as an independent risk factor for *JAK2*V617F mutation and myeloproliferative neoplasm and indicates that therapeutics designed to block interleukin-6 receptor signaling might prevent or retard progression of myeloproliferative neoplasm.

Funding: Karen Elise Jensen Foundation.

Introduction

Philadelphia chromosome-negative myeloproliferative neoplasms (myeloproliferative neoplasm) comprises essential thrombocythaemia, polycythaemia vera, and myelofibrosis and are clonal stem cell neoplasms characterized by chronic inflammation, clonal myeloproliferation, and myelo-accumulation leading to elevated blood cell counts [1,2]. Etiologically, myeloproliferative neoplasm involves a complex interplay between genetic and environmental risk factors, and the precise time of disease onset is unknown. Since patients with myeloproliferative neoplasm often have elevated biomarkers of inflammation in the blood and deregulation of inflammatory- and immunomodulatory genes [3,4], chronic low-grade inflammation has been suggested to be

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https://doi.org/10.1016/j.eclinm.2020.100280

Research in context

Evidence before this study

Chronic low-grade inflammation might be an initiator and driver or a consequence of myeloproliferative neoplasm. We searched PubMed for previous human studies published in English until October 5, 2019 using the following search terms: "myeloproliferative neoplasm", "*JAK2*V617F", "chronic inflammation", "interleukin-6″, "Mendelian randomization", and "risk factors". No study had investigated the causal association between inflammation and risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm.

Added value of this study

Using a Mendelian randomization approach in a Danish contemporary population-based cohort of 107,969 individuals, we found that an anti-inflammatory loss-of-function polymorphism in *IL6R* reduced risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. Associations were primarily observed for polycythaemia vera and myelofibrosis, and for *JAK2*V617F-positive myeloproliferative neoplasm.

Implications of all the available evidence

These findings support that chronic low-grade inflammation is an independent risk factor for *JAK2*V617F somatic mutation and myeloproliferative neoplasm and indicates that therapeutics designed to block interleukin-6 receptor signaling might prevent or retard progression of myeloproliferative neoplasm.

an initiator and driver or a consequence of disease development [5]. Thus, it is unclear whether the malignant clone triggers an inflammatory response, or whether an underlying chronic low-grade inflammation initiates and drives development of the malignant clone, i.e. a chicken and egg situation [6]. Similarly, it has been proposed that chronic low-grade inflammation could result in acquisition of the driver mutation Janus kinase 2 V617F (*JAK2*V617F); a somatic mutation that leads to constitutive activation of the kinase and is present in the majority of individuals with myeloproliferative neoplasm [2,3,5,7].

The gold standard in establishing causal relationships in research is randomized, double-blinded, placebo-controlled trials. However, some exposures are unethical, impractical, or impossible to study in humans using such a study design. Although cell or animal studies could answer this question, extrapolating to humans can be difficult. In contrast, a Mendelian randomization approach takes advantage of genetic variants as proxies for modifiable risk factors to infer causality [8]. Due to the random distribution of genes at conception, genetic variants should not be associated with potential confounders and, since genes are present at birth, genetic variants are not susceptible to reverse causation [8].

In European populations the genetic variant rs4537545 in the interleukin-6 receptor (*IL6R*) gene on chromosome 1 is in high linkage disequilibrium with the genetic variant rs2228145 (D'=0·996, R^2=0·934, Distance=8091 bp) (Fig. 1) [9]. The latter results in a change from aspartic acid to alanine at position 358 of the *IL6R* gene [10], which leads to a reduction in the number of membrane-bound interleukin-6 receptors due to membrane-cleavage and hence an impairment of the interleukin-6 receptor signaling and dampened inflammation [10,11]. Impairment of the interleukin-6 receptor signaling pathway leads in turn to lower concentrations of acute-phase reactants such as C-reactive protein and higher concentrations of soluble interleukin-6 receptor and interleukin-6 [10]. Thus, genetic variation in *IL6R* rs4537545 and *IL6R* rs2228145 can be used to study consequences of variations in chronic low-grade inflammation and using one of these instruments the independent relationship with *JAK2*V617F somatic mutation and myeloproliferative neoplasm can therefore be assessed.

By using a Mendelian randomization approach in the Copenhagen General Population Study with 107,969 individuals, we tested the hypothesis that an anti-inflammatory loss-of-function polymorphism in the *IL6R* gene (marked by rs4537545) reduces risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm.

Methods

Study population

We included 107,969 individuals aged 20−100 from the Copenhagen General Population Study, an ongoing population-based cohort since 2003 (response rate 43%) [12]. Non-responders were more often men (48% versus 45%) and slightly younger (median age: 56 versus 58) compared to responders. In Denmark, all individuals are assigned a unique identification number at birth or immigration and registered in the national Danish Civil Registration System since 1968 [13]. Thus, individuals were randomly selected and invited from the national Danish Civil Registration System to reflect the adult white population of Danish descent. All participants completed a comprehensive questionnaire, underwent a physical examination, and gave blood for biochemical and genetic analyses. Questionnaires were reviewed in detail at the day of attendance by a healthcare professional together with the participant.

Ethics

The study was approved by Herlev and Gentofte Hospital and a Danish ethical committee (approval number: H-KF-01-144/01) and was conducted according to the Declaration of Helsinki. Written informed consent was obtained from all participants.

