JAK2V617F somatic mutation in the general population: myeloproliferative neoplasm development and progression rate

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**JAK2V617F somatic mutation in the general population: myeloproliferative neoplasm development and progression rate**

*Running head: Myeloproliferative neoplasm progression rate*

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Abstract

Clinical significance of the JAK2V617F mutation in patients with a myeloproliferative neoplasm has been the target of intensive research in recent years. However, there is considerably uncertainty about prognosis in JAK2V617F positive individuals without overt signs of myeloproliferative disease.

In this study we tested the hypothesis that increased JAK2V617F somatic mutation burden is associated with myeloproliferative neoplasm progression rate in the general population. Among 49,488 individuals from the Copenhagen General Population Study, 63(0.1%) tested positive for the JAK2V617F mutation in the time period 2003-2008. Of these, 48 were available for re-examination in 2012.

Level of JAK2V617F mutation burden was associated with myeloproliferative neoplasm progression rate, consistent with a biological continuum of increasing JAK2V617F mutation burden across increasing severity of myeloproliferative neoplasm from no disease (n=8 at re-examination) through essential thrombocytemia (n=20) and polycythemia vera (n=13) to primary myelofibrosis (n=7). Among those diagnosed with a myeloproliferative neoplasm only at re-examination in 2012, in the preceding years JAK2V617F mutation burden increased by 0.55%/year, erythrocyte volume fraction increased by 1.19%/year, and erythrocyte mean corpuscular volume increased by 1.25%/year, while platelet count and erythropoietin levels did not change. Furthermore, we established a JAK2V617F mutation burden cut off point of 2% indicative of disease versus no disease; however, individuals with a mutation burden less than 2% may suffer from a latent form of myeloproliferative disease revealed by a slightly larger spleen and/or slightly higher lactic acid dehydrogenase concentration compared to controls. Of all 63 JAK2V617F positive individuals, 48 eventually were diagnosed with a myeloproliferative neoplasm.
**Introduction**

The JAK2V617F somatic mutation has a central role in the pathogenesis of Ph negative myeloproliferative neoplasms, i.e. essential thrombocythemia, polycythemia vera, and primary myelofibrosis.\(^1\) This mutation is also found in patients with different types of venous thromboses but without an overt chronic myeloproliferative neoplasm,\(^2\) and in otherwise healthy individuals.\(^3, 4\)

The JAK2V617F mutation has a prevalence of 0.1-0.2% in the general population,\(^5, 6\) but its clinical implications are still unknown for those individuals harboring the mutation without overt signs of a myeloproliferative neoplasm. These individuals, who often have less than 10% mutation burden,\(^6\) may suffer from a latent form of myeloproliferative neoplasm; however, a mutation burden cut-off point indicative of disease versus no disease has not been established for JAK2V617F mutation positives. For these individuals it is also unknown whether the JAK2V617F mutation burden will change over time, and if such alterations in mutation burden are reflected in an altered hematological phenotype. Among patients with a chronic myeloproliferative neoplasm, a biological continuum of phenotypic presentation has been described, partly influenced by increasing JAK2V617F mutation burden.\(^7-9\) However, a similar correlation between JAK2V617F mutation burden and blood counts or other laboratory tests has not yet been demonstrated among individuals from the general population without overt signs of a myeloproliferative neoplasm.

In this study we tested the hypothesis that JAK2V617F somatic mutation burden is associated with myeloproliferative neoplasm development and evolution in the general population. Among 49,488 individuals from the Copenhagen General Population Study, 63 tested positive for the JAK2V617F mutation in the time period 2003-2008. Of these, 48 were available for re-examination in 2012, which gave us the opportunity to examine increase of allelic burden, changes in clinical phenotype, hematological parameters, splenic volume, and morbidity.

