The *JAK2* V617F somatic mutation, mortality and cancer risk in the general population

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ABSTRACT

JAK2 V617F is present in the majority of patients with myeloproliferative cancer; however, its prevalence and clinical significance in the general population is unknown. We screened for presence of the mutation in 10,507 participants from the Copenhagen City Heart Study with up to 17.6 years of follow up.

Prevalence of the mutation was 0.2% (n=18). All 18 mutation positives died during follow up corresponding to a multifactorially adjusted hazard ratio for early death of 3.0 (95%CI:1.9-4.9). Corresponding hazard ratios for men *versus* women and 1-year age increases were 1.4 (1.1-1.9) and 1.1 (1.1-1.1). Multifactorially adjusted hazard ratios for any cancer, hematologic cancer and myeloproliferative cancer were 3.7 (1.7-8.0), 58 (13-261) and 161 (12-2,197), respectively. Corresponding hazard ratios were 1.2 (0.8-2.0), 2.3 (0.2-25), 1.3 (0.3-5.4) for men *versus* women, and 1.0 (1.0-1.1), 1.1 (0.9-1.2), 0.9 (0.8-1.1) for 1-year age increases. In the general population, *JAK2* V617F is associated with increased morbidity and mortality, although only present in 18 of 10,507 (0.2%).

Key words: *JAK2* V617F, mortality, cancer risk.

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Introduction

The *JAK2* V617F mutation is an acquired, somatic mutation present in the majority of patients with myeloproliferative cancer (myeloproliferative neoplasms) i.e. nearly 100% of patients with polycythemia vera and in about 50% of patients with essential thrombocytosis and primary myelofibrosis.[1-5] The *JAK2* V617F mutation may also be prevalent in individuals without overt signs of myeloproliferative cancer.[6-9] However, the significance of the mutation in this setting is unknown, leading us to examine whether presence of the *JAK2* V617F mutation in individuals from the general population is associated with poor health.

We measured the prevalence of the *JAK2* V617F mutation and tested the hypothesis that presence of the mutation is associated with overall mortality, risk of any cancer, hematologic cancer, and myeloproliferative cancer in individuals in the general population. For this purpose, we screened DNA isolated from whole blood samples of 10,507 participants from the Copenhagen City Heart Study who were followed for up to 17.6 years after blood sampling. After screening, presence of the mutation was confirmed on 2 independent assays using different analytical techniques.

Design and Methods

The study was approved by a Danish scientific ethical committee (KF V.100 2039/91, KF 01-144/01) and by Herlev Hospital, Copenhagen University Hospital. The study population comprised 10,507 participants from the Copenhagen City Heart Study. This is an ongoing population-based study initiated in 1976 including individuals aged 20-95 years. More than 99% were Caucasians of Danish descent. Dates of death were obtained from the national Danish Civil Registration System.[10] Diagnosis dates and diagnosis of any cancer were obtained from the Danish Cancer Registry.[11,12] Diagnoses were classified according to the World Health Organization International Classification of Diseases (ICD).[13,14]

We quantified the *JAK2* V617F mutation using a PCR-based Taqman assay (ABI PRISM® 7900 Sequence Detection System) on DNA isolated from peripheral blood from all 10,507 participants. Sequences of primers and probes and the reaction conditions are available upon request. The 1% of participants (n=107) with the highest signal from the screening assay were also tested using the allele-specific semi-quantitative PCR described by Baxter *et al.*[1] and additionally by a highly sensitive real-time quantitative PCR assay based on a previously published assay,[15] the latter quantitating the mutation burden.

To reduce the possibility of confounding the risk analyses due to sex and age at the time of blood sampling, we selected 30 mutation negative participants matched for sex and age at blood sampling for each of the 18 mutations positive subjects. Analyses were performed in the matched subset as well as in all mutation positives versus all negatives. Further details of Design and Methods are described in the *Online Supplementary Appendix*.

The online version of this article has a Supplementary Appendix.
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Results and Discussion

Among 10,507 participants screened, we detected 18 individuals who were positive for the *JAK2* V617F somatic mutation on 3 independent assays using different analytical techniques. This corresponds to a prevalence of 0.2% in this sample of the general population with a median age of 59 years at the time of blood sampling. The present prevalence of *JAK2* V617F positive individuals is considerably lower than the 1% estimated prevalence in the only other large study which examined 3,935 consecutive Chinese hospital patients with a variety of diagnoses.[8]

Among all participants, the presence of the mutation was positively associated with increasing age ($P<0.0001$), male sex ($P=0.02$), and cumulated smoking ($P=0.005$) (*Online Supplementary Table S1*). In the matched subset, cancer risk factors, i.e. age, current and cumulative tobacco consumption, alcohol consumption and body mass index, were similar in mutation positive and negative individuals (*Online Supplementary Table S1*).