Myeloproliferative neoplasms

Information on myeloproliferative neoplasm was obtained from the national Danish Patient Registry, which records medical diagnoses from all public- and private hospitals in Denmark since 1977 [14]. Cases of myeloproliferative neoplasms included essential thrombocythaemia (International Classification of Diseases [ICD]-8: 287·29 and ICD-10: D47·3, D75·2), polycythaemia vera (ICD-8: 208·99 and ICD-10: D45), myelofibrosis (ICD-8: 209 and ICD-10: D47·4, C94·5), and unclassifiable myeloproliferative neoplasm (ICD-10: D47·1) and were defined as hospital contacts recorded at haematological departments with the mentioned ICD codes as the main cause, recorded until April 10, 2018 (see Fig. S1 for a flowchart of case ascertainment). Initially, only diagnoses from inpatient hospital admissions were reported to the national Danish Patient Registry but since 1994 diagnoses from all outpatient hospital specialist clinics have also been reported. Denmark used ICD-8 codes until January 1, 1994 and proceeded directly to ICD-10 codes after this date. All included cases first recorded with a myeloproliferative neoplasm diagnosis using ICD-8 codes also had an ICD-10 code recorded. Date of diagnosis was defined as the initial day of hospital contact at the haematological department with a diagnosis of myeloproliferative neoplasm. The subtype of myeloproliferative neoplasm was determined at diagnosis and was not changed during follow-up in the statistical analyses.

In Denmark, all patients with myeloproliferative neoplasm are diagnosed according to the World Health Organization criteria based on clinical information together with pathological diagnosis using bone marrow biopsy and aspiration; such patients are exclusively followed and treated at specialized haematological departments, as healthcare utilization including treatment for these patients are free of charge [15]. The national Danish Patient Registry has previously shown high validity of recorded ICD codes for haematological neoplasms [16].

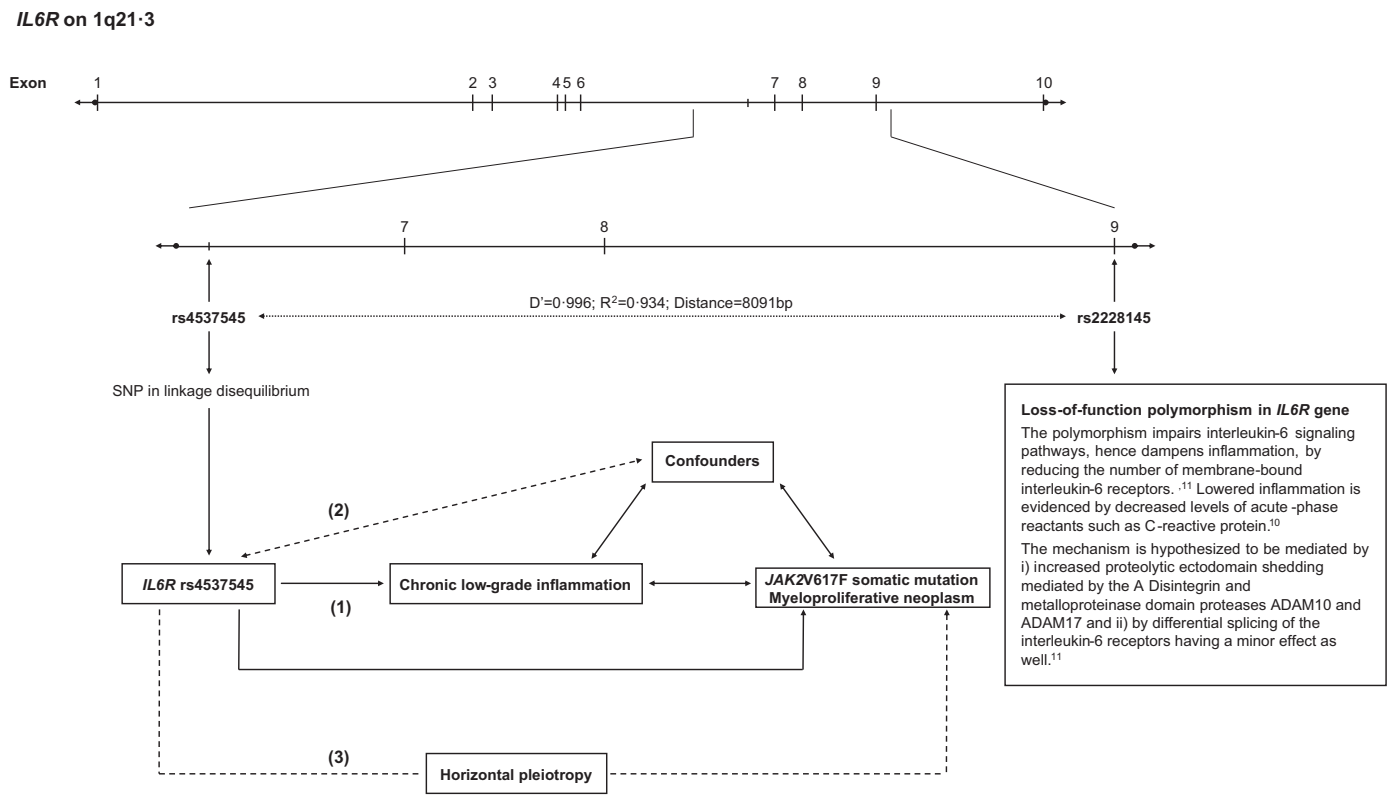


Fig. 1. Genomic location of the *IL6R* gene and directed acyclic graph for Mendelian randomization including underlying assumptions. In this Mendelian randomization study, *IL6R* rs4537545 is used as a proxy for impaired interleukin-6 receptor signaling without being influenced by confounding or reverse causation due to the random distribution of genes at conception and since genes are present at birth. Thus, the independent relationship between chronic low-grade inflammation and the *JAK2*V617F somatic mutation and myeloproliferative neoplasm can be tested. Assumption 1) the genotype is robustly associated with the risk factor i.e. the *IL6R* rs4537545 genotype is associated with chronic low-grade inflammation. Assumption 2) the genotype is independent of confounding factors that confound the association between the risk factor and outcome, i.e. no association between the *IL6R* rs4537545 genotype and potential confounders. Assumption 3) the genotype is related to the outcome only by its association with the risk factor, i.e. no horizontal pleiotropy in the association between the *IL6R* rs4537545 genotype with the *JAK2*V617F somatic mutation and myeloproliferative neoplasm. *D'*, R^2, and distance between single nucleotide polymorphisms are obtained from the National Cancer Institute in individuals of European ancestry (https://ldlink.nci.nih.gov/?tab=ldmatrix). rs4537545 represents a *C*>*T* exchange; rs2228145 represents Asp358Ala.

