the addition of cladribine seems a reasonable option to be considered for all *FLT3*-ITD$^+$ patients.

In recent years, targeted therapy has been proposed as the most promising treatment option for *FLT3*-ITD$^+$ patients. Recent data, however, does not show convincing results regarding their clinical application.[2] In summary, our data demonstrate that cladribine could abolish the negative effect of *FLT3*-ITD on survival of NK-AML patients and thus might present an interesting and safe solution for *FLT3*-ITD$^+$ AML patients.

The retrospective nature of our study, as well as the relatively small number of *FLT3*-ITD$^+$ AML patients included in the analysis, is the most serious limitation of the obtained data. Our results, however, provide a rationale to carry out prospective studies on the role of cladribine in the treatment of different genetic subgroups of NK-AML patients.

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References

1. Schlenk RF, Döhner K, Krauter J, et al; German-Austrian Acute Myeloid Leukemia Study Group. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med.* 2008;358(18):1909-1918.
2. Baker SD, Zimmerman EI, Wang YD, et al. Emergence of polyclonal FLT3 tyrosine kinase domain mutations during sequential therapy with sorafenib and sunitinib in FLT3-ITD-positive acute myeloid leukemia. *Clin Cancer Res.* 2013;19(20):5758-5768.
3. Robak T. Purine nucleoside analogues in the treatment of myeloid leukemias. *Leuk Lymphoma.* 2003;44(3):391-409.
4. Holowiecki J, Grosicki S, Robak T, et al; Polish Adult Leukemia Group (PALG). Addition of cladribine to daunorubicin and cytarabine increases complete remission rate after a single course of induction treatment in acute myeloid leukemia. Multicenter, phase III study. *Leukemia.* 2004;18(5):989-997.
5. Holowiecki J, Grosicki S, Giebel S, et al. Cladribine, but not fludarabine, added to daunorubicin and cytarabine during induction prolongs survival of patients with acute myeloid leukemia: a multicenter, randomized phase III study. *J Clin Oncol.* 2012;30(20):2441-2448.
6. Libura M, Asnafi V, Tu A, et al. FLT3 and MLL intragenic abnormalities in AML reflect a common category of genotoxic stress. *Blood.* 2003;102(6):2198-2204.
7. Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). *Blood.* 2006;107(10):4011-4020.
8. Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood.* 2001;98(6):1752-1759.
9. Boissel N, Cayuela JM, Preudhomme C, et al. Prognostic significance of FLT3 internal tandem repeat in patients with de novo acute myeloid leukemia treated with reinforced courses of chemotherapy. *Leukemia.* 2002;16(9):1699-1704.
10. Fröhling S, Schlenk RF, Breitruck J, et al; AML Study Group Ulm. Acute myeloid leukemia. Prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML Study Group Ulm. *Blood.* 2002;100(13):4372-4380.
11. Sehgal AR, Gimotty PA, Zhao J, et al. DNMT3A mutational status affects the results of dose-escalated induction therapy in acute myelogenous leukemia. *Clin Cancer Res.* 2015;21(7):1614-1620.
12. Luskin MR, Lee J-W, Fernandez HF, et al. High dose daunorubicin improves survival in AML up to age 60, across all cytogenetic risk groups including patients with unfavorable cytogenetic risk, and FLT3-ITD mutant AML: updated analyses from Eastern Cooperative Oncology Trial E1900 [abstract]. *Blood.* 2014;124(21). Abstract 373.
13. Larson SM, Campbell NP, Huo D, et al. High dose cytarabine and mitoxantrone: an effective induction regimen for high-risk acute myeloid leukemia (AML). *Leuk Lymphoma.* 2012;53(3):445-450.
14. Bhatla D, Gerbing RB, Alonzo TA, et al. Cytidine deaminase genotype and toxicity of cytosine arabinoside therapy in children with acute myeloid leukemia. *Br J Haematol.* 2009;144(3):388-394.
15. Mahlknecht U, Dransfeld CL, Bulut N, et al. SNP analyses in cytarabine metabolizing enzymes in AML patients and their impact on treatment response and patient survival: identification of CDA SNP C-451T as an independent prognostic parameter for survival. *Leukemia.* 2009;23(10):1929-1932.

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To the editor:

Germline *RBBP6* mutations in familial myeloproliferative neoplasms

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Myeloproliferative neoplasms (MPNs) comprise a heterogeneous group of hematologic disorders characterized by clonal overproduction of differentiated myeloid cells, propensity to thrombosis, hemorrhage, and increased risk of leukemia. Three MPN subtypes, polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are considered as the "classical BCR-ABL1–negative MPNs," 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[1-3] and increased risk of MPN in relatives.[4] Somatic mutations have been associated with MPN, most notably disease causing, mutually exclusive mutations in Janus kinase 2 (*JAK2*), calreticulin *(CALR)*, and myeloproliferative leukemia *(MPL)* genes. Somatic *JAK2* mutations are present in ~60% of MPN cases,[5,6] *MPL* mutations occur somatically in ~1% to 5% of cases,[7] and somatic *CALR* mutations are found in ~20% to 30% of ET and PMF.[8,9] Familial MPN is clinically indistinguishable from sporadic MPN, and displays frequent somatic *JAK2* and *CALR* mutations.[2,3,10-12] Other genes that frequently mutate somatically in MPN, such as *TET2*,[13] *DNMT3A*,[14] and *ASXL1*,[15] do not significantly contribute to familial MPN and in the majority of familial cases the causative germline mutation is unknown.[16,17]

