

# Common germline variation at the *TERT* locus contributes to familial clustering of myeloproliferative neoplasms

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The C allele of the rs2736100 single nucleotide polymorphism located in the second intron of the *TERT* gene has recently been identified as a susceptibility factor for myeloproliferative neoplasms (MPN) in the Icelandic population. Here, we evaluate the role of *TERT* rs2736100\_C in sporadic and familial MPN in the context of the previously identified *JAK2* GGCC predisposition haplotype. We have confirmed the *TERT* rs2736100\_C association in a large cohort of Italian sporadic MPN patients. The risk conferred by *TERT* rs2736100\_C is present in all molecular and diagnostic MPN subtypes. *TERT* rs2736100\_C and *JAK2* GGCC are independently predisposing to MPN and have an additive effect on disease risk, together explaining a large fraction of the population attributable fraction (PAF = 73.06%). We found *TERT* rs2736100\_C significantly enriched ( $P = 0.0090$ ) in familial MPN compared to sporadic MPN, suggesting that low-penetrance variants may be responsible for a substantial part of familial clustering in MPN.

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## ■ Introduction

Myeloproliferative neoplasms (MPN) constitute a group of phenotypically diverse chronic myeloid malignancies including three major disease entities: polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). Mutually exclusive oncogenic somatic mutations in three genes have been identified in more than 90% of MPN cases [1]. Mutations in *JAK2*, most notably *JAK2*-V617F are present in all three disease subtypes, more frequently in PV [2–5], while *MPL* and *CALR* mutations are exclusively found in ET and PMF patients [6–8]. The same somatic mutations are present also in familial MPN, which account for 5–10% of MPN cases [9–11]. Previously, a common haplotype (GGCC or 46/1) at the *JAK2* locus has been found to predispose to *JAK2* mutation positive sporadic and familial MPN [12–15]. Genome-wide association studies have successfully revealed risk loci for a series of cancers [16]. A recent study identified the germline sequence variant rs2736100\_C located in the second intron of the *TERT* gene as risk variant for MPN in the Icelandic population [17]. In this study, we were seeking to confirm the *TERT* association in an independent cohort from a different ethnic background and evaluate the role of the *TERT* predisposition locus in familial clustering of MPN. Furthermore, we were testing the possibility of an interaction of *TERT* and *JAK2* susceptibility loci in sporadic and familial MPN.

## ■ Methods

Blood samples from sporadic MPN ( $n = 717$ ), familial MPN ( $n = 121$ ) and control ( $n = 202$ ) subjects from Italy were obtained after written informed consent. The study was approved by the institutional ethics committee (Comitato di Bioetica, Fondazione IRCCS Policlinico San Matteo) and procedures were in accordance with the Helsinki declaration. Details on patient characteristics and sample collection have been described in a previous study [15]. Patients were defined as familial cases if two or more individuals within the same pedigree were affected. For each family, the proband was identified as the first affected family member seeking medical attention.

*Additional Supporting Information may be found in the online version of this article.*

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**Conflict of interest:** Nothing to report.

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**TABLE 1.** Association of *TERT* rs2736100 with Sporadic and Familial MPN and Molecular Subtypes

Case population	Genotype frequency (%) case population				Genotype frequency (%) control population				Odds ratio (95% CI)				P value
	A/A	A/C	C/C	n	A/A	A/C	C/C	n	A/A	A/C	C/C	n	
Sporadic MPN (n = 717)	11.3 (81)	46.2 (331)	42.5 (305)	23.3 (47)	43.6 (88)	33.2 (67)	1	2.18 (1.42–3.35)	2.64 (1.69–4.13)	1.15 × 10 <sup>-4</sup>			
Sporadic MPN <i>JAK2</i> + (n = 516)	10.7 (55)	44.8 (231)	44.6 (230)	23.3 (47)	43.6 (88)	33.2 (67)	1	2.24 (1.42–3.55)	2.93 (1.82–4.72)	5.55 × 10 <sup>-5</sup>			
Sporadic MPN <i>CALR</i> + (n = 126)	11.9 (15)	46.8 (59)	41.3 (52)	23.3 (47)	43.6 (88)	33.2 (67)	1	2.10 (1.08–4.10)	2.43 (1.23–4.82)	0.0270			
Familial MPN (n = 121)	5.0 (6)	39.7 (48)	55.4 (67)	23.3 (47)	43.6 (88)	33.2 (67)	1	4.27 (1.7–10.72)	7.83 (3.14–19.55)	1.10 × 10 <sup>-6</sup>			
Familial MPN probands (n = 75)	5.3 (4)	36.0 (27)	58.7 (44)	23.3 (47)	43.6 (88)	33.2 (67)	1	3.61 (1.19–10.92)	7.72 (2.60–22.94)	2.65 × 10 <sup>-5</sup>			
Familial MPN (n = 121)	5.0 (6)	39.7 (48)	55.4 (67)	11.3 (81)	46.2 (331)	42.5 (305)	1	1.96 (0.81–4.73)	2.97 (1.24–7.08)	0.0090			
Familial MPN probands (n = 75)	5.3 (4)	36.0 (27)	58.7 (44)	11.3 (81)	46.2 (331)	42.5 (305)	1	1.65 (0.56–4.85)	2.92 (1.02–8.37)	0.0180			