Genotyping and inflammation

Genotyping of *IL6R* rs4537545 was conducted blind to information on degree of inflammation in blood, *JAK2*V617F somatic mutation status, and diagnosis of myeloproliferative neoplasm. DNA from all individuals were isolated from whole blood and stored at −45 °C. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems Inc) was used to genotype *IL6R* rs4537545 with TaqMan assays. Genotyping was verified by DNA sequencing. As we performed reruns, call rates were >99%. Primers and probe sequences are available from the authors upon request.

*JAK2*V617F somatic mutation status was determined at baseline examination in a subset of the study population (n = 49,143). In brief, the *JAK2*V617F somatic mutation was first detected using a polymerase chain reaction-based TaqMan screening assay. Subsequently, using a highly sensitive real-time quantitative polymerase chain reaction assay, the one percent of the individuals with the highest signal from the screening assay was tested (lower detection limit of 0·8%), as described previously [17,18].

C-reactive protein plasma concentration was used as a marker for degree of inflammation in the blood and measured with a high-sensitivity assay using latex-enhanced turbidimetry or nephelometry. Analyses were subjected to daily precision testing using an internal quality control material and monthly accuracy testing using an external control quality programme.

Statistical analyses

STATA/SE 13·1 (StataCorp LP, StataCorp, College Station, TX) was used. Deviation from Hardy–Weinberg equilibrium was examined using the $\chi 2$ test; deviation may suggest genotyping or population sampling errors [8]. The association between *IL6R* rs4537545 and C-reactive protein concentration including F-statistics and R^2 were investigated using multiple linear regression analyses [8]. The association between *IL6R* rs4537545 and potential confounders, chosen as they *a priori* could be associated with inflammation and/or *JAK2*V617F somatic mutation and myeloproliferative neoplasm, was investigated using linear and logistic regression models as appropriate. The association between *IL6R* rs4537545 and risk of *JAK2*V617F somatic mutation at baseline examination was investigated using logistic regression and myeloproliferative neoplasm was investigated using Cox regression analysis and competing-risk regression analysis according to Fine and Gray with death (n = 10,372) and emigration (n = 477) as competing events. Risk of myeloproliferative neoplasm was also stratified according to *JAK2*V617F somatic mutation status, i.e. positive or negative, determined at baseline examination. Risk of myeloproliferative neoplasm was assessed by setting the entry date to the beginning of the national Danish Patient Registry (=left truncation) and choosing age as the underlying time scale (=age adjustment). Thus, all individuals diagnosed from the beginning of the national Danish Patient Registry in 1977 until end of follow-up in April 2018 were included in the analyses, since genes are present at birth. Nonetheless, in sensitivity analysis risk was also assessed by setting the entry date to the baseline examination date, thereby excluding individuals with myeloproliferative neoplasm at baseline examination from the analysis. Risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm was also investigated according to potential risk factors, i.e. age, sex, smoking, body mass index, and family history of cancer, and the likelihood ratio test was used to assess effect modification (=interaction).

To investigate if the association between impaired interleukin-6 receptor signaling and risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm is mediated through C-reactive protein, we used the genetic variants rs3091244, rs1130864, rs1205, and rs3093077 in the C-reactive protein (*CRP*) gene [19]. The four genetic variants were combined and ranked according to the nine most common combinations with increasing concentrations of C-reactive protein [19].

A two-sample Mendelian randomization approach with summary statistics on the association of rs4537545 with soluble interleukin-6 receptor concentration in blood was used in an instrumental variable analysis with the Wald ratio [32], to quantify the effect of genetically impaired interleukin-6 receptor signaling on risk of myeloproliferative neoplasm, i.e. presented as a doubling in soluble interleukin-6 receptor concentration. A detailed description of potential confounders and instrumental variable analysis is provided in the supplementary material.

Role of the funding source

This study was funded by the Danish Karen Elise Jensen Foundation. YÇ was funded by the Lundbeck Foundation. The funders had no role in the design, conduct of study, collection, management, analysis, or interpretation of the data, or in the preparation, review, or approval of the manuscript. KMP, YÇ, and BGN had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

Among 107,969 individuals from the Copenhagen General Population Study, 352 (0·3%) had or developed myeloproliferative neoplasm. Among a subset of 49,143 individuals, 62 (0·1%) were tested positive for the *JAK2*V617F somatic mutation at baseline examination. Among 352 individuals with myeloproliferative neoplasm, 205 were tested for the *JAK2*V617F somatic mutation, of whom 46 were tested positive. Among 107 969 individuals, 36,871 were non-carriers, 52,500 were heterozygotes, and 18,598 were homozygotes for the T-allele of the *IL6R* rs4537545 genotype (Table 1). There was no evidence of deviation from Hardy–Weinberg equilibrium (*P*-value=0·70).

The *IL6R* rs4537545 genotype was associated with lower C-reactive protein concentration; compared to non-carriers, C-reactive protein concentration was −6·4% (95% confidence interval [CI]: −7·7%; −5·1%) in heterozygotes and −13·7% (−15·3%; −12·1%) in homozygotes (*P*-value for trend=3×10^{-56}); *F* statistics were 117 and R^2 was 0·2%. In contrast, the *IL6R* rs4537545 genotype was not associated with any potential confounders after Bonferroni correction (Table 1).

