Germline *RBBP6* mutations in familial myeloproliferative neoplasms

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To the editor:

Myeloproliferative neoplasms (MPN) comprise a heterogeneous group of hematological disorders characterized by clonal overproduction of differentiated myeloid cells, propensity to thrombosis, hemorrhage and increased risk of leukemia. Three of MPN subtypes, polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) are considered as the “classical BCR-ABL1-negative myeloproliferative neoplasms” and they share many clinical and molecular features. Although most cases of MPN are sporadic, several previous studies have shown familial clustering of the disease\textsuperscript{1-3} and increased risk of MPN in relatives\textsuperscript{4}. Somatic mutations have been associated with MPN, most notably disease causing, mutually exclusive mutations in \textit{JAK2}, \textit{CALR} and \textit{MPL}. Somatic \textit{JAK2} mutations are present in about 60\% of MPN cases\textsuperscript{5, 6}, \textit{MPL} mutations occur somatically in about 1-5\% of cases\textsuperscript{7}, and somatic \textit{CALR} mutations are found in about 20-30\% of ET and PMF\textsuperscript{8, 9}. Familial MPN is clinically indistinguishable from sporadic MPN, and displays frequent somatic \textit{JAK2} and \textit{CALR} mutations\textsuperscript{2, 3, 10-12}. Other genes that frequently mutate somatically in MPN, such as \textit{TET2}\textsuperscript{13}, \textit{DNMT3A}\textsuperscript{14}, \textit{ASXL1}\textsuperscript{15}, etc., do not significantly contribute to familial MPN and in the majority of familial cases the causative germline mutation is unknown\textsuperscript{16, 17}.

The role of common germline variants in MPN predisposition has been established. Specifically, the \textit{JAK2} ‘GGCC’ haplotype increases the risk to develop \textit{JAK2}-mutant MPN by several fold\textsuperscript{18}. Another germline variant in \textit{TERT} (rs2736100) increases the risk of all molecular subtypes of MPN by ~2-fold\textsuperscript{19, 20}. Both of these single nucleotide polymorphisms contribute to familial clustering of the disease, although they can explain
only a minor proportion of it\textsuperscript{19}. Recently several additional common variants conferring susceptibility to MPN have been identified\textsuperscript{21}.

In order to identify the germline mutation predisposing to MPN, we studied an Australian pedigree of English ancestry (Northern England) with five members in four generations diagnosed with MPN. DNA was available from three affected members (Figure 1A). Each of the three members of the family has a different MPN-specific somatic mutation (\textit{JAK2-V617F, MPL-W515L, CALR-Type 1}, Figure 1A). To map the candidate disease loci in the pedigree, we applied a nonparametric algorithm SEGEX (Supplementary Figure 1) and identified 12 shared genomic regions with a total size of 217.87 Mb amongst the three affected subjects (Figure 1B; Supplementary Table 1). As one of these genomic regions was likely to carry the disease-causing mutation, we next applied exome sequencing. After reference alignment, a number of filtering criteria were applied to the detected variants (Supplementary Figure 2). We performed Sanger sequencing of all the 18 final candidate variants and confirmed DNA variants segregating with the disease in three genes (\textit{RBBP6, C20orf3, and ARMC5}) (Figure 1C; Supplementary Table 2).

To identify which of the \textit{RBBP6, C20orf3, and ARMC5} variants is the one predisposing to MPN, we examined healthy subjects for the presence of the candidate variants. Based on this analysis, \textit{ARMC5-P507L} was excluded due to 7\% frequency in healthy controls while \textit{RBBP6-R1569H} and \textit{C20orf3-D395N} were not found in any of over 700 healthy controls.
Next we sequenced the exons carrying the identified \textit{RBBP6} and \textit{C20orf3} mutations in an additional 66 MPN families. This analysis yielded two unique mutations in \textit{RBBP6} (E1654G and R1451T, Figure 1D) and one polymorphism in \textit{C20orf3} (P406L) present in 4\% of the healthy subjects. Unfortunately, for both familial cases with \textit{RBBP6} mutation, DNA sample was available from only one affected member, therefore we could not obtain data on the mutation segregation with MPN for these two additional families. We used Polyphen 2 and SIFT mutation prediction tools to assess the possible effects of the mutations on protein function. \textit{RBBP6}-R1569H is predicted to be damaging by both tools, while \textit{C20orf3}-D395N mutation is benign (Supplementary Table 3). In summary, \textit{C20orf3} is unlikely to be involved in familial MPN, whereas \textit{RBBP6} mutations remain strong candidates for familial predisposition to MPN.

Due to the low penetrance associated with \textit{RBBP6} mutations, establishing the family history of MPN may often be difficult. Therefore, we screened for \textit{RBBP6} mutations in 490 sporadic MPN cases. In this analysis we identified two unique germline mutations (S1444F and A1673V) in three apparently unrelated subjects and one polymorphism (I1661V) present in 0.5\% of healthy controls (Table 1; Figure 1E). Overall, we identified 5 different germline \textit{RBBP6} mutations associated with MPN and not detected in the general population (Table 1; Figure 1F).

