To the editor:

LNK mutations in familial myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPNs), including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis, have in most instances a sporadic occurrence, but familial clustering of MPNs has been reported and familial cases are about 7% to 8% of all MPN patients.[1,2] Driver mutations in *JAK2*, *CALR*, or *MPL* are somatically acquired in familial cases as they are in sporadic patients.[1,3,4] Common single-nucleotide polymorphisms in the *JAK2* and *TERT* genes confer susceptibility to MPNs and contribute to the familial clustering of MPNs.[5-7] Recently, germ line *RBBP6* mutations have been identified in about 5% of familial MPN cases[8] and germ line duplication of *ATG2P* and *GSKIP* genes has been reported in 4 families from the French West Indies.[9]

The SH2B adaptor protein 3 (SH2B3) gene, also known as the *LNK* gene, encodes a negative regulator of cytokine signaling. In mouse

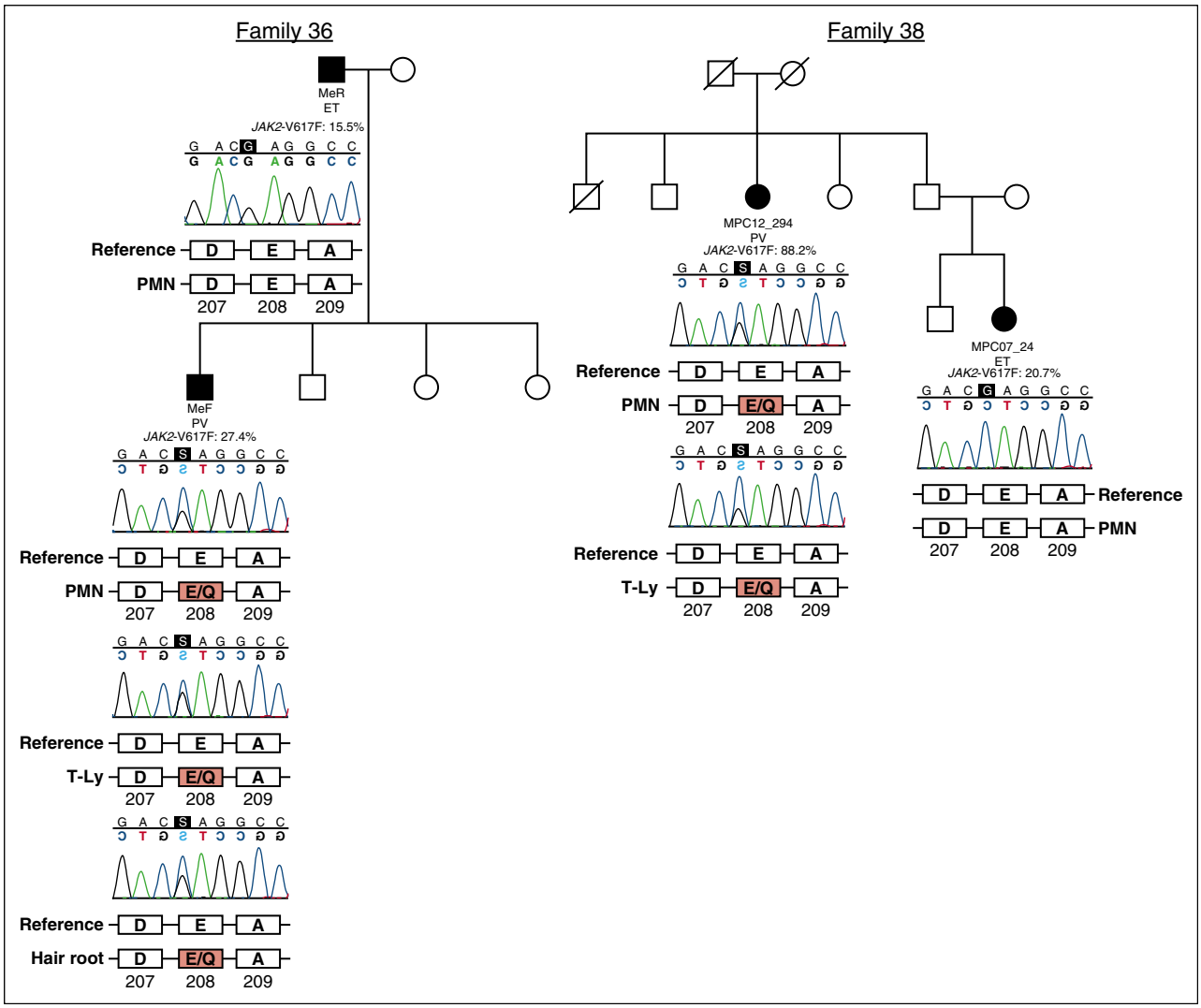

Figure 1. Pedigrees and DNA sequences of the 2 families with germ line *LNK* (E208Q) mutation. Filled symbols represent affected patients. The diagnosis (PV or ET) and the *JAK2* (V617F) allele burden are reported below each symbol. DNA sequences of granulocytes (polymorphonuclear cells [PMN]), T lymphocytes (T-Ly), and hair roots, reference amino acid sequence, and amino acid substitution (E instead of Q) are reported. MeF had 70% of mutant alleles in both PMN and T cells; MPC12_294 had 70% of mutant alleles in PMN and 75% in T cells.

models, Lnk negatively regulates erythropoietin receptor signaling and thrombopoietin receptor signaling by attenuating Jak2 activation, and thus negatively modulating erythropoiesis and megakaryopoiesis, respectively.[10,11]

LNK mutations have been described in some patients with sporadic MPNs[12,13] and in a small number of cases with idiopathic erythrocytosis and subnormal Epo levels.[14] *LNK* mutations mainly affect exon 2 and may occur concurrently with the *JAK2* (V617F) mutation.[12,14]

In an attempt to identify the germ line genetic factors that underlie familial clustering of MPNs, we applied next-generation sequencing to our MPN families. All samples were collected after subjects gave their written informed consent and the study was approved by the local ethics committee.