**Methods**

*Study population*
Among 49,488 individuals from the Copenhagen General Population Study\textsuperscript{10-12} we found 63 individuals harboring the \textit{JAK2V617F} mutation. Of these, 52 were still alive and were re-invited in 2012, and 48 of them were re-examined (Fig 1). Control groups were selected as described in the \textit{Online Supplementary Appendix}.

The study was approved by a Danish ethical committee (H-KF 01-144/01) and by Herlev Hospital, Copenhagen University Hospital. Written informed consent was obtained from all study participants.

\textit{Covariates}

At the general population examination in 2003-2008, the 63 \textit{JAK2V617F} mutation positives and all mutation negatives filled in a self-administered questionnaire concerning present and past life-style and health status. This was completed together with an investigator during the visit, prior to physical examination and blood sampling; participants were unaware of their mutation status at the time of examination.

At their re-examination visit, the 48 \textit{JAK2V617F} mutation positives filled in an additional self-administered questionnaire concerning known hematological diagnoses, symptoms, and manifestations. This was completed together with CN and HSB during the visit, prior to physical examination and blood sampling. Diagnostic criteria for myeloproliferative neoplasms were in accordance with the World Health Organization’s criteria for myeloproliferative neoplasms.\textsuperscript{1}

\textit{Somatic mutation detection assays}

A highly sensitive real-time quantitative PCR assay, using DNA isolated from whole blood, including all leukocytes. This assay, based on a previously published assay,\textsuperscript{8} briefly described in the \textit{Online Supplementary Appendix}, was used to re-quantify the mutation burden in the 63 individuals from the general population examination in 2003-2008 as well as the 48 individuals from the re-examination in 2012; this assay was previously validated against the Baxter assay,\textsuperscript{5} where all participants with a mutant allele burden \textgreater{}2\% were also positive on the Baxter assay.
**Genotyping**

The rs10974944 germline genotype was chosen as a marker of the \( \text{JAK2} \) haplotype designated 46/1, associated with risk of developing the \( \text{JAK2V617F} \) mutation.\textsuperscript{14-16} Genotyping was performed as reported in our previously published paper.\textsuperscript{6}

**Hematological phenotype**

Hematological parameters were measured with a flow cytometer-based hematology analyzer, ADVIA\textsuperscript{TM}120 (Siemens, Healthcare Diagnostics, Deerfield, IL, USA), in the routine laboratory of Herlev Hospital, Copenhagen University Hospital.

**Erythropoietin**

Serum samples were analyzed using an Immulite autoanalyzer (Siemens, Healthcare Diagnostics, Deerfield, IL, USA) in the routine laboratory of Herlev Hospital, Copenhagen University Hospital.

**Lactic acid dehydrogenase**

Lactic acid dehydrogenase was determined in plasma by using a colorimetric assay performed on a Konelab 60i autoanalyzer (Helsinki, Finland).

**Spleen imaging**

At re-examination in 2012 all 48 \( \text{JAK2V617F} \) mutation positives were offered a CT scan of the spleen, and 31 of them accepted. Splenomegaly was defined as splenic volumes higher than the 97.5 percentile in the control group, corresponding to splenic volumes above 353 cubic centimetres.

**Registries**

Living status until 2012 was obtained from the national Danish Civil Registration System.\textsuperscript{20} This information is 100% complete for the participants of the Copenhagen General Population Study.

**Statistical analyses**

The statistical software package STATA release 12.1 was used for all analyses. We used linear regression, Wilcoxon rank-sum test, and Cuzick’s trend test. Two-sided \( P \) values below 0.05 were considered significant.

