SUPPLEMENTARY APPENDIX

*JAK2*V617F somatic mutation in the general population: myeloproliferative neoplasm development and progression rate

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Supplementary Appendix

Material and Methods

Study population

From the 48,425 *JAK2V617F* mutation negatives from the Copenhagen General Population Study, we selected three control groups, each including a total of 4 or more mutation negative participants for each of the mutation positive subjects examined. Individuals in the splenic volume control group were matched for sex, age, and body mass index, because these covariates may influence splenic volume. Individuals in the common hematological parameters and lactic acid dehydrogenase control group were matched for sex, age, current tobacco consumption, body mass index, and alcohol intake, because each of them may influence one or more of the parameters examined. Finally, individuals in the erythropoietin control group were matched for sex, age, current tobacco consumption, body mass index, and forced expiratory volume in 1 second, because these covariates may influence plasma erythropoietin levels.

Covariates

Information about tobacco consumption was obtained through the questionnaire, while body mass index was calculated as measured weight in kilograms divided by measured height in meters squared. Forced expiratory volume in 1 second was determined without inhalation of a bronchodilator using a spirometer.

All individuals diagnosed with a myeloproliferative neoplasm prior to re-examination had undergone a bone marrow examination and their patient files were obtained for information on histological diagnosis. At re-examination diagnosis of a myeloproliferative neoplasm was based on hematological parameters; however, individuals diagnosed with a myeloproliferative neoplasm were later offered bone marrow examination as part of their diagnostic work-up.

Somatic mutation detection assays

Briefly, two real-time quantitative PCR reactions were performed in parallel with a common forward primer and probe and two reverse primers: one specific for the normal *JAK2* allele and the other specific for the aberrant *JAK2V617F* somatic mutation. DNA extracted from the UKE1 cell line which has a *JAK2V617F* somatic mutation burden of 100%[13] (generously provided by Prof. Dr. W. Fiedler, Universitätsklinikum, Hamburg-Eppendorf, Germany) was mixed with normal DNA extracted from the K-562 cell line (purchased from DSMZ-German Collection of Microorganisms and Cell Cultures, Niedersachsen, Germany) to produce controls and dilution series with known and decreasing fractions of mutated DNA. Two controls were used containing 25% and 3% mutation burden. The mutation burden was calculated from the K-562/UKE1 dilution series and their standard curves, included in each plate. Individuals were run in triplicates and those with a mutation burden of 0.8% or above were categorized as positive for the *JAK2V617F* somatic mutation, as this was the lower detection limit of the assay. In our previous study[6] we found 68 individuals positive for the *JAK2V617F* mutation among the 49 488 individuals from the Copenhagen General Population Study.[10-12] As we re-tested all 68 original blood samples drawn in the general population examination in 2003-2008 for the *JAK2V617F* mutation in 2012, we found 5 individuals individuals whose previously detected mutation burden was within 0.8 and 1.1%, who had now a mutation burden below the detection limit, indicating that these individuals were false positives and therefore they were no longer considered as *JAK2V617F* mutation positive cases.

Hematological phenotype, erythropoietin, and lactic acid dehydrogenase

The measurement precision of the hematological parameters was monitored daily using internal controls and the coefficients of variation were: erythrocyte volume fraction 2.9%, mean corpuscular volume 2.3%, and platelet count 4.2%. All internal control levels were within the corresponding

reference interval of each analysis. The measurement accuracy was monitored monthly by the use of external controls from UKNEQAS (United Kingdom National External Quality Assessment Service, Sheffield, UK).

The measurement precision of the erythropoietin analysis was monitored daily using 17.7 mIU/mL and 72.0 mIU/mL internal controls with coefficients of variations of 4.4% and 2.1%

The measurement precision of the lactic acid dehydrogenase analysis was monitored daily using internal controls and the coefficient of variation was 4.5% at the level of 203 U/L.

Spleen imaging

Splenic volumes were calculated by the summation-of-volumes technique,[17] which has been shown to represent actual splenic volume within 3-5% accuracy.[18, 19] This was achieved using the software package Vitrea 6.5.0.

Statistical analyses

JAK2V617F mutation burden was grouped into 0.8-2.0%, 2.1-5.0%, 5.1-10.0%, and >10%. These intervals were chosen with the intention to depict phenotypic distribution in the lower range of *JAK2V617F* mutation burden. Cuzick's trend test was used in the setting of the now generally accepted progression of myeloproliferative neoplasms from no disease to essential thrombocythemia over polycythemia vera to primary myelofibrosis alongside an increase in *JAK2V617F* mutation burden, otherwise known as the biological continuum.[7-9] A X^2-test was used to examine if rs10974944 genotype was differently distributed among those with and without a myeloproliferative neoplasm.

Suppl. Table 1: Hematological parameters of 33 *JAK2V617F* mutation positive individuals not diagnosed with MPN at the general population examination in 2003-2008.

Erythrocyte count, 10^{12}/L	4.9 (4.8-5.1)
Platelet count, 10^{9}/L	465.9 (393.1-538.1)
Leukocyte count, 10^{9}/L	9.1 (8.2-9.9)
Erythrocyte volume fraction, %	42.4 (40.9-43.9)
Erythrocyte mean corpuscular volume, fL	85.7 (83.9-87.6)

Values are mean (2.5%-97.5% percentile). MPN=myeloproliferative neoplasm.