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 Policlínico 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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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 “scirr.” 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 I). The size of our sporadic MPN cohort allowed for comparison of genotype distributions in different molecular and diagnostic subgroups (Table I). Significant associations were detected for both JAK2-positive and CALR-positive MPN at similar strength (Table I), 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 I), indicating a role of the TERT locus in MPN familial clustering. Proband-based analysis confirmed this observation (P = 0.018; Table I). 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).

### Table 1: Association of TERT rs2736100 with Sporadic and Familial MPN and Molecular Subtypes

| Case population | Control(n = 202) | Control(n = 717) | Sporadic MPN(n = 202) | Sporadic MPN(n = 717) | Familial MPN(n = 717) |
|-----------------|-----------------|-----------------|----------------------|----------------------|---------------------|
| Sporadic MPN    | 11.7(61)        | 10.7(65)        | 12.3(67)             | 12.3(67)             | 12.3(67)            |
| JAK2 positive   | 10.7(65)        | 10.7(65)        | 12.3(67)             | 12.3(67)             | 12.3(67)            |
| JAK2 negative   | 12.3(67)        | 12.3(67)        | 11.9(65)             | 11.9(65)             | 11.9(65)            |
| Families with JAK2-positive cases | 5.0(60) | 5.0(60) | 5.0(60) | 5.0(60) | 5.0(60) |

Odds ratio (95% CI) 2.10 (0.98–4.50) 2.10 (0.98–4.50) 1.65 (0.65–4.68) 1.65 (0.65–4.68) 1.65 (0.65–4.68)
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 red 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 cell counts.

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