**Results**
**Myeloproliferative neoplasm progression rate**

Of 49,488 individuals from the Copenhagen General Population Study, 63 were found positive for the \textit{JAK2V617F} somatic mutation at the examination from 2003 through 2008 (Fig 1). This corresponds to a prevalence of 0.1% in this sample of the general population with a median age of 63 years at the time of blood sampling (Table 1). Of these, 30 individuals had already been diagnosed with a myeloproliferative neoplasm (5 essential thrombocythemia, 17 polycythemia vera, 7 primary myelofibrosis, and 1 acute myeloid leukemia), while the remaining 33 individuals (6 dead, 1 non-responder, and 26 attending re-examination) were undiagnosed (Table 2 and Fig 2A). Hematological parameters of these 33 \textit{JAK2V617F} mutation positive individuals not diagnosed with a myeloproliferative neoplasm at the general population examination in 2003-2008 are shown in Suppl. Table 1.

In 2012, the 52 individuals still alive were invited for a re-examination, and 48 individuals attended, their median follow-up time being 5.4 years. Of these 48 re-examined individuals, 22 had already been diagnosed with a myeloproliferative neoplasm before 2012 (5 essential thrombocythemia, 11 polycythemia vera, 6 primary myelofibrosis) while 26 individuals were still undiagnosed (Fig 1). As a consequence of the re-examination in 2012, 18 of these 26 individuals were diagnosed with a myeloproliferative neoplasm based on hematological parameters (15 essential thrombocythemia, 2 polycythemia vera, and 1 primary myelofibrosis) (Fig 1). These individuals were subsequently offered further medical examination, including a bone marrow examination and this was performed on 14 individuals, confirming their diagnosis. The remaining 8 \textit{JAK2V617F} mutation positive individuals (“No MPN after diagnostic tests” in Fig 2B) did not have hematological parameters indicative of a myeloproliferative disorder; however, 1 had splenomegaly.

The majority of the \textit{JAK2V617F} mutation positives had mutation burden levels below 10% at the general population examination in 2003-2008 as well as at re-examination in 2012 (Fig 2). Of the 8 \textit{JAK2V617F} mutation positive individuals with no hematological parameters indicative
of a myeloproliferative neoplasm at re-examination, 7 had mutation burden levels below 5% whereas the only case with splenomegaly as the only indicator of myeloproliferative disorder had a mutation burden of 5.7%. No individuals with a myeloproliferative neoplasm were found in the group with allele burden below 2%, neither at the general population examination in 2003-2008, nor at re-examination in 2012. At mutation levels of 2.1% and higher, individuals were diagnosed with essential thrombocythemia and polycythemia vera, while individuals with primary myelofibrosis had mutation burden levels of 6.2% or higher, at both examinations. One single individual, only attending the general population examination, was diagnosed with acute myeloid leukemia one year after the examination and had a mutation burden of 11.2% at examination. Medical records of this patient did not provide information on the preceding myeloproliferative neoplasm.

Taken together, these results suggest that the JAK2V617F mutation burden level was associated with myeloproliferative neoplasm development and progression rate, consistent with a biological continuum of increasing JAK2V617F mutation burden across increasing severity of myeloproliferative neoplasm from no disease (n=8 at re-examination) through essential thrombocythemia (n=20) and polycythemia vera (n=13) to primary myelofibrosis (n=7).

JAK2V617F somatic mutation progression rate

Figure 3 shows the progression rate of the JAK2V617F mutation burden among the 26 individuals undiagnosed with a myeloproliferative neoplasm until re-examination in 2012. In the 18 individuals diagnosed with a myeloproliferative neoplasm at re-examination, the JAK2V617F mutation burden increased by 0.55% per year (P = 0.01) during their follow-up time from the general population examination in 2003-2008 through to the re-examination in 2012; if the person with a 4 time increase in mutation burden was excluded, the consequently increase was 0.31% per year (P = 0.09). In the 8 individuals without a hematologically proven myeloproliferative neoplasm, the JAK2V617F mutation burden was unchanged (P = 0.98) during their follow-up time.