Presence of the *JAK2*-V617F mutation, *JAK2* exon 12 mutations, *MPL* exon 10 mutations, and *CALR* exon 9 mutations was assessed in granulocyte DNA as previously described [7]. Genotyping for rs2736100 (*TERT*) and rs10974944 (*JAK2*) was performed using commercially available TaqMan SNP genotyping assays (C\_1844009 and C\_31941696, respectively; Applied Biosystems, Foster City, CA).

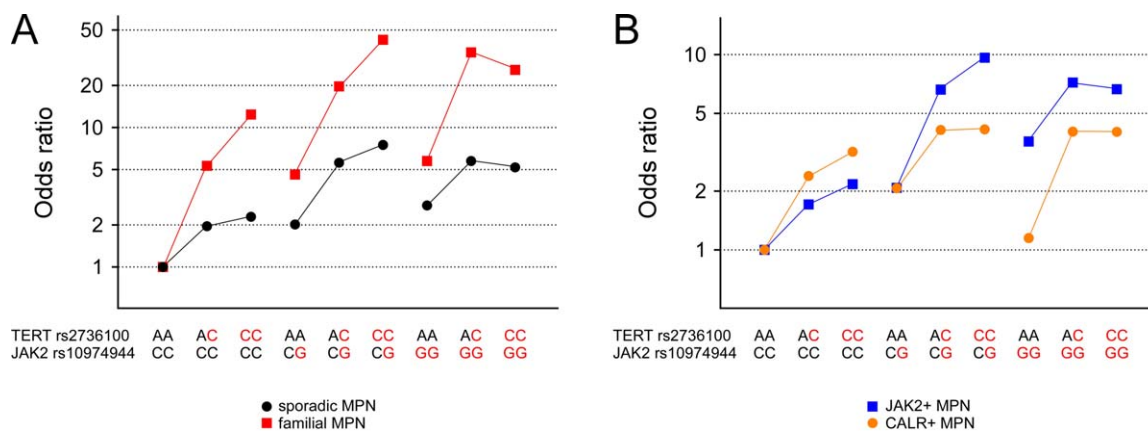
Statistical analyses were performed using the R statistical software (version 3.0.3) [18] in conjunction with the R-packages “SNPassoc” and “scrim.” Population attributable fraction (PAF) and proportions of familial relative risk (FRR) explained by *TERT* and *JAK2* loci were calculated as previously described by others [19,20], using an estimate of 5.6 for the overall MPN FRR [21]. The Cochran–Armitage test of trend was applied to study differences in distribution of risk allele numbers per individual in the different cohorts. The absolute risk for developing MPN in different *TERT*/*JAK2* genotypic classes was calculated using logistic regression.

## Results

We confirmed the previously reported [17] association of rs2736100\_C with the MPN phenotype in a cohort of sporadic MPN patients (n = 717) of a different ethnic background (Table 1). The size of our sporadic MPN cohort allowed for comparison of genotype distributions in different molecular and diagnostic subgroups. Significant associations were detected for both *JAK2*-positive and *CALR*-positive MPN at similar strength (Table 1), suggesting that there is no preferential susceptibility to any molecular subtype. A similar trend was observed for *MPL*-positive and triple negative MPN patients (lacking *JAK2*, *MPL*, and *CALR* mutations), however, statistical significance is absent, possibly due to low sample sizes (Supporting Information Table 1). As in the prior study [17], the *TERT* rs2736100\_C association was present in PV, ET, and PMF, implying a general role in MPN pathogenesis (Supporting Information Table 1).