Our cohort of 94 MPN families was analyzed with 2 strategies. First, we applied whole-exome sequencing (HiSeq2000 system; Illumina) in a subgroup of 16 families with MPNs. This approach resulted in the identification of the *LNK* (E208Q) mutation in a patient with familial PV belonging to family 36; the variant was then validated by Sanger sequencing.

We next screened for exon 2 *LNK* mutations by Sanger sequencing in the remaining 93 families. All affected and healthy members for whom DNA was available were studied (149 patients and 89 healthy relatives). Of 149 patients affected with familial MPNs, 2 patients (1.4%) carried the *LNK* (E208Q) mutation (including the initial case, identified through exome sequencing). None of the 89 healthy relatives carried mutations in exon 2 of the *LNK* gene.

The pedigrees of the 2 mutated cases (MeF and MPC12_294, belonging to family 36 and family 38, respectively) are reported in Figure 1. Both patients were affected with *JAK2* (V617F)-mutant PV, diagnosed at 42 years (MeF) and 66 years (MPC12_294). The 2 patients carried *LNK* (E208Q) both in granulocyte and T-lymphocyte DNA. To confirm the germ line nature of the mutation, we analyzed DNA extracted from hair roots of patient MeF, detecting *LNK* (E208Q) also in this nonhematopoietic tissue. In both families, the other family member affected with MPNs (MeR and MPC07_24) did not carry any mutation in the *LNK* gene, thus excluding segregation of the *LNK* (E208Q) mutation with the disease phenotype.

In conclusion, germ line *LNK* mutations rarely occur in familial MPNs and do not segregate with the disease phenotype. Our findings suggest that mutations in *LNK*, either germ line or acquired, may cooperate with acquired driver mutations in *JAK2*, *CALR*, or *MPL* to determine disease phenotype in MPNs. In the study by Oh et al, the patient with the missense mutation (E208Q) in the pleckstrin homology domain of *LNK* had an ET phenotype.[13] This patient was negative for *JAK2* (V617F) and *MPL* (W515) mutations; however, *CALR* mutations had not yet been described at the time of this report, and we cannot exclude that a *CALR* mutation was responsible for this ET. Two additional patients reported for their *LNK* mutations had idiopathic erythrocytosis and not a myeloproliferative disorder.[14] Overall, it appears unlikely that *LNK* alterations may act as driver mutations in MPNs.

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