Hematological and erythropoietin progression rate
Hematological phenotypic changes among the 26 individuals undiagnosed with a myeloproliferative neoplasm until re-examination in 2012 are shown in Figure 4. In the 18 individuals diagnosed with a myeloproliferative neoplasm at re-examination, erythrocyte volume fraction increased by 1.19% per year ($P = 0.001$) during their follow-up time, but this parameter was unchanged in the 8 individuals without a hematologically proven myeloproliferative neoplasm. Erythrocyte mean corpuscular volume increased by 1.25 fL per year in both diagnosed ($P = 0.001$) and undiagnosed ($P = 0.02$) individuals. Platelet counts and erythropoietin levels did not change during follow-up time in diagnosed or undiagnosed individuals.

**JAK2V617F mutation burden, splenic volume, and lactic acid dehydrogenase**

*JAK2V617F* mutation burden increased across the severity of myeloproliferative neoplasm diagnoses (Fig 5A). Individuals with no hematologically proven myeloproliferative neoplasm had a median *JAK2V617F* mutation burden of 3.1% (2.5th – 97.5th percentiles: 0.9-5.7%), individuals with essential thrombocythemia had a median *JAK2V617F* mutation burden of 5.5% (3.1-62%), individuals with polycythemia vera had a median *JAK2V617F* mutation burden of 5.9% (2.1-27%), while individuals with primary myelofibrosis had a median *JAK2V617F* mutation burden of 13% (6.9-24%); the trend test across the 4 groups had a $P = 3*10^{-4}$.

Splenic volumes were measured in 31 *JAK2V617F* positive individuals at re-examination in 2012 and were higher compared to controls (Fig 5B). Six of the 8 individuals without a hematologically proven myeloproliferative neoplasm had a splenic volume measurement. All six presented with splenic volumes higher than the median control ($P = 2*10^{-4}$). One individual with no sign of a myeloproliferative neoplasm had a splenic volume of 636 cubic centimetres (pink dot in Fig 5B). Even when omitting this single individual with splenomegaly, the “No MPN” individuals had larger splenic volumes than the controls ($P = 8*10^{-4}$). Individuals diagnosed with a myeloproliferative neoplasm all had larger splenic volumes compared to controls: for essential thrombocythemia $P = 0.04$, for polycythemia vera $P = 2*10^{-4}$, and for primary myelofibrosis $P = 3*10^{-3}$; the trend test across the 5 groups had a $P = 1*10^{-7}$. 
Lactic acid dehydrogenase concentrations exceeded control levels among individuals diagnosed with essential thrombocythemia ($P = 2 \times 10^{-5}$), primary myelofibrosis ($P = 1 \times 10^{-4}$), and among individuals with no hematological parameters indicative of a myeloproliferative neoplasm ($P = 0.03$) (Fig 5C). Although individuals with polycythemia vera did not have higher lactic acid dehydrogenase concentration compared to controls, the overall trend test across the 5 groups had a $P = 6 \times 10^{-7}$.

Influence of rs10974944 on myeloproliferative neoplasm status

Of all 63 JAK2V617F positive individuals, 48 eventually were diagnosed with a myeloproliferative neoplasm, 8 had no myeloproliferative neoplasm after diagnostic tests, and 7 were undiagnosed for a myeloproliferative neoplasm as they did not attend the re-examination in 2012 (1 non-responder and 6 died with no medical record of a myeloproliferative neoplasm diagnosis) (Table 2 and Fig 1). The rs10974944 genotype was not differently distributed among those with and without a diagnosis of a myeloproliferative neoplasm ($X^2: P = 0.45$, Fig 6).

Discussion

In this study of 63 and 48 JAK2V617F somatic mutation positive individuals found among 49,488 individuals from the Danish general population, we observed that JAK2V617F mutation burden was associated with myeloproliferative neoplasm development and progression rate. Furthermore, we propose a cut-off point of 2% for disease versus no disease for JAK2V617F mutation positive individuals; however, even individuals with JAK2V617F mutation burden <2% should receive medical attention as our results suggest that in time, many of them will develop a myeloproliferative neoplasm.