The T-allele of the *IL6R* rs4537545 genotype was associated with a reduced risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm (Fig. 2). Compared to non-carriers, age- and sex-adjusted odds ratios (OR) for risk of *JAK2*V617F somatic mutation were 0·55 (95% CI: 0·32–0·94) in heterozygotes and 0·51 (0·24–1·12) in homozygotes. Corresponding ORs were 0·54 (0·33–0·89) in carriers versus non-carriers and 0·66 (0·45–0·96) per T-allele. Compared to non-carriers, age- and sex-adjusted hazard ratios (HR) for risk of myeloproliferative neoplasm were 0·82 (95% CI: 0·65–1·02) in heterozygotes and 0·65 (0·47–0·91) in homozygotes. Corresponding HRs were 0·77 (0·63–0·96) in carriers versus non-carriers and 0·81 (0·70–0·94) per T-allele. The decreased risk was most pronounced for polycythaemia vera and myelofibrosis (Fig. S2). Results were similar when the competing events death and emigration were taken into account (Fig. 3). Risk estimates were also similar when stratified according to potential risk factors for myeloproliferative neoplasm without any evidence of effect modification (all *P* for interaction ≥0·05) (Fig. 4). For *JAK2*V617F somatic mutation, analyses showed sign of effect modification concerning age, smoking status, and family history of cancer. However, *P*-values were all ≥0·05 after Bonferroni correction. And if examining effect modification according to non-carriers versus carriers instead of per T-allele, all *P*-values were ≥0·05 (data not shown).

Compared to non-carriers, age- and sex-adjusted HRs for *JAK2*V617F-positive myeloproliferative neoplasm were 0·51 (0·27–0·95) in heterozygotes, 0·50 (0·20–1·23) in homozygotes, 0·51 (0·28–0·90) in carriers, and 0·64 (0·40–1·02) per T-allele (Fig. 5). Corresponding HRs for *JAK2*V617F-negative myeloproliferative neoplasm were 0·90

Table 1
Potential confounders for individuals in the Copenhagen general population study according to the *IL6R* rs4537545 genotype.

	IL6R rs4537545			
	C/C *n* = 36 871	C/T *n* = 52 500	T/T *n* = 18 598	*P* for trend
Age, years	58·1 (48·2–67·4)	58·2 (48·2–67·5)	58·4 (48·3–67·7)	0·020[a]
Men, no. (%)	16,463 (45)	23,685 (45)	8424 (45)	0·11
Body mass index, kg/m^2	25·6 (23·2–28·4)	25·5 (23·2–28·4)	25·6 (23·2–28·4)	0·83
Never-smokers, no. (%)	15,341 (42)	21,903 (42)	7867 (42)	0·15
Former smokers, no. (%)	15,095 (41)	21,460 (41)	7469 (40)	0·12
Current smokers, no. (%)	6296 (17)	8964 (17)	3193 (17)	0·82
Cumulative tobacco consumption, pack-years[b]	16 (6–30)	15 (6–30)	16 (6–30)	0·30
Alcohol consumption, units/week[c]	8 (4–15)	8 (4–15)	8 (4–15)	0·40
Low leisure-time physical activity, no. (%)[d]	2272 (6·2)	3279 (6·3)	1103 (6·0)	0·41
Low education, no. (%)[e]	5762 (16)	8228 (16)	2989 (16)	0·25
Low annual household income, no. (%)[f]	4494 (12)	6590 (13)	2396 (13)	0·018[a]
Any chronic disease, no. (%)	6297 (17)	9080 (17)	3185 (17)	0·73
Cancer, no. (%)[g]	2487 (6·8)	3653 (7·0)	1295 (7·0)	0·25
Ischaemic heart disease, no. (%)	2129 (5·8)	3043 (5·8)	1043 (5·6)	0·52
Diabetes, no. (%)	1619 (4·4)	2202 (4·2)	769 (4·1)	0·11
Rheumatoid arthritis, no. (%)	322 (0·9)	513 (1·0)	148 (0·8)	0·73
Chronic obstructive pulmonary disease, no. (%)	762 (2·1)	1108 (2·1)	415 (2·2)	0·23

Data are summarized as medians with the 25th and 75th percentiles, or numbers with percent.
P-values were derived from Wald's test in linear regression models for continuous covariates and logistic regression models for dichotomous covariates.

[a] When P for trend is adjusted for 17 individual trend analyses according to the Bonferroni method, *P* = 0·05 is equivalent to *P* = 0·05/17=0·003.

[b] Included only former and current smokers.

[c] 12 g = 1 unit of alcohol.

[d] Being completely sedentary or doing light physical activity less than 2 h/week.

[e] <9 years of school attendance.

[f] Annual household income <200,000 DKK (approximately 30,000 USD).

[g] Cancer was based on all cancer forms except for nonmelanoma skin cancer.