For overall survival with up to 17.6 years of follow up, the 18 mutation positives *versus* negatives had a lower cumulative survival (log rank, $P=0.00003$, Figure 1A). This corresponds to a multifactorially adjusted hazard ratio for early death in mutation positives *versus* negatives of 3.0 (95%CI: 1.9-4.9) (Table 1). Corresponding hazard ratios for men *versus* women and 1-year increase in age were 1.4 (1.1-1.9) and 1.1 (1.1-1.1).

For any cancer, the 11 mutation positives without any cancer prior to the time of blood sampling had a higher cumulative incidence of any cancer than that of the matched negatives (log rank, $P=0.0001$, Figure 1B), corresponding to a multifactorially adjusted hazard ratio of 3.7 (1.7-8.0). Corresponding hazard ratios for men *versus* women and 1-year increase in age were 1.2 (0.8-2.0) and 1.0 (1.0-1.1).

For hematologic cancer, the 15 mutation positives without hematologic cancer prior to the time of blood sampling had a higher cumulative incidence than the matched mutation negatives (log rank, $P=2*10^{-32}$, Figure 1C), corresponding to a multifactorially adjusted hazard ratio of 58 (13-261). Corresponding hazard ratios for men *versus* women and 1-year increase in age were 2.3 (0.2-25) and 1.1 (0.9-1.2).

For myeloproliferative cancer (myeloproliferative neoplasms), the 15 mutation positives without myeloproliferative cancer prior to the time of blood sampling, had a higher cumulative incidence than the matched mutation negatives (log rank, $P=7*10^{-22}$, Figure 1D), corresponding to an age and sex adjusted hazard ratio of 161 (12-2,197). Corresponding hazard ratios for men *versus* women could not be calculated in the matched subset since no women developed myeloproliferative cancer after blood sampling; however, when all participants were included the hazard ratios for men *versus* women were 1.3 (0.3-5.4). For 1-year increase in age, hazard ratio was 0.9 (0.8-1.1). Risk estimates for all four end-points remained stable after including all mutation negatives in the calculation.

The V617F mutant JAK2 protein exerts its action in bone marrow hematopoietic stem cells and causes autonomous expansion of several hematologic lineages.[16] Thus, dependent on the nature of the *JAK2* V617F positive stem cell, the patient presents with either polycythemia vera, essential thrombocytosis, or primary myelofibrosis,[16] i.e. the three diseases referred to by the term myeloproliferative cancer in this manuscript.

Mortality and cancer morbidity before and after the time of blood sampling for each of the 18 mutation positives are shown in Table 2. Fourteen developed any cancer, 7 developed hematologic cancer, 5 developed myeloproliferative cancer and all 18 died during follow up. Four of the 18 did not develop any kind of cancer in their entire life-span although they were mutation positive for at least 2-12 years. For 2 participants, the mutation burden was even very high at 83% (Patient 4) and 94% (Patient 1). No single non-hematologic cancer subtype seemed to have a higher incidence in mutation positives *versus* negatives than expected in explorative *post hoc* analyses. These results document that, in some cases, presence of the mutation precedes the clinical diagnosis of myeloproliferative cancer.

We did not have sufficient statistical power to pinpoint a single cause of death as being responsible for the observed 3-fold mortality, but it is probably mainly caused by an underlying, sometimes occult, myeloproliferative cancer rather than by a general effect of the *JAK2* V617F mutation

Table 1. Mortality and morbidity in the general population according to *JAK2* V617F somatic mutation status, gender and age at the time of blood sampling.

Event Population	Mutation positive participants/ incident events	Mutation negative participants/ incident events	Hazard ratio for presence *vs.* absence of mutation (95%CI)	Hazard ratio for men *vs.* women (95%CI)	Hazard ratio per year increase in age (95%CI)
Death					
All participants	18/18	10,489/3,544	3.0 (1.9-4.7)	1.6 (1.5-1.7)	1.1 (1.1-1.1)
Age matched subset	18/18	540/348	3.0 (1.9-4.9)	1.4 (1.1-1.9)	1.1 (1.1-1.1)
Any cancer					
All participants	11/7	9,503/1,681	3.8 (1.8-8.0)	1.0 (0.9-1.1)	1.0 (1.0-1.1)
Age matched subset	11/7	473/129	3.7 (1.7-8.0)	1.2 (0.8-2.0)	1.0 (1.0-1.1)
Hematologic cancer					
All participants	15/4	10,467/78	55 (19-159)	1.6 (1.0-2.6)	1.1 (1.0-1.1)
Age matched subset	15/4	538/6	58 (13-261)	2.3 (0.2-25)	1.1 (0.9-1.2)
Myeloproliferative cancer					
All participants	15/2	10,485/8	384 (53-2774)	1.3 (0.3-5.4)	1.0 (1.0-1.1)
Age matched subset	15/2	540/1	161 (12-2197)*	Not possible	0.9 (0.8-1.1)