Our findings are consistent with the hypothesis of a biological continuum from JAK2V617F mutation positive individuals from no disease through essential thrombocythemia and polycyhtemia vera to primary myelofibrosis, like that described in recent studies,\textsuperscript{7-9} as we did find an increase in JAK2V617F mutation burden across the severity of myeloproliferative neoplasm diagnoses. Furthermore, a novel observation was that no individuals with a myeloproliferative
neoplasm were found in cases with an allele burden below 2%, which may represent a cut-off point of disease versus no disease for JAK2V617F mutation positive individuals. Also, as there was a median time span of 5.4 years between the first JAK2V617F mutation burden measurement at the general population examination in 2003-2008 and at re-examination in 2012, we were able to describe the increase rate of allelic burden in JAK2V617F mutation positive individuals from the general population undiagnosed of myeloproliferative neoplasms. This is also a novel observation. Among individuals diagnosed with a myeloproliferative neoplasm at re-examination, the JAK2V617F mutation burden increased by 0.55% per year between the general population examination and the re-examination. This is unlikely to be explained by error of the mutation detection technique as our assay had coefficient of variation of 1.4% at 3% mutation burden and 2.5% at 30% mutation burden; nevertheless, if we excluded the person with 4 times increase in mutation burden over time, the mutation burden increase was lower at 0.31% per year. In comparison, Theocharides et al.21 found a 9% increase in mutation burden among 16 JAK2V617F positive patients without cytoreductive therapy, during a follow-up of 36±13 months. Our study is however not directly comparable to the study performed by Theocharides et al. as they also included JAK2 exon 12 allele burden and used DNA from purified granulocytes. Nevertheless, our and their findings together support the present knowledge of the natural course of myeloproliferative neoplasms as diseases developing over several years.5, 21, 22 The fact that JAK2V617F mutation burden was unchanged in individuals without a hematologically proven myeloproliferative neoplasm also indicates a long subclinical, and accordingly, undiagnosed phase before myeloproliferative neoplasms become clinically overt. Since individuals without hematological parameters indicative of a myeloproliferative neoplasm at the time of re-examination had higher splenic volumes and plasma lactic acid dehydrogenase concentrations compared to their respective control groups, it seems likely that all individuals tested positive for the JAK2V617F mutation will ultimately develop a myeloproliferative neoplasm.
In this study, we also showed changes during follow-up of clinically relevant hematological parameters in JAK2V617F positive individuals diagnosed with a myeloproliferative neoplasm at re-examination versus individuals without a hematologically proven myeloproliferative neoplasm. As expected, erythrocyte volume fraction increased during follow-up in individuals with a myeloproliferative neoplasm. This is probably the natural course of the disease and indicates that without treatment, the disease will progress. Erythrocyte mean corpuscular volume also increased during follow-up in both those with and without a diagnosis of a myeloproliferative neoplasm at re-examination. This however, is a bit surprising, as individuals in both groups were untreated. Platelet counts did not change during follow-up in either group, which however could be explained by the fact that individuals in both groups already had high platelet counts at the general population examination. Therefore, the measurements might represent stability of the high platelet count during the observation period. Similarly, the low erythropoietin concentrations did not decrease further during follow-up, which might be due to an already suppressed erythropoietin level at the general population examination that might not decrease any further despite disease progression.

Finally, we analysed presence of the rs10974944 polymorphism as an expression of the 46/1 haplotype among JAK2V617F mutation positive individuals with and without a diagnosis of myeloproliferative neoplasm showing that genotype was not differently distributed in those with and without a myeloproliferative neoplasm.

Strengths of our study include that we identified JAK2V617F mutation positive individuals in the general population, thus avoiding ascertainment bias. Also, because we re-examined 48 mutation positive individuals 5.4 years after the initial examination, we could study the natural history of myeloproliferative neoplasm progression rate. Finally, we had 100% complete follow-up concerning hospital diagnoses, as the Danish registries do not lose track of any persons living in Denmark.