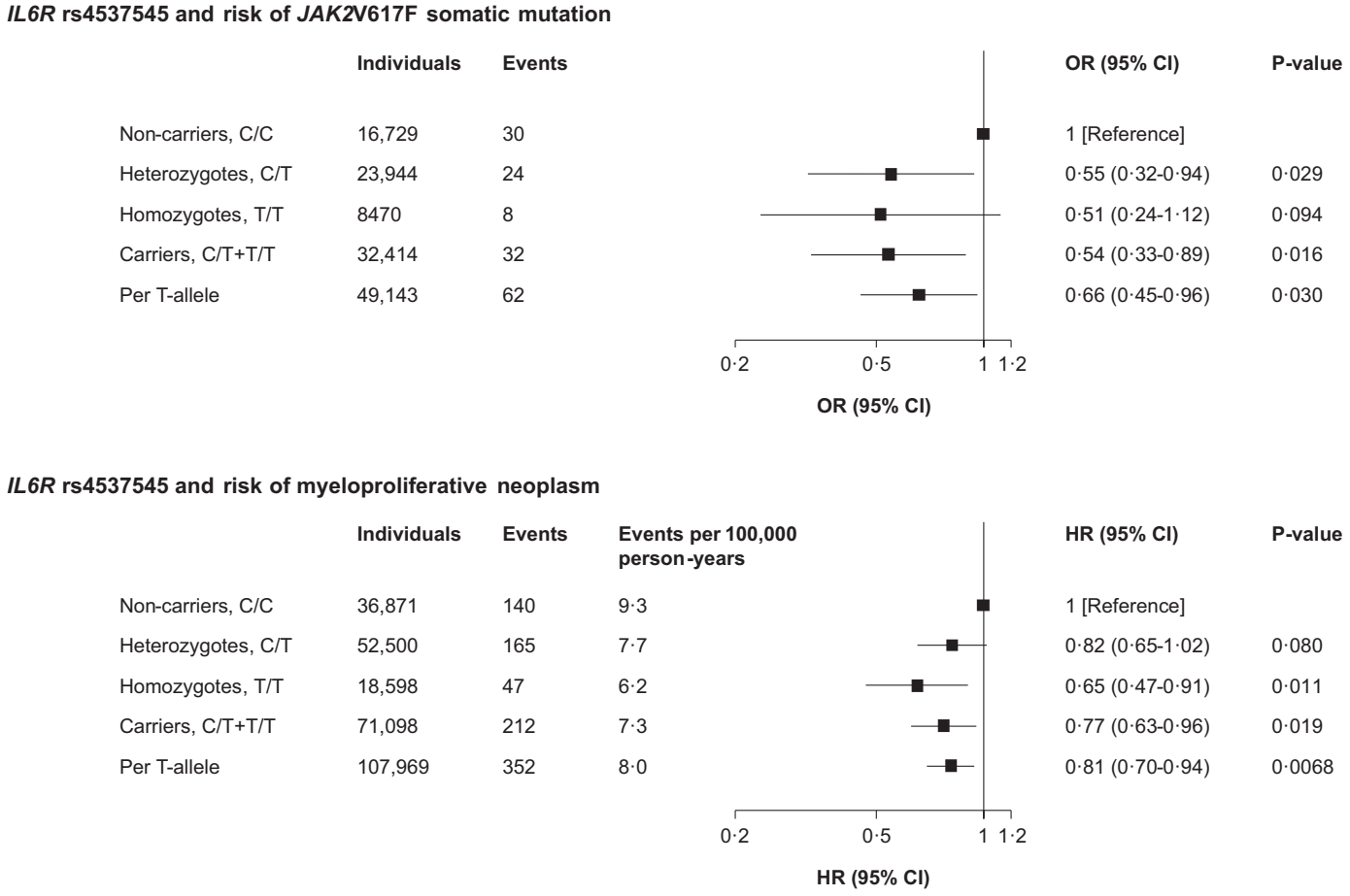


Fig. 2. Association of the *IL6R* rs4537545 genotype and risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. Odds ratios were obtained from logistic regression analysis adjusted for age and sex. Hazard ratios were obtained from Cox regression analysis adjusted for age and sex. CI = confidence interval. HR = hazard ratio. OR = odds ratio.

(0·64–1·26) in heterozygotes, 0·63 (0·38–1·05) in homozygotes, 0·83 (0·60–1·14) in carriers, and 0·82 (0·66–1·03) per T-allele, respectively.

None of the genetic variants in the *CRP* gene were associated with *JAK2*V617F somatic mutation or myeloproliferative neoplasm separately or combined (Table S1 and Figs. S3 and S4), suggesting that the associations between impaired interleukin-6 receptor signaling and reduced risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm are not independently mediated through C-reactive protein. In instrumental variable analysis, a doubling in soluble interleukin-6 receptor concentration yielded a causal risk ratio of 0·60 (0·41–0·88) for risk of myeloproliferative neoplasm.

In sensitivity analyses, results were similar when adjusting for potential confounders of inflammation (Figs. S5 and S6). Results were also similar after excluding individuals with prevalent myeloproliferative neoplasm from the analyses (Fig. S7) or when using logistic regression

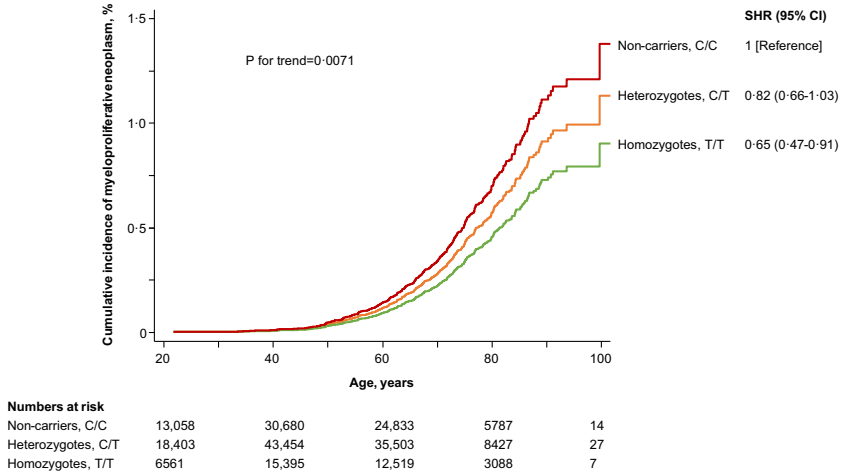
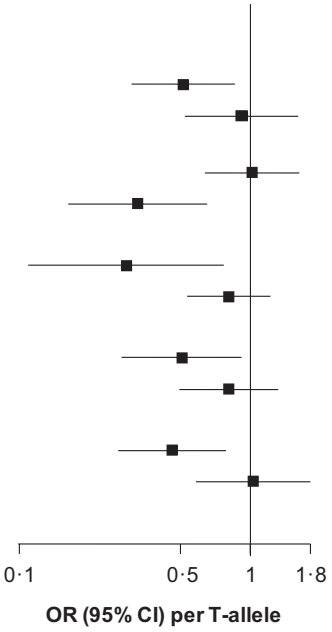


Fig. 3. Cumulative incidence of myeloproliferative neoplasm according to the *IL6R* rs4537545 genotype. Cumulative incidence and subdistribution hazard ratios were obtained from competing-risk analysis according to Fine and Gray with death and emigration as competing events, adjusted for age and sex. *P* for trend was obtained from Wald's test. CI = confidence interval. SHR = subdistribution hazard ratio.

