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

It has been increasingly recognized that a subset of myeloproliferative neoplasms (MPN) aggregates within families, suggesting a role of germline mutations in disease etiology. Relatives of MPN patients were shown to be at 5- to 7-fold increased risk of developing MPN.[1] Moreover, we previously reported that 7.6% of apparently sporadic MPN in fact exhibit familial clustering.[2] Causative germline variants underlying familial MPN (fMPN) remain largely unknown. Of note, the phenotypic driver mutations established in MPN (*JAK2*, *CALR*, *MPL*) are acquired somatically also in fMPN.[2-4] The co-existence 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.[5] 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.[5-7] 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.[8] 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 *TERT* rs2736100_C allele in conjunction with the *JAK2* 46/1 (GGCC) predisposition haplotype.[9]

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

In order to evaluate the role of *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 *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. In order to screen for the presence of *ATM* L2307F, we developed an amplicon-based next-generation sequencing assay to derive DNA sequences of *ATM* exon 47 (NM_000051.4), subsequently allowing for genotyping of rs56009889.

We did not observe *ATM* L2307F in any of our 180 patients constituting 121 fMPN families from Northern Italy.

Table1. Clinical and molecular characteristics of our cohort of patients with familial myeloproliferative neoplasms and unrelated healthy controls.

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

*One hundred and forty-four patients carried *JAK2* V617F mutations, 2 patients carried *JAK2* exon 12 mutations, and 1 patient carried a *JAK2* H608N mutation. fMPN: familial myeloproliferative neoplasms; ET: essential thrombocythemia; PV: polycythemia vera; MF: myelofibrosis; CML: chronic myeloid leukemia.

This stands in contrast to the study by Braunstein *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.[13] In the gnomAD database (v4.0.0),[14] the *ATM* L2307F variant (rs56009889; single-nucleotide variant: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.[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%).[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.[12] While Braunstein *et al.*[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[15] might be present at consistent frequencies across populations. Thus, further cohort studies using targeted resequencing-based analyses or alternative genotyping approaches, possibly focused on well-selected candidate genes, are warranted.

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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. RJ and ER finalized the manuscript. All authors contributed to data analysis, participated in the revision of the draft, and provided final approval.

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Data-sharing statement

Data are available upon request to the corresponding author.

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