Limitations of our study should also be considered. First, it might seem that statistical power provided by the 63 and 48, respectively, mutation positives among 49,488 individuals from
the general population is limited; however, despite these relatively low numbers our results were mostly highly significant suggesting sufficient statistical power. Second, individuals with the highest JAK2V617F mutation burden may not have attended the Copenhagen General Population Study, as they may already have died or may have been too sick to attend. Such a scenario would however bias our results towards the null hypothesis, and thus cannot explain our results. Third, shortfalls of the screening assay used by us should also be considered. In our initial study, we found 68 mutation positive individuals,6 of which 5 subsequently were found likely to be false positives upon an independent analysis performed 2 years later, using the same assay and the same person performing the analysis (CN). At first hand, this scenario suggest that the assay is not sufficiently robust; however, the 5 persons originally found positive and later negative, only had a mean mutation burden of 0.9% at the original examination. Also, the R² between the first and the second measurement was 0.84%. Nevertheless, the suitability of a 2% mutant allele burden cut-off point for likely disease manifestation may depend on the individual assay used, as our assay like other assays are not standardized internationally. Importantly, we chose to screen DNA samples from whole blood rather than from purified granulocytes. This means that the mutant allele burden is lower than if lymphoid and mononuclear cells (which are generally not part of the malignant clone) were excluded from measurement. This may be a potential problem in the analysis of samples with an initial low mutant allele burden as fluctuations in the clone size over time may influence the apparent change in allelic burden (positively or negatively). Finally, as we studied Whites only, our results may not necessarily apply to other ethnic groups. On the other hand, we are not aware of data suggesting that our results should not be applicable to all races, particularly as the JAK2V617F somatic mutation has been found in people from different races.4, 23, 24

In conclusion, we demonstrated that increased JAK2V617F mutation burden is associated with myeloproliferative neoplasm progression rate in the general population. Furthermore, we established a JAK2V617F mutation burden cut-off point of 2% indicative of disease versus no disease. However, our results also suggest that individuals with a mutation burden...
less than 2% and no clinical phenotype suggestive of a myeloproliferative neoplasm, may still
suffer from a latent form of myeloproliferative neoplasm that may only be traced through a slightly
larger spleen and/or slightly higher lactic acid dehydrogenase concentrations compared to controls.
Acknowledgements

The authors would like to thank the staff and participants of the Copenhagen General Population Study for their important contribution and their willingness to participate. We also thank Prof. Dr. W. Fiedler, Universitätsklinikum, Hamburg-Eppendorf, Germany, for providing us with the UKE1 cell line.

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Authorship and disclosures

CN, SEB, BGN, and HSB planned the study. CN, SEB, and BGN were responsible for re-inviting participants. CN and HSB were responsible for re-examination. CN, BGN and KFK were responsible for the CT-measurements. CN was responsible for the laboratory work, supervised by SEB and BGN. CN and SEB analyzed data. All authors interpreted data. CN, SEB, BGN, and HSB wrote the report. CN prepared the figures. All authors contributed to the final approved version of this report. The authors reported no potential conflict of interest.
References


Table 1: Characteristics of JAK2V617F somatic mutation positives and negatives from the Danish general population.