Per T-allele of *IL6R* rs4537545 and risk of *JAK2*V617F somatic mutation

Characteristic	Individuals	Events	OR (95% CI)	P-value for interaction
Males	21,931	37	0·51 (0·31-0·86)	0·12
Females	27,212	25	0·91 (0·52-1·61)	
Age ≤65·5*	35,551	36	1·01 (0·63-1·62)	0·0056
Age >65·5*	13,592	26	0·33 (0·16-0·65)	
Never-smokers	19,260	14	0·29 (0·11-0·77)	0·047
Ever-smokers	29,711	48	0·80 (0·53-1·22)	
BMI ≤25 kg/m^2	21,615	28	0·50 (0·28-0·91)	0·24
BMI >25 kg/m^2	27,445	34	0·81 (0·49-1·32)	
No family history of cancer	28,140	36	0·46 (0·27-0·78)	0·043
Family history of cancer	20,180	25	1·02 (0·58-1·80)	



Per T-allele of *IL6R* rs4537545 and risk of myeloproliferative neoplasm

Characteristic	Individuals	Events	Events per 100,000 person-years	HR (95% CI)	P-value for interaction
Males	48,572	168	8·5	0·72 (0·57-0·90)	0·15
Females	59,397	184	7·6	0·90 (0·73-1·11)	
Age ≤65·5*	75,414	176	5·7	0·79 (0·63-0·99)	0·72
Age >65·5*	32,555	176	13	0·83 (0·68-1·03)	
Never-smokers	45,111	117	6·4	0·92 (0·72-1·17)	0·24
Ever-smokers	62,477	235	9·3	0·76 (0·62-0·92)	
BMI ≤25 kg/m^2	47,499	154	8·0	0·80 (0·63-1·02)	0·94
BMI >25 kg/m^2	60,308	198	8·1	0·81 (0·67-0·99)	
No family history of cancer	59,714	193	8·0	0·78 (0·64-0·96)	0·51
Family history of cancer	46,914	157	8·2	0·87 (0·69-1·09)	

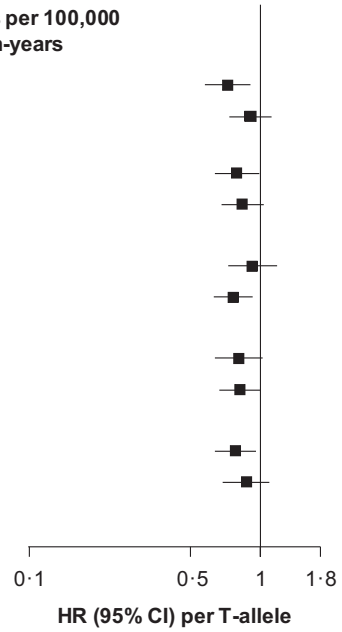


Fig. 4. Association between the *IL6R* rs4537545 genotype and risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm according to potential risk factors. Odds ratios were obtained from logistic regression analysis adjusted for age and sex. Hazard ratios were obtained from Cox regression analysis adjusted for age and sex. *P*-values for interaction were obtained from the likelihood ratio test. *Corresponds to the median age of individuals with myeloproliferative neoplasm at baseline examination. CI = confidence interval. HR = hazard ratio. OR = odds ratio.

analyses (Fig. S8). Results were also similar after adjusting for or excluding individuals with asthma, allergy, coronary heart disease, and rheumatoid arthritis in the analyses (data not shown); as shown before, all these phenotypical traits were associated with the *IL6R* rs4537545 genotype (*P*-value$<1 \times 10^{-5}$) and its proxies ($R^2 > 0{\cdot}80$) [20], and therefore could in theory represent horizontal pleiotropy (Fig. 1). Lastly, the *IL6R* rs4537545 genotype was not found to be in linkage disequilibrium with any known functional variants on chromosome 1 associated with myeloproliferative neoplasm in a prior genome-wide association study [9,21].

Discussion

In a large Mendelian randomization study with 107,969 individuals from the Danish general population, we found that an anti-inflammatory loss-of-function polymorphism in *IL6R* (marked by rs4537545) reduces risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. Associations were primarily observed for polycythaemia vera and myelofibrosis, and for *JAK2*V617F-positive myeloproliferative neoplasm. These findings support that inflammation is an independent risk factor for

