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

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Citation: Nielsen C, Birgens HS, Nordestgaard BG, Kjær L, and Bojesen SE. The JAK2 V617F somatic mutation, mortality and cancer risk in the general population. Haematologica 2011;96(03):450-453. doi:10.3324/haematol.2010.033191

Online Supplementary Appendix

Design and Methods

Study population

Participants gave written informed consent. 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 Copenhagen City Heart Study is a population-based study initiated in 1976-78. Participants aged 20 years or above were randomly drawn from the Copenhagen Population Register and re-examined in 1981-83, 1991-94 and 2001-2003.[1,2] Whole blood samples from the last two examinations were used for DNA isolation. We included 10,507 participants with available DNA samples. More than 99% were Caucasians of Danish descent.

End-points

Dates of death until 9th of May 2009 were obtained from the national Danish Civil Registration System.[3] Diagnosis dates and diagnosis of any cancer from 1947 through December 31st 2006 were obtained from the Danish Cancer Registry.[4,5] Diagnoses were classified according to the World Health Organization International Classification of Diseases (ICD),[6,7] seventh revision (ICD-7 codes 200-205;207;209;404;503-504;514;900;903-904;914 for hematologic cancer and 207;209 for myeloproliferative cancer) until December 31st 1977 or 10th revision (ICD-10 codes C81;C84-C85;C88;C90-96;D45-47;D60-61;D70-77;D85 for hematologic cancer and C94;D45;D47;D75 for myeloproliferative cancer) thereafter. Thus, the end-point myeloproliferative cancer is included in the end-point hematologic cancer, which in turn is included in the end-point any cancer. All registry information was collected using each participant's unique Central Person Registry number and follow up was 100% complete: we did not lose track of even a single individual.

Somatic mutation detection assay

We quantified the mutation using a PCR-based Taqman assay (ABI PRISM® 7900 Sequence Detection System) on isolated DNA from all 10,507 participants, calibrators or controls. Sequences of primers and probes and the reaction conditions are available upon request. The quantitative signals from the wild-type and mutant probes were called after the PCR in the Taqman system. Sequences of primers and probes and the reaction conditions are available upon request. DNA extracted from the HEL erythroleukemia cell line, which has a very high *JAK2* V617F mutation burden[8] (purchased from DSMZ-German Collection of Microorganisms and Cell Cultures, Niedersachsen, Germany) were mixed with normal DNA without the mutation to produce samples containing 100%, 50%, 10% and 0% mutation burden. The mutation burdens of these samples were verified by the quantitative real-time PCR assay. The 100%, 50% and 0% mutation samples were used as calibrators and the 10% mutation sample was used as an internal control to measure plate-to-plate variation. Each 384-well plate was analyzed individually and included one of each of the three calibrators and the internal control. The quantitative estimate of the mutation burden in each participant DNA and the internal control was calculated as shown below.

Quantitative estimate (in %):

$100*(Y_{participant}-Y_0)*50/Y_{50}/(((Y_{participant}-Y0)*50/Y_{50})+(X_{participant}-X_{100})*50/X_{50})$

$X_{Participant}$: Signal of the normal probe from the participant DNA or 10% control DNA

$Y_{Participant}$: Signal of the mutation probe from the participant DNA or 10% control DNA

Y_0: Signal of the mutation probe from the 0% calibrator

Y_{50}: Signal of the mutation probe from the 50% calibrator

X_{50}: Signal of the normal probe from the 50% calibrator

X_{100}: Signal of the normal probe from the 100% calibrator

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.*[9] and additionally by a highly sensitive real-time quantitative PCR assay based on a previously published assay,[10] the latter quantitating the mutation burden.

Covariates

Participants filled in a self-administered questionnaire concerning present and past life-style and health status. This was completed by medical staff on the day of examination, prior to physical examination and blood sampling. Current and cumulative tobacco consumption and alcohol consumption were calculated from information in the questionnaire and body mass index was calculated as measured weight in kilograms divided by squared measured height in meters.

Statistical analyses

The statistical software package STATA, release 10.1 was used for all analyses. All statistical tests were two-sided. We used Pearson's χ^2 test for categorical data and Mann-Whitney U test for continuous data to test for differences between participants. Two-sided *P* values below 0.05 were considered statistically significant.

To reduce 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 mutation positives. Analyses were performed in the matched subset as well as in all mutation positives *versus* all negatives.

The log rank test was used for differences of cumulative incidence. Incidence rates were calculated per 10,000 person years. We used Cox's proportional hazard regression with time since blood sampling as time scale to calculate hazard ratios. All analyses were adjusted for sex, age (continuous), current (0, 0.1-20, or above 20 cigarettes/day) and cumulative (0, 0.1-20, or above 20 pack-years) tobacco consumption, alcohol consumption (0, 0.1-168, or above 168 g/week for women; 0, 0.1-252, or above 252 g/week for men), and body mass index (below 18, 18-24.9, 25.0-29.9, or above 30 kg/m^2) at the time of blood sampling. For all end-points, follow up began at the time of blood sampling. Participants with diagnosis prior to the time of blood sampling were excluded from the specific analysis. For overall survival, follow up ended at death, emigration, or May 9th 2009, whichever came first. For the end-points any cancer, hematologic cancer and myeloproliferative cancer, follow up ended at first incident diagnosis, death, emigration, or December 31st 2006.

Online Supplementary Table S1. Characteristics of participants by JAK2 V617F somatic mutation status at the time of blood sampling

Characteristics	Positives	Matched negatives	*P* value *vs.* positives	All negatives	*P* value *vs.* positives
Number	18	540	-	10,489	-
Women (%)	28	28	1.0 (matched)	56	0.02
Age (years)	70 (66-77)	71 (66-77)	1.0 (matched)	59 (44-69)	<0.001
Current smoking (cigarettes/day)	5 (0-20)	0 (0-15)	0.54	0 (0-15)	0.46
Cumulated smoking (pack-years)	33 (25-45)	32 (16-47)	0.82	16 (0-35)	0.005
Alcohol (g/week)	54 (12-132)	84 (12-180)	0.41	60 (12-144)	0.65
Body mass index (kg/m2)	24 (23-25)	26 (23-29)	0.01	25 (22-28)	0.16

Values are median (interquartile range) for continuous variables and frequencies for gender. P values are from Mann-Whitney U test and Pearson's χ^2 test.

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