<table>
<thead>
<tr>
<th></th>
<th>All JAK2V617F mutation negatives</th>
<th>All JAK2V617F mutation positives</th>
<th>MPN diagnosed at general population examination</th>
<th>No MPN at general population examination, No Re-examination</th>
<th>MPN diagnosed at re-examination</th>
<th>No MPN at re-examination</th>
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<tr>
<td>No. of individuals</td>
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<td>63</td>
<td>30</td>
<td>7</td>
<td>18</td>
<td>8</td>
</tr>
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<td>Men, %</td>
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<td>60</td>
<td>50</td>
<td>43</td>
<td>67</td>
<td>100</td>
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<tr>
<td>Age, years</td>
<td>56 (46-66)</td>
<td>63 (57-74)</td>
<td>63 (55-70)</td>
<td>86 (61-89)</td>
<td>71 (64-81)</td>
<td>60 (56-65)</td>
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<td>Body mass index, kg/m²</td>
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<td>26 (23-29)</td>
<td>26 (23-29)</td>
<td>26 (25-27)</td>
<td>25 (22-26)</td>
<td>29 (28-30)</td>
</tr>
<tr>
<td>Current smokers, %</td>
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<td>17</td>
<td>7</td>
<td>14</td>
<td>28</td>
<td>38</td>
</tr>
<tr>
<td>Daily tobacco consumption* (g/day)</td>
<td>15 (10-20)</td>
<td>15 (14-18)</td>
<td>15 (15-15)</td>
<td>15 (15-15)</td>
<td>18 (18-20)</td>
<td>6 (2-15)</td>
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<tr>
<td>Alcohol** (g/week)</td>
<td>96 (84-120)</td>
<td>108 (84-144)</td>
<td>96 (84-132)</td>
<td>144 (60-156)</td>
<td>120 (96-144)</td>
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<td>Forced expiratory volume in 1 second (mL)</td>
<td>288 (232-353)</td>
<td>293 (225-355)</td>
<td>270 (225-356)</td>
<td>182 (129-225)</td>
<td>319 (283-341)</td>
<td>364 (324-377)</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) for continuous variables or frequencies. *Among current smokers. **1 unit alcohol ~ 12g. MPN=myeloproliferative neoplasm.
Table 2: Clinical diagnoses of 63 $JAK2V617F$ mutation positives from the Danish general population.

<table>
<thead>
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<th>Diagnosis</th>
<th>General examination 2003-2008</th>
<th>Re-examination</th>
<th>Final diagnostic</th>
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<td>No MPN after diagnostic</td>
<td>—</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Undiagnose</td>
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<td>Polycythemia</td>
<td>17 MPN n=3</td>
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<td>Dea</td>
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</tr>
<tr>
<td>Total</td>
<td>63</td>
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Figure Legends

Figure 1. Flow chart of study population. * Individuals without hematological parameters indicative of a myeloproliferative neoplasm.


Figure 2. JAK2V617F mutation positive individuals at the general population examination and at re-examination across JAK2V617F mutation burden. The term “No MPN” designates individuals without hematological parameters indicative of a myeloproliferative neoplasm at the time of re-examination. The term “Undiagnosed for MPN” designates individuals without a diagnosis of a myeloproliferative neoplasm at the time of the general population examination. MPN: myeloproliferative neoplasm.

Figure 3. Progression rate of JAK2V617F mutation burden in individuals undiagnosed with a myeloproliferative neoplasm until re-examination. Of the JAK2V617F mutation positive individuals without diagnosis of a myeloproliferative neoplasm at the time of the general population examination in 2003-2008, 26 could be re-examined in 2012. The left side panels show the 18 individuals diagnosed with a myeloproliferative neoplasm at re-examination. The right side panels show the 8 individuals without hematological parameters indicative of a myeloproliferative neoplasm at re-examination. In the upper panels the JAK2V617F mutation burden measurements at the general population examination and at re-examination are shown for each individual separately, while lower panels show corresponding linear regression analyses with 95% confidence intervals and $P$ values for the combined group.

Figure 4. Progression rate of clinically relevant hematological parameters in individuals undiagnosed with a myeloproliferative neoplasm at re-examination. (A) Erythrocyte volume fraction. (B) Erythrocyte mean corpuscular volume. (C) Platelet count. (D) Erythropoietin plasma concentration. For each panel, the left side shows the 18 individuals diagnosed with a myeloproliferative neoplasm
at re-examination while the right side shows the 8 individuals without hematological parameters indicative of a myeloproliferative neoplasm at re-examination.