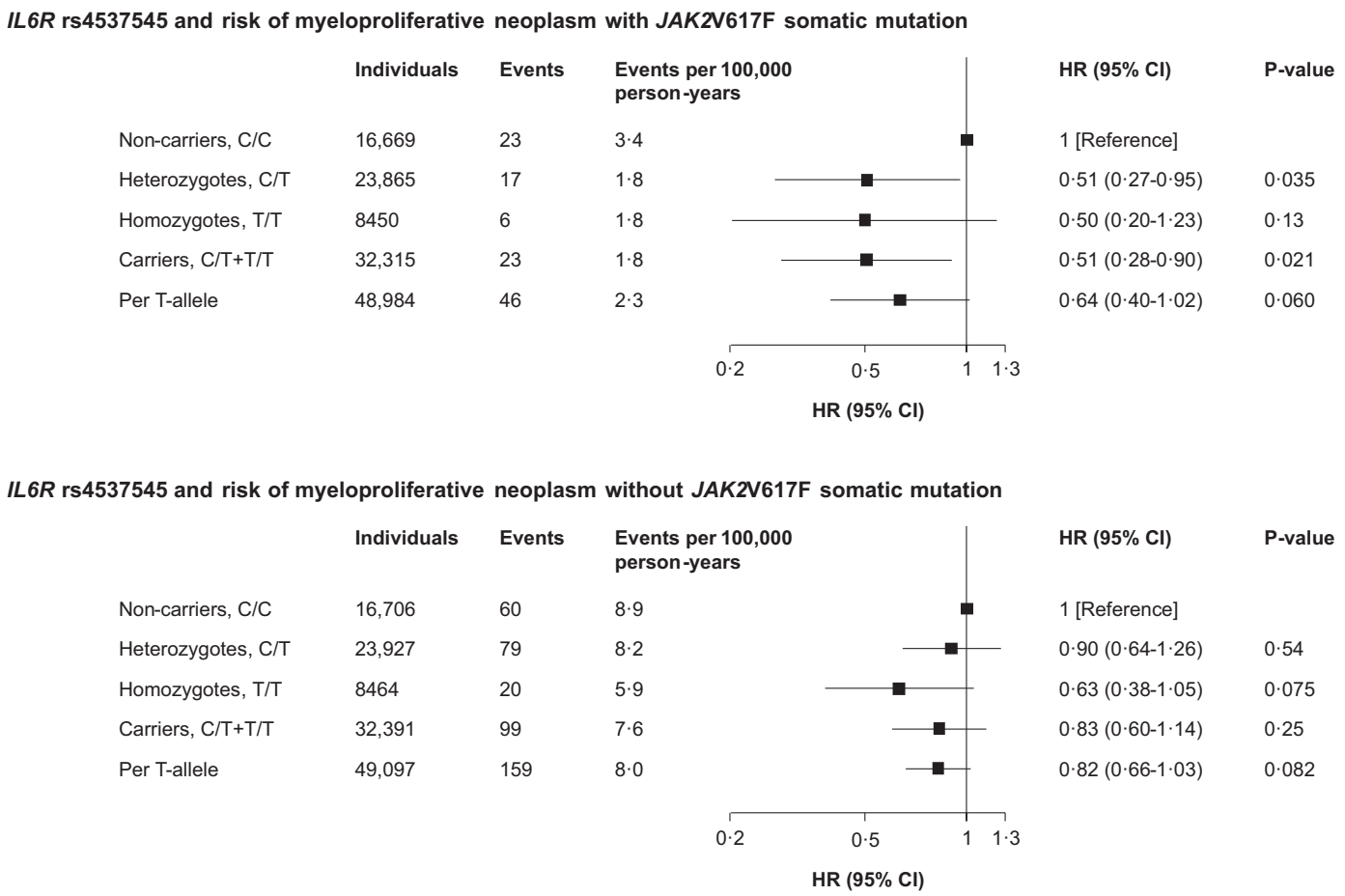


Fig. 5. Association of the *IL6R* rs4537545 genotype and risk of *JAK2*V617F somatic mutation positive and negative myeloproliferative neoplasm. Hazard ratios were obtained from Cox regression analysis adjusted for age and sex. *JAK2*V617F status was determined at baseline examination. CI = confidence interval. HR = hazard ratio.

*JAK2*V617F somatic mutation and myeloproliferative neoplasm and indicate that therapeutics designed to block interleukin-6 receptor signaling might prevent or retard progression of myeloproliferative neoplasm.

Interleukin-6 is a proinflammatory cytokine exerting its biological effects through different pathways: (i) classical signaling through membrane-bound interleukin-6 receptors primarily on hepatocytes and certain types of leukocytes, (ii) trans-signaling by binding to soluble forms of the interleukin-6 receptor and subsequently to ubiquitously expressed membrane-bound transducer glycoprotein-130, and (iii) trans-presentation which constitutes a cell-to-cell mechanism with presentation of a membrane-bound interleukin-6 receptor with bound interleukin-6 on one cell to a membrane-bound transducer glycoprotein-130 on another cell [22]. An increased interleukin-6 receptor signaling is believed to be associated with mutagenesis and tumorigenesis by affecting the hallmarks of cancer such as apoptosis, proliferation, and angiogenesis [23]. Accumulation of reactive oxygen species due to chronic low-grade inflammation may lead to genetic instability and DNA oxidative damage in the cells of the rapidly dividing bone marrow with acquisition of *JAK2*V617F somatic mutation, subclone formation, progression, and in the end development of myeloproliferative neoplasm [3,5,23]. In contrast, the genetic loss-of-function variant rs2228145 (marked by rs4537545) in the *IL6R* gene mimics a blockade of the classical interleukin-6 receptor signaling pathway through membrane-cleavage of the interleukin-6 receptor, thereby damping chronic low-grade inflammation and reducing the risk of coronary heart disease [10], and now also shown to reduce the risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. Although interleukin-6 receptor signaling could hypothetically be activated through trans-signaling and/or trans-presentation, buffering of secreted interleukin-6 by the soluble interleukin-6 receptor/soluble glycoprotein-130 systems is suggested to hinder such alternative signaling pathways [24]. Alternatively, the loss-of-function polymorphism could also hypothetically slow down the progression and expansion of a malignant myeloproliferative neoplastic clone in the presence of driver-mutations such as the *JAK2*V617F, thereby leading to disease alleviation and/or postponing disease onset.

In this study, we did not observe an association between the *IL6R* rs4537545 genotype and risk of essential thrombocythaemia. One possible explanation is that essential thrombocythaemia occurs in much younger individuals, predominantly females, and many without *JAK2*V617F somatic mutation compared to polycythaemia vera and myelofibrosis. In contrast, we found an association between the T-allele of the *IL6R* rs4537545 genotype and decreased risk of *JAK2*V617F somatic mutation, and the T-allele also seemed to be associated with decreased risk of *JAK2*V617F-positive and not *JAK2*V617F-negative myeloproliferative neoplasm. In this context, individuals with polycythaemia vera and myelofibrosis also have higher *JAK2*V617F allele burden compared to those with essential thrombocythaemia, and this may reflect inflammation being operative in expanding *JAK2*V617F-positive myeloproliferative neoplasm clones. Thus, essential thrombocythaemia might develop in a different causal context with a smaller role for IL6-mediated inflammation.

Another interesting aspect to consider is the interaction analysis. It seemed that the T-allele was more protective against myeloproliferative neoplasm in males and ever-smokers compared to their counterparts. This raises the question as to whether the protection through the T-allele of *IL6R* rs4537545 is more apparent in individuals who have a higher inflammatory drive. For the *JAK2*V617F somatic mutation; having a higher age, being a never-smoker, and having no family history of cancer were all associated with larger protection by

the T-allele of the *IL6R* rs4537545 genotype. However, these interaction results should be interpreted with caution, as all *P*-values were ≥0·05 after Bonferroni correction.