We next studied the combined effects of the two known MPN risk loci at *TERT* and *JAK2* as well as their possible interaction. Conditional logistic regression analysis revealed that *TERT* and *JAK2* loci are independently predisposing to MPN, the total combined risk approximating the sum of the two genotypes (Fig. 1; Supporting Information Table 2). Direct testing for interaction was negative (Supporting Information Table 3). The combined effects of *TERT* and *JAK2* risk loci are stronger in *JAK2*-positive MPN (Fig. 1). This finding is compatible with the *JAK2* risk haplotype predisposing primarily for the acquisition of somatic mutations in the *JAK2* gene.

To evaluate the role of *TERT* in familial MPN, we determined the rs2736100 genotype in 121 affected members of 75 Italian families with two or more MPN cases within first and/or second degree relatives. The *TERT* rs2736100\_C association is significantly stronger in familial MPN compared to sporadic MPN (P = 0.009; Table 1), indicating a role of the *TERT* locus in MPN familial clustering. Proband-based analysis confirmed this observation (P = 0.018; Table 1). As previously reported on a smaller familial MPN cohort [15], such a significant difference in association strength is absent for *JAK2* GGCC when directly comparing sporadic and unfiltered familial *JAK2*-positive MPN cases (Supporting Information Table 4). However, MPN families consisting of both *JAK2*-positive and *JAK2*-negative members might be confounding the possible association, as *JAK2* GGCC predisposes mainly to *JAK2*-positive MPN. Indeed, when restricting analysis to families exclusively consisting of *JAK2*-positive members, we observed a significant trend toward *JAK2* GGCC enrichment in familial MPN compared to sporadic MPN (P = 0.046; Supporting Information Table 4). Moreover, the familial MPN cohort exhibits enrichment for individuals carrying more than three risk alleles (counting one for heterozygotes and two for homozygotes; Supporting Information Figure 1) and a trend for odds ratios increasing toward double positive (*TERT*/*JAK2*) individuals (Supporting Information Table 2), further supporting the possibility of *JAK2* GGCC contributing to MPN familial clustering. Independence of *TERT* and *JAK2* loci in mediating MPN susceptibility, as observed in sporadic MPN, is also true for familial MPN (Fig. 1).



**Figure 1.** Combined effects of *TERT* and *JAK2* MPN predisposition loci. Genotypic odds ratios for MPN in respect to the nine genotypic combinations at *TERT* (rs2736100) and *JAK2* (rs10974944) loci are shown for (A) sporadic and familial total cohorts as well as for (B) sporadic *JAK2*-positive and *CALR*-positive subcohorts. Risk alleles are marked in red. [Color figure can be viewed in the online issue, which is available at [wileyonlinelibrary.com](http://wileyonlinelibrary.com).]

Similar to that reported in the Icelandic study [17], in this study cohort *TERT* rs2736100\_C is estimated to account for 51.89% of PAF. In concert with *JAK2* GGCC (PAF = 44.01%), they explain a large part (combined PAF = 73.06%) of the population susceptibility for MPN. The absolute risk for developing MPN in different *TERT*/*JAK2* genotypic classes is calculated in Supporting Information Table 5. As the risk variant rs2736100\_C is present at high frequency (55%; Table I) in the general population, only 2.01% of the FRR can be attributed to the *TERT* rs2736100\_C variation. In contrast, 5.15% of FRR can be explained by the *JAK2* GGCC risk haplotype which is present at lower frequency (27%; Supporting Information Table 4) in the general population.

## Discussion

The *TERT* gene encodes the reverse transcriptase of the telomerase complex, essential for maintaining telomere length [22]. The C allele of rs2736100 has been linked to longer telomeres [23,24], compatible with a direct regulatory effect of rs2736100 genotype on *TERT* expression. *TERT* rs2736100\_C was previously shown to also associate with elevated risk for several other cancers, albeit with lower effect [25–28]. Furthermore, rs2736100\_C is linked to increased blood cell count values [17,29], a hallmark of MPN. The fact that *TERT* rs2736100\_C predisposes to all MPN subtypes implies a generic role in MPN predisposition, possibly through affecting blood cells counts.

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