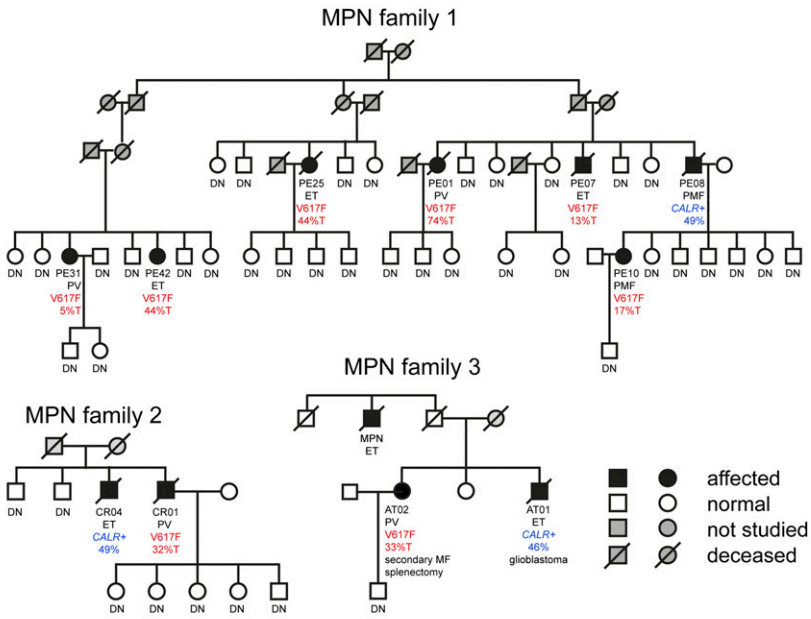
To the editor:

Somatic mutations in calreticulin can be found in pedigrees with familial predisposition to myeloproliferative neoplasms

JAK2-V617F is the most frequently observed somatic mutation in patients with myeloproliferative neoplasms (MPNs).[1,2] Recently, mutations in *CALR* were described in patients with essential thrombocythemia (ET) and primary myelofibrosis (PMF) that were negative for *JAK2*-V617F.[3,4] All *CALR* mutations associated with MPNs described to date are either insertions or

A Pedigrees of MPN families with mutations in *CALR*



B Summary of *CALR* and *JAK2*-V617F mutations in MPN and HT families

	family members analyzed	affected			unaffected		
		V617F	*CALR*	double negatives	V617F	*CALR*	double negatives
MPN family 1	44	6	1	0	0	0	37
MPN family 2	10	1	1	0	0	0	8
MPN family 3	3	1	1	0	0	0	1
MPN family 4	26	5	0	0	0	0	21
MPN family 5	29	4	0	0	1	0	24
MPN family 6	7	3	0	0	0	0	4
MPN family 7	3	3	0	0	0	0	0
MPN family 8	2	2	0	0	0	0	0
MPN family 9	11	2	0	0	0	0	9
MPN family 10	3	2	0	0	0	0	1
MPN family 11	2	2	0	0	0	0	0
MPN family 12	8	2	0	0	0	0	6
Total	**148**	**33**	**3**	0	**1**	**0**	111
HT family 1	9	0	0	3	0	0	6
HT family 2	13	0	0	3	0	0	10
HT family 3	2	0	0	2	0	0	0
HT family 4	4	0	0	3	0	0	1
HT family 5	5	0	0	2	0	0	3
HT family 6	2	0	0	2	0	0	0
HT family 7	3	0	0	2	0	0	1
HT family 8	9	0	0	3	0	0	6
HT family 9	10	0	0	5	0	0	5
HT family 10	12	0	0	4	0	0	8
HT family 11	23	0	0	5	0	0	18
Total	**92**	**0**	**0**	34	**0**	**0**	58

Figure 1. Somatic *CALR* mutations in familial myeloproliferative diseases. (A) The 3 pedigrees with somatic *CALR* mutations are shown in detail. Screening for *CALR* mutations was performed by using the sizing polymerase chain reaction (PCR) assay described by Klampfl et al.[3] The *JAK2*-V617F mutation was screened by allele-specific PCR.[6] Percentages in blue represent the *CALR* mutant allele burden. %T (red), percentage of G>T *JAK2*-V617F mutant allele burden; *CALR*+, presence of a 52-base deletion in exon 9 of the *CALR* gene; DN, double-negative (ie, absence of detectable mutations in *CALR* exon 9 and *JAK2*-V617F); V617F, presence of the *JAK2*-V617F mutation. (B) Summary of families analyzed. Affected and unaffected family members from families with familial predisposition to MPN (upper part) or HT (lower part) were screened for the presence or absence of mutations in CALR.

deletions and result in a frameshift into the alternative reading frame 1. Familial predisposition to MPNs also frequently leads to somatic *JAK2*-V617F mutations that are found in the majority of the affected family members.[5] Because polycythemia vera (PV), ET, and PMF phenotypes can be observed in these pedigrees, we tested whether mutations in *CALR* also occur in familial MPNs.

We studied 12 pedigrees with familial MPNs in which at least 1 affected family member carried a somatic *JAK2*-V617F mutation, and we also examined 11 pedigrees with hereditary thrombocytosis (HT), in which we excluded mutations in the *THPO*, *MPL*, and *JAK2* genes (data not shown). We found a *CALR* mutation in 3 of the 12 familial MPN pedigrees (Figure 1A). In each of these 3 pedigrees, 1 affected family member carried a 52-base deletion in exon 9 of the *CALR* gene (not shown), which represents the most frequent form of the *CALR* mutations (type 1). One of these patients was initially diagnosed with ET[7] and later progressed to PMF (MPN family 1), and the two remaining patients had ET (MPN families 2 and 3). All *CALR* mutations occurred in patients with an MPN diagnosis, whereas a somatic *JAK2*-V617F mutation was found in 1 healthy family member with platelets in the upper normal range but otherwise normal blood counts (MPN family 5; data not shown). A similar finding was reported previously.[8] We did not detect *CALR* mutations in any of the 11 families with HT and a total of 44 affected and family members (Figure 1B).

In the 12 pedigrees with familial MPNs, all affected family members carried either a somatic *JAK2*-V617F mutation or a mutation in *CALR*. In MPN family 1, the underlying predisposition is inherited as an autosomal dominant trait with low penetrance (Figure 1A), whereas in other pedigrees, the mode of transmission is more difficult to determine because too few family members in too few generations were available (eg, MPN families 2 and 3). Two mechanistic models have been proposed regarding how such a germline predisposition may result in MPNs with clonal hematopoiesis.[9,10] First, the germline mutation could increase the mutation rate in *JAK2* and *CALR* genes (hypermutability hypothesis), or second, it could synergize with *JAK2*-V617F or *CALR* in MPN disease initiation (fertile ground hypothesis). Because the G>T transversion in *JAK2*-V617F and the 52-base deletion in *CALR* are mechanistically very different, it seems unlikely that they could be promoted by the same germline mutation through a hypermutability mechanism. Therefore, our findings favor the fertile ground hypothesis of germline predisposition to MPN.

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To the editor:

Physical activity limits pulmonary endothelial activation in sickle cell SAD mice

Vaso-occlusion (VOC) in sickle cell disease (SCD) results from many pathophysiological mechanisms including sickling of red blood cells, hemolysis, inflammation, vascular adhesion, and reduced nitric oxide (NO) bioavailability.[1] All these mechanisms interact together to trap blood cells and to accentuate the local blood flow decrease and subsequent local hypoxia.

In healthy and pathological conditions, physical training results in physiological and molecular adaptations including anti-inflammatory