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Received: April 4, 2024.
Accepted: April 12, 2024.


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Evaluation of the *ATM* L2307F germline variant in 121 Italian pedigrees with familial myeloproliferative neoplasms

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Running title: *ATM* germline variant in familial MPN

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Contributions: OB, RJ, RK and ER designed research and wrote the paper; IF, RJ and DP performed molecular investigations; GR collected clinical data; RK and ER finalized the manuscript. All Authors participated to analysis of data, revision of the draft and final approval.

All Authors do not have any competing financial interests.

Data are available upon request to the corresponding author.

This work was supported by grants from Associazione Italiana per la Ricerca sul Cancro (AIRC; Milan, Italy) to ER: AIRC IC 2021 ID 25703, Minerva project AIRC award number 21267.
To evaluate the role of ATM L2307F in germline genetic predisposition to familial myeloproliferative neoplasms (fMPN), we screened our large cohort of fMPN for the presence of ATM L2307F. We did not observe ATM L2307F in any of our 121 fMPN families from Northern Italy, thus excluding a role of ATM L2307F as predisposing factor to fMPN.

It has been increasingly recognized that a subset of MPN aggregates within families, suggesting a role of germline mutations in disease etiology. Relatives of MPN patients were shown to be at five- to seven-fold increased risk of developing MPN. Moreover, we previously reported that 7.6% of apparently sporadic MPN in fact exhibit familial clustering. Causative germline variants underlying fMPN remain largely unknown. Of note, the phenotypic driver mutations established in MPN (JAK2, CALR, MPL) are acquired somatically also in fMPN. The coexistence of JAK2, CALR, and MPL somatic mutations in relatives within the same pedigree has led to the hypothesis that what is truly inherited is a genetic predisposition to acquire one of the three MPN drivers. Underlying germline variants do not drive the disease per se, but rather predispose for the acquisition of oncogenic mutations.

In recent years, a few highly penetrant susceptibility variants for fMPN have been reported. However, these variants are rare in the general population or regionally restricted and therefore do not explain most of the hereditability observed in fMPN. To date, a unique predisposing gene accounting for familial clustering of MPN has not been identified. This led to the hypothesis that a part of the inherited risk might depend on common, low penetrance risk alleles each representing a small fraction of MPN heritability, jointly contributing to familial clustering. Thus, common germline susceptibility alleles, each slightly increasing the risk of developing sporadic MPN, might be enriched in fMPN, as
previously demonstrated for the \textit{TERT} \textit{rs2736100\_C} allele in conjunction with the JAK2 46/1 (GGCC) predisposition haplotype.\textsuperscript{9}

Most recently, the germline variation \textit{ATM} L2307F, caused by a single nucleotide variant (\textit{rs56009889}) in the coding region of the \textit{ATM} gene was reported to occur in nearly 8\% of individuals with fMPN in a single-center study.\textsuperscript{10} Braunstein et al. reported an increased prevalence of \textit{ATM} L2307F in fMPN as compared to sporadic MPN (7.8\% vs 2.3\%, \textit{P}=0.05) at borderline statistical significance. While the authors classified \textit{ATM} L2307F as variant of uncertain significance based on ACMG guidelines for interpretation of sequence variants,\textsuperscript{11} they presented functional data suggesting that \textit{ATM} L2307F stabilizes the ATM dimer in a closed conformation, thereby decreasing the phosphorylation of the downstream tumor suppressor \textit{CHECK2}, subsequently altering the cellular response to DNA damage.\textsuperscript{10} In a different study, \textit{in vitro} experiments showed increased rates of apoptosis for cells carrying \textit{ATM} L2307F after exposure to DNA damaging agents, suggesting \textit{ATM} L2307F to be functionally hypomorphic.\textsuperscript{12}

To evaluate the role of \textit{ATM} L2307F in germline genetic predisposition to familial clustering of MPN, we screened our cohort of 121 fMPN families, defined by two or more affected relatives per family. DNA was available for 180 affected individuals with fMPN. Additionally, a control cohort of 111 unrelated subjects was screened for the presence of \textit{ATM} L2307F. These unrelated controls were recruited from the same hospital and showed normal hemograms or reactive hematological conditions. The clinical and molecular characteristics of our fMPN cohort and controls are detailed in Table 1. To screen for the presence of \textit{ATM} L2307F, we developed an amplicon-based next-generation sequencing assay to derive DNA sequences of \textit{ATM} exon 47 (NM\_000051.4), subsequently allowing for genotyping of \textit{rs56009889}.