In the upper part of the panels A-D, measurements of hematological parameters at the general population examination and at re-examination are shown for each individual separately, while the lower part of these panels shows corresponding regression analyses with 95% confidence intervals and $P$ values for the combined group.

Figure 5. $JAK2V617F$ mutation burden, splenic volume, and lactic acid dehydrogenase concentrations in $JAK2V617F$ positive individuals and in controls. The term “No MPN” designates individuals without hematological parameters indicative of a myeloproliferative neoplasm at the time of re-examination. One of these 6 individuals had splenomegaly (pink dot). ET: essential thrombocythemia, PV: polycythemia vera, PMF: primary myelofibrosis. The numbers are slightly lower for splenic volumes because not all $JAK2V617F$ mutation positive individuals were examined with a CT scan. Among individuals diagnosed with essential thrombocythemia, one individual had a $JAK2V617F$ mutation burden of 62%, which in the figure is plotted at 30% (+ arrow). $P$ values are from Wilcoxon rank-sum test of the comparison with the control group and from Cuzick’s trend test across the 5 groups.

Figure 6. Rs10974944 genotype by myeloproliferative neoplasm status in $JAK2V617F$ mutation positive individuals from the Danish general population. The 48 individuals diagnosed with myeloproliferative neoplasm include 30 individuals (5 dead, 3 non-responders, and 22 with a known myeloproliferative neoplasm) diagnosed before the re-examination in 2012 and 18 individuals diagnosed at the re-examination. MPN: myeloproliferative neoplasm.
Figure 1

49,488 individuals from the general population, examined year 2003-2008

63 JAK2V617F mutation positives

52 re-invited year 2012

48 re-examined year 2012

26 undiagnosed

18 MPN diagnosed at re-examination (15 ET, 2 PV, 1 PMF)

8 no MPN (1 splenomegaly only)

48,425 JAK2V617F mutation negatives excluded

11 dead before re-examination (5 MPN, 6 undiagnosed)

4 non responders (3 MPN, 1 undiagnosed)

22 MPN diagnosed (5 ET, 11 PV, 6 PMF)
Figure 3

Myeloproliferative neoplasm diagnosed at re-examination in 2012

![Graphs showing JAK2V617F mutation burden over follow-up time for patients with and without diagnosis.](image-url)
Myeloproliferative neoplasm diagnosed at re-examination in 2012

A  Yes  n = 18

B  Yes  n = 18

C  No  n = 8

D  No  n = 8

Figure 4
Figure 5
Figure 6

$X^2: P = 0.45$

<table>
<thead>
<tr>
<th>Category</th>
<th>No MPN after diagnostic tests</th>
<th>Diagnosed with MPN</th>
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</thead>
<tbody>
<tr>
<td>Non-carriers (CC)</td>
<td>3</td>
<td>11</td>
</tr>
<tr>
<td>Heterozygotes (CG)</td>
<td></td>
<td>31</td>
</tr>
<tr>
<td>Homozygotes (GG)</td>
<td></td>
<td>6</td>
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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 study6 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 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,\(^1^7\) which has been shown to represent actual splenic volume within 3-5% accuracy.\(^1^8\),\(^1^9\) 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.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
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<tbody>
<tr>
<td>Erythrocyte count, $10^{12}$/L</td>
<td>4.9 (4.8-5.1)</td>
</tr>
<tr>
<td>Platelet count, $10^9$/L</td>
<td>465.9 (393.1-538.1)</td>
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<tr>
<td>Leukocyte count, $10^9$/L</td>
<td>9.1 (8.2-9.9)</td>
</tr>
<tr>
<td>Erythrocyte volume fraction, %</td>
<td>42.4 (40.9-43.9)</td>
</tr>
<tr>
<td>Erythrocyte mean corpuscular volume, fL</td>
<td>85.7 (83.9-87.6)</td>
</tr>
</tbody>
</table>

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