Apart from the three affected members, eight additional healthy family members carried \textit{RBBP6}-R1569H in the Australian pedigree, consistent with the expected low penetrance\textsuperscript{16}. Since common germline SNPs have been shown to contribute to familial
MPN\textsuperscript{19}, we checked all members of the family for \textit{JAK2} GGCC haplotype and the \textit{rs2736100-C} risk variant in \textit{TERT} (Supplementary Table 4). As expected, from the affected members only the one with \textit{JAK2}-V617F mutation was heterozygous for \textit{JAK2} GGCC haplotype, while two affected members were heterozygous for \textit{TERT} risk variant and the third one was homozygous for the \textit{TERT} risk allele. Although it is not possible to draw any solid conclusions based on a single family, we noticed a trend in \textit{JAK2} GGCC haplotype and \textit{TERT} SNP distribution similar to the pattern we observed in our previous study\textsuperscript{19}. The risk allele frequencies for both \textit{JAK2} GGCC haplotype and \textit{TERT} \textit{rs2736100} were higher in \textit{RBBP6}-R1569H mutant MPN patients compared to \textit{RBBP6}-R1569H mutant unaffected members (16.67\% vs. 6.25\% for \textit{JAK2} GGCC haplotype, 66.67\% vs. 50.00\% for \textit{TERT} SNP).

We have identified germline \textit{RBBP6} mutations in about 5\% of familial MPN cases (3/67) and in about 0.6\% of sporadic cases (3/490) where family history is unknown. The low penetrance present in MPN pedigrees suggests that the disease is triggered by some stochastic factors, perhaps the acquisition of somatic mutations. In addition, common germline predisposition factors, such as \textit{JAK2} GGCC haplotype and \textit{TERT} \textit{rs2736100} SNP seem to have an additive effect on the MPN risk in \textit{RBBP6} mutation carriers.

\textit{RBBP6} is a RING finger E3 ubiquitin ligase located in the nucleus. It has been reported to ubiquitinate and degrade p53, in association with MDM2.\textsuperscript{22} As the \textit{RBBP6} mutations identified in our study were all located in the vicinity of its p53-binding domain (Figure 1F), they may affect p53 functions. It is likely that mutant \textit{RBBP6} causes an elevation in
somatic mutagenesis rates through inhibition of p53 function and deregulation of cell cycle. The existence of somatic mutations in three hallmark MPN genes (JAK2, MPL and CALR) in a single family might be due to elevated mutagenesis in RBBP6 mutation carriers. Alternatively, RBBP6 mutations might enhance JAK-STAT signaling by a yet unknown mechanism, providing “fertile ground” for MPN development.

We cannot completely exclude the possibility that another unidentified mutation might be segregating with MPN phenotype in the family we studied due to insufficient coverage of some genomic regions by exome sequencing or the mutation being located in a noncoding region. However, our data suggests that RBBP6 is a candidate gene for MPN susceptibility in a subset of pedigrees with familial MPN. The question how RBBP6 mutations predispose predominantly MPN phenotype remains elusive. There are examples of germline mutations in cancer associated genes causing a specific familial phenotype, e.g. retinoblastoma23 (RB1), neurofibromatosis24 (NF1), melanoma25 (CDKN2A) and others. Similarly, germline RBBP6 mutations may predominantly predispose towards myeloproliferative phenotypes.

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**Author Contributions**


**Competing Financial Interests**

The authors declare no competing financial interests.

**REFERENCES**


**FIGURE LEGENDS**

**Figure 1. Identification of the germline mutation causing myeloproliferative neoplasms in the Australian family and screening in other MPN cases.**

A. Family tree of the Australian family. The patients with mutations in JAK2, MPL and CALR are marked. Below the mutations, the age at diagnosis is indicated. 

B. Genomic regions shared by the three affected members in the family identified by the SEGEX analysis (red horizontal bars). Arrows indicate the physical position of the candidate genes RBBP6, ARMC5, and C20orf3.

C. Validation of the mutations in RBBP6, ARMC5, and C20orf3 segregating with the disease in the pedigree. The locations of mutations are marked with an arrow.

D. The RBBP6 mutations found in familial cases of myeloproliferative neoplasms. The respective family trees are shown. For both families DNA was available only from one member and the segregation of the mutation with MPN was not possible to establish.

E. The two unique RBBP6 mutations found in three sporadic cases of myeloproliferative neoplasms.

F. The schematic structure of the RBBP6 protein with known and predicted domains. The locations of the detected mutations that are not observed in healthy controls are shown with stars. Mb, mega
base pair; AAS, absence of allele-sharing; BR, binding region; Znf, zinc finger domain; DWNN, domain with no name.
Table 1. Summary of unique *RBBP6* variants in familial and sporadic MPN cases.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Sample</th>
<th>diagnosis</th>
<th>JAK2/MPL/CALR</th>
<th>cDNA change</th>
<th>amino acid change</th>
<th>Polyphen 2 Score</th>
<th>Polyphen 2 prediction</th>
<th>in healthy controls</th>
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<td>R1569H</td>
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<td></td>
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<td>S1444F</td>
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Abbreviations: MPN, myeloproliferative neoplasm; Ex12del – exon 12 deletion E543-D544; del, deletion; ET, essential thrombocythemia; PV, polycythemia vera; PMF, primary myelofibrosis.
Figure 1
Germline \textit{RBBP6} mutations in familial myeloproliferative neoplasms


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