*Hazard ratios are adjusted for gender, age, current and cumulative tobacco consumption, alcohol consumption, body mass index, and JAK2 V617F somatic mutation status at the time of blood sampling, excluding the covariate in question. *Only adjusted for sex and age due to lack of other covariate information. For any cancer, hematologic cancer and myeloproliferative cancers, some participants had event prior to date of blood sampling and were, therefore, excluded from the analysis.*

Table 2. Characteristics of the *JAK2* V617F somatic mutation positives from the general population (18 of 10,507 screened).

Participant #	Mutation burden (Q-PCR) (%)	Sex (F/M)	Age at blood sampling (years)	Any cancer prior to blood sampling/age (years)	Any cancer after blood sampling/age (years)	Age at death (years)
1	94	M	79	None	None	83
2	85	F	60	Polycythemia vera/55	None	75
3	84	M	68	Non-melanoma skin*/67	Polycythemia vera/70	85
4	83	M	71	None	None	73
5	78	M	78	None	Basal cell carcinoma/79	82
6	67	M	77	None	Myelodysplastic syndrome/82	82
7	36	F	77	None	None	83
8	33	M	76	Myelofibrosis/72	Lung/82	82
9	29	M	66	None	Acute myelofibrosis/75	75
10	25	M	76	Non-melanoma skin*/62 Breast/65	None	80
11	25	M	60	None	Myelofibrosis/65	73
12	23	M	56	Seminoma testis/52	None	57
13	17	M	68	None	None	80
14	14	M	72	None	Brain/80	81
15	13	F	60	None	Lung/62	62
16	13	F	68	Myelofibrosis/61	None	77
17	8	F	73	Breast/46	Bladder/77	77
18	8	M	69	None	Liver/76	76

*Histological type not specified. Q-PCR=Quantitative polymerase chain reaction.