Previous studies have shown that patients with myeloproliferative neoplasm exhibit elevated biomarkers of inflammation in blood and deregulation of inflammatory- and immunomodulatory genes [3]. Some studies have also associated different inflammatory sources such as tobacco smoking and autoimmune disorders with risk of myeloproliferative neoplasm and *JAK2*V617F somatic mutation [25–27]. Likewise, mathematical modeling studies have been carried out supporting an association between inflammation, acquisition of somatic mutations, and myeloproliferative neoplasm [7]. However, no study has to date investigated the genetic independent relationship between chronic low-grade inflammation and risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. In the present study, we found that an anti-inflammatory loss-of-function polymorphism in *IL6R* reduces risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm, supporting chronic low-grade inflammation as an independent risk factor in the development of myeloproliferative neoplasm [3,5,6].

Strengths of the present study include a large number of genotyped individuals from the general population with validated cases of myeloproliferative neoplasm and no loss to follow-up. That essentially all cases in Denmark with myeloproliferative neoplasm have the diagnosis confirmed using bone marrow biopsy and aspiration is an additional strength.

Potential limitations in Mendelian randomization studies include population stratification bias and horizontal pleiotropy [8]. Since the population studied is ethnically homogenous and since genotype distributions did not deviate from Hardy–Weinberg equilibrium, population stratification and genotyping errors are less likely to have distorted the results.

Regarding horizontal pleiotropy two mechanisms should be addressed. The first mechanism is where a genetic variant influences the outcome through a pathway other than the exposure. However, only mRNA is separating the genetic sequence rs2228145 (marked by rs4537545) from the change in protein structure i.e. Asp358Ala, implying that the likelihood of horizontal pleiotropy due to this mechanism is diminished in the present study [28]. Likewise, results were similar after exclusion of individuals with other traits associated with the *IL6R* rs4537545 genotype [20]. The second mechanism is where a genetic variant is statistically associated with two traits, e.g. inflammation and myeloproliferative neoplasm, simply because it causally relates to one trait while also being in linkage disequilibrium with a causal variant for the other trait. Yet, the *IL6R* rs4537545 genotype did not seem to be in linkage disequilibrium with any functional variants associated with myeloproliferative neoplasm in a prior genome-wide association study [9,21]. Thus, horizontal pleiotropy is deemed unlikely, although, cannot be fully excluded.

Another potential limitation is that the *JAK2*V617F somatic mutation status was only determined at baseline examination and not at time of myeloproliferative neoplasm diagnosis. Thus, some individuals diagnosed with myeloproliferative neoplasm after baseline examination may acquire *JAK2*V617F somatic mutation. Yet, this potential limitation cannot explain the positive finding regarding *JAK2*V617F-positive myeloproliferative neoplasm but would bias the estimates concerning *JAK2*V617F-negative myeloproliferative neoplasm towards a protective effect of the T allele of *IL6R* rs4537545, and hence away from the null hypothesis. Thus, *JAK2*V617F mutation-specific analyses should be interpreted with some caution and further studies are needed to fully determine whether the protective effect of the T-allele of *IL6R* rs4537545, i.e. dampened chronic low-grade inflammation, also applies for mutation-specific myeloproliferative neoplasm. To examine this, high sensitivity assays testing for *JAK2*V617F somatic mutation [27], and other driver-mutations in relevant populations, e.g. individuals with clonal haematopoiesis of indeterminate potential, might be useful.

Another potential limitation is that our results may not necessarily apply to other ethnicities; however, we are not aware of data to suggest that the present results should not be generally applicable.

The clinical implications of the present study include a potential new drug target for the treatment of myeloproliferative neoplasm. The *IL6R* rs2228145 genotype (marked by *IL6R* rs4537545) mimics a pharmacological interleukin-6 receptor blockade. A life-long genetic doubling in soluble interleukin-6 receptor concentration due to impaired interleukin-6 receptor signaling yielded a 40% reduced risk of myeloproliferative neoplasm. As a point of reference, the monoclonal antibody tocilizumab that targets the interleukin-6 receptor increases soluble interleukin-6 receptor concentration by approximately 10-fold and could easily be considered as a candidate drug for retarding or treating overt myeloproliferative neoplasm [10,29].

Another new drug for the treatment of myeloproliferative neoplasm might be the monoclonal antibody canakinumab that targets the interleukin-1β, which is a more central regulator in the inflammatory response and which, *inter alia*, induces interleukin-6 receptor signaling [30]. Thus, canakinumab directly inhibits the interleukin-1β to interleukin-6 to C-reactive protein axis [30], which would imply a reduced risk of myeloproliferative neoplasm. Furthermore, canakinumab has recently been shown to reduce cardiovascular events through modulation of the interleukin-6 receptor signaling pathway in patients with stable atherosclerosis in the Canakinumab Anti-Inflammatory Thrombosis Outcomes Study [31]. Importantly, patients with myeloproliferative neoplasm suffer a huge cardiovascular disease burden. Thus, canakinumab could principally not only be used to treat the disease but also reduce the risk of associated cardiovascular complications in patients with myeloproliferative neoplasm.

In conclusion, an anti-inflammatory loss-of-function polymorphism in *IL6R* reduces risk of *JAK2*V617F somatic mutation and myeloproliferative neoplasm. This finding supports that inflammation is an independent risk factor for *JAK2*V617F somatic mutation and myeloproliferative neoplasm and indicates that therapeutics designed to block interleukin-6 receptor signalling might prevent or retard progression of myeloproliferative neoplasm.

Declaration of Competing Interest

KMP reports grants from the Danish Karen Elise Jensen Foundation during the conduct of the study. YÇ reports grants from the Lundbeck Foundation and personal fees from Boehringer Ingelheim, AstraZeneca, and Sanofi Genzyme outside the submitted work. HCH reports grants from Novartis Denmark and personal fees from AOP Orphan Pharmaceuticals AG and PharmaEssentia outside the submitted work. CE, SEB, and BGN have nothing to disclose in relation to this study.

Acknowledgment

We are indebted and thankful to all participants and staff from the Copenhagen General Population Study for their valuable contributions. This work was supported by the Danish Karen Elise Jensen Foundation through a PhD programme for KMP. YÇ was funded by the Lundbeck Foundation.

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.eclinm.2020.100280.

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