We did not observe \textit{ATM} L2307F in any of our 180 patients constituting 121 fMPN families from Northern Italy. This stands in contrast to the study by Braunstein \textit{et al.} conducted on
a single-center cohort assembled in North America. Moreover, the ATM L2307F variant was not detected in any of the 111 unrelated control individuals collected locally at our center, in line with data reported in large population-based studies.\textsuperscript{13} In the gnomAD database (v4.0.0),\textsuperscript{14} the ATM L2307F variant (rs56009889; SNV:11-108326169-C-T (GRCh38)) is reported at an overall minor allele frequency of 0.013%, presenting at 0.014% in the European (non-Finnish) sub-cohort. In contrary, ATM L2307F is reported at a significantly higher frequency in the Ashkenazi Jewish population (3.017%). Accordingly, associations of ATM L2307F with cancer susceptibility were previously shown to be influenced by the ethno-geographic origin of the populations investigated. Specifically, Ji et al. reported an association of ATM L2307F with lung adenocarcinoma risk that was observed at higher effect size in Israeli (odds ratio 6.74) as compared to North American (odds ratio 3.36) populations, but was absent in Europeans due to the lack of variant carriers.\textsuperscript{13} A different study by Lampson et al., involving patients from the Dana-Farber Cancer Institute located in Boston (MA), USA, demonstrated an enrichment of germline ATM L2307F in chronic lymphocytic leukemia (CLL) (2.78%) and in other non-CLL lymphoid disorders (1.47%) as compared to myeloid disorders (0.67%).\textsuperscript{12} While only a fraction of the latter was diagnosed with MPN, in comparison with Braunstein et al. this study suggests low frequencies of ATM L2307F also in some North American MPN patient cohorts. Moreover, the same study reported an absence of ATM L2307F in a local control cohort, arguing for a limited potential of ATM L2307F as genetic marker also outside of Europe.\textsuperscript{12} While Braunstein et al.\textsuperscript{10} reported ATM L2307F frequencies of 7.8% and 2.3% for fMPN and sporadic MPN, respectively, a control cohort recruited at the same center may allow separating population-specific effects from general applicability with regard to the disease.

In conclusion our data do not support a role of ATM L2307F as predisposing factor for fMPN in our cohort from Northern Italy representing a European population. However,
other germline variants affecting DNA repair pathway genes recently implicated in fMPN susceptibility \textsuperscript{15} might be present at consistent frequencies across populations. Thus, further cohort studies using targeted re-sequencing-based analyses or alternative genotyping approaches, possibly focused on well-selected candidate genes, are warranted.
References


Table 1: Clinical and molecular characteristics of our cohort of patients with familial myeloproliferative neoplasms and unrelated healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>fMPN (n=180)</th>
<th>Unrelated controls (n=111)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at sampling, years (range)</td>
<td>54.0 (16-87)</td>
<td>48.9 (13-86)</td>
</tr>
<tr>
<td>Gender n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80 (44.4)</td>
<td>61 (55.0)</td>
</tr>
<tr>
<td>Female</td>
<td>100 (55.6)</td>
<td>50 (45.0)</td>
</tr>
<tr>
<td>Phenotype at sampling n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ET</td>
<td>82 (45.6)</td>
<td>Not applicable</td>
</tr>
<tr>
<td>PV</td>
<td>69 (38.3)</td>
<td></td>
</tr>
<tr>
<td>MF</td>
<td>28 (15.6)</td>
<td></td>
</tr>
<tr>
<td>CML</td>
<td>1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Driver mutation n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>JAK2*</td>
<td>147 (81.7)</td>
<td>Wild-type</td>
</tr>
<tr>
<td>CALR</td>
<td>19 (10.6)</td>
<td></td>
</tr>
<tr>
<td>MPL</td>
<td>1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Bcr/Abl</td>
<td>1 (0.5)</td>
<td></td>
</tr>
<tr>
<td>Triple-negative</td>
<td>12 (6.7)</td>
<td></td>
</tr>
</tbody>
</table>

fMPN, familial myeloproliferative neoplasms; ET, essential thrombocythemia; PV, polycythemia vera; MF, myelofibrosis; CML, chronic myeloid leukemia

* 144 patients carried JAK2 V617F mutation, 2 patients carried exon 12 JAK2 mutations and 1 patient carried a H608N JAK2 mutation