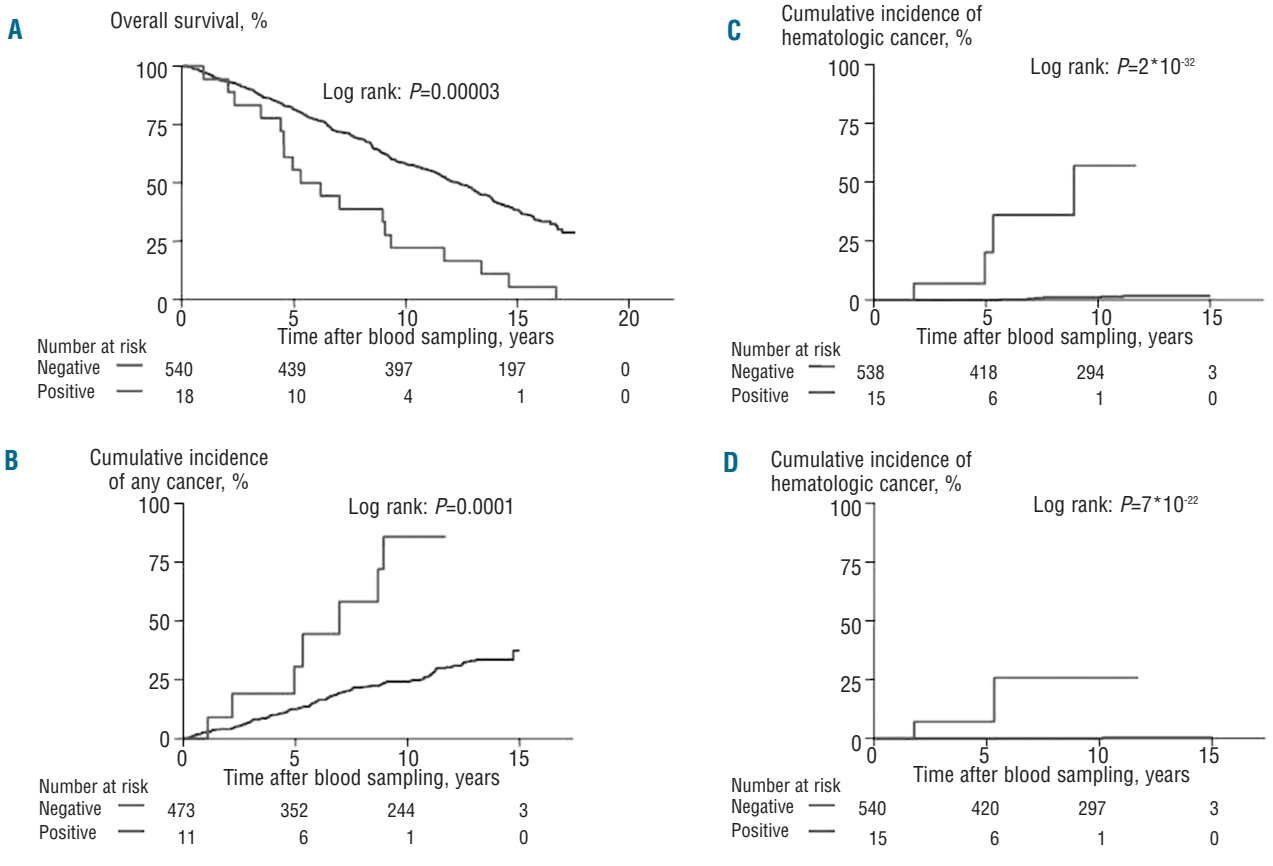

Figure 1. The *JAK2* V617F somatic mutation, mortality and cancer risk in the Danish general population. (A) Cumulative survival as a function of time after blood sampling. (B) Cumulative incidence of any cancer as a function of time after blood sampling. (C) Cumulative incidence of hematologic cancer as a function of time after blood sampling. (D) Cumulative incidence of myeloproliferative cancer as a function of time after blood sampling. The numbers at risk at time=0 vary due to varying numbers of participants with disease prior to the time of blood sampling.

and/or any associated genetic predisposition linked to the *JAK2* locus.

Our study has several limitations: first, the small statistical power provided by the 18 mutation positives out of 10,507 participants. This, however, does not weaken the findings on mortality and cancer morbidity, since increased power in this case would likely only contribute to narrowing the confidence intervals of our findings, but not to changes in the point estimates. Second, our endpoints are based on registry data and not collected with the specific intent of studying myeloproliferative cancer. Also, visits to a general practitioner which did not result in a histological diagnosis are not registered in the Danish Cancer Registry used in the present study. It would not have been surprising that, even considering the older age, many of the 18 mutation positives actually went un/misdiagnosed, given that many myeloproliferative cancers can have a rather smooth course and a variety of manifestations attributable to many other causes. Nevertheless, this potential misclassification of patients into healthy participants would, however, only tend to reduce risk estimates and thus cannot explain the risk estimates observed. Third, unfortunately, the hematologic phenotype of the 18 mutation positive participants at the time of blood sampling is unknown. This information would have been highly interesting and might have identified any immediate clinical implications of this study. Fourth, survival bias prior to blood sampling could distort estimates, especially since we found an association with increased mortality during follow up. Such a survival bias would then tend to underestimate our risk estimates because a larger proportion of the mutation positives *versus* negatives would not have blood samples taken. Accordingly, the true mortality and cancer morbidity might be even higher than those reported here. Finally, as we only studied Caucasians, our results may not necessarily apply to other ethnic groups.

Perspectives from the huge size of the risk estimate in mutation positives *versus* negatives, the efficacy of the present treatment of myeloproliferative cancer, the apparent latency time of up to nine years and the relatively easy diagnostics make it tempting to speculate whether screening for this mutation in the general population would provide a reasonable cost/benefit efficient analysis. This would be even more relevant if the 3-fold increased mortality in mutation positives *versus* negatives in this study was mainly driven by un/misdiagnosed myeloproliferative cancers. However, there are several arguments against such a screening: the prevalence of myeloproliferative cancer is very low and our finding that not all *JAK2* V617F mutation positive individuals will develop myeloproliferative or other cancers is not confirmed by other studies. At any rate, future studies of the clinical relevance of a *JAK2* V617F mutation screening in the general population should also include measurement of the hematologic phenotype.

In conclusion, in this study of 10,507 individuals, the prevalence of the *JAK2* V617F mutation in the general population was very low, but mutation positives *versus* negatives had increased mortality, and increased risk of any cancer, hematologic cancer, and myeloproliferative cancer.

Authorship and Disclosures

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