The role of common germline variants in MPN predisposition has been established. Specifically, the *JAK2* "GGCC" haplotype increases the risk to develop *JAK2*-mutant MPN by several fold.[18] Another germline variant in telomerase reverse transcriptase (*TERT*) (rs2736100) increases the risk of all molecular subtypes of MPN by approximately twofold.[19,20] Both of these single nucleotide polymorphisms (SNPs) contribute to familial clustering of the disease, although they can explain only a minor proportion of it.[19] Recently several additional common variants conferring susceptibility to MPN have been identified.[21]

In order to identify the germline mutation predisposing to MPN, we studied an Australian pedigree of English ancestry (Northern England) with 5 members in 4 generations diagnosed with MPN. DNA was available from 3 affected members (Figure 1A). Each of the 3 members of the family had a different MPN-specific somatic mutation (*JAK2*-V617F, *MPL*-W515L, and *CALR*-type 1; Figure 1A). To map the candidate disease loci in the pedigree, we applied a nonparametric algorithm, segregation exclusion analysis

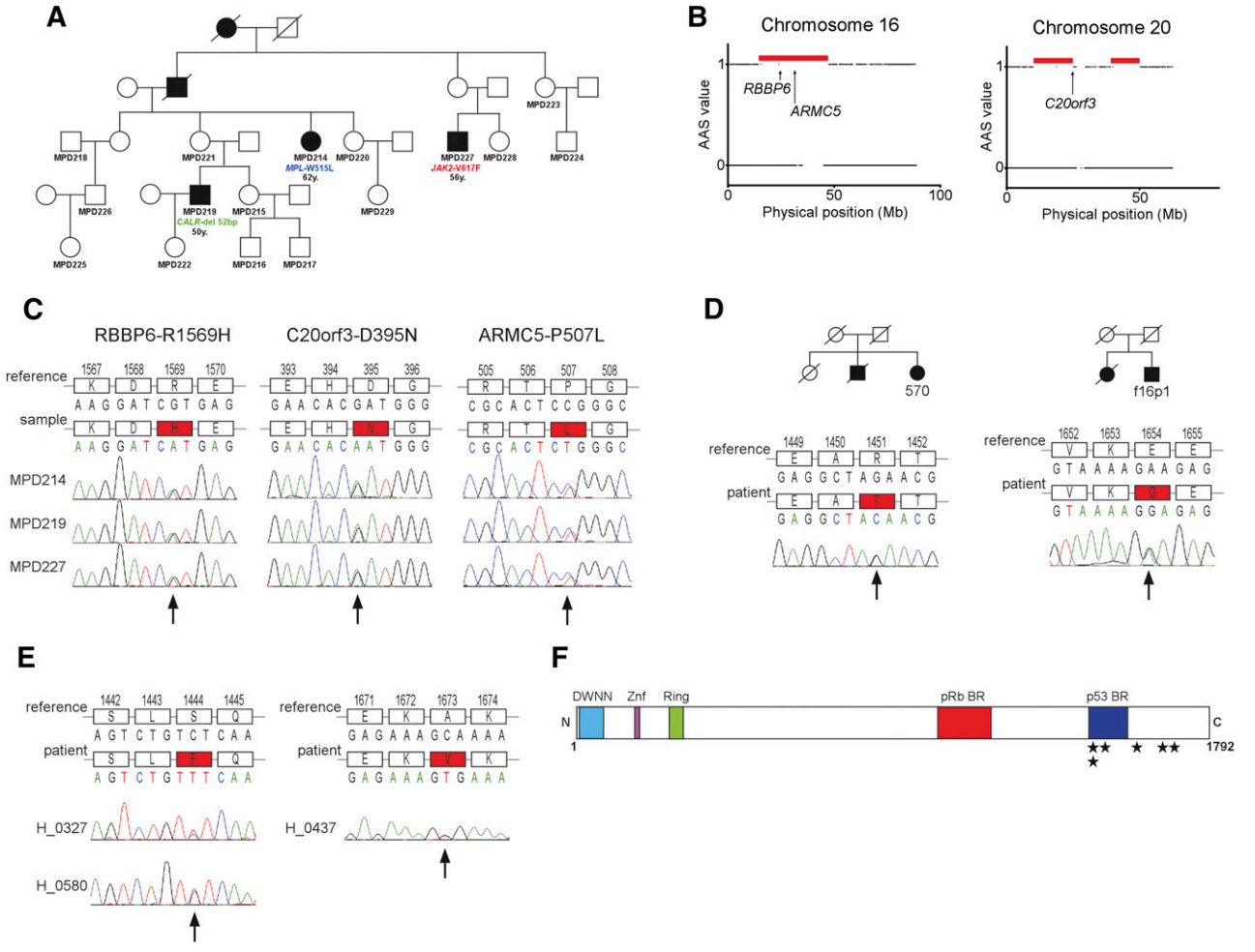

Figure 1. Identification of the germline mutation causing MPNs in the Australian family and screening in other MPN cases. (A) Family tree of the Australian family. The patients with mutations in *JAK2*, *MPL*, and *CALR* are marked. Below the mutations, the age at diagnosis is indicated. (B) Genomic regions shared by the 3 affected members in the family identified by the segregation exclusion 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 MPNs. The respective family trees are shown. For both families, DNA was available only from 1 member and the segregation of the mutation with MPN was not possible to establish. (E) The 2 unique *RBBP6* mutations found in 3 sporadic cases of MPNs. (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. AAS, absence of allele-sharing; BR, binding region; DWNN, domain with no name; Mb, megabase pair; Znf, zinc finger domain.

Table 1. Summary of unique *RBBP6* variants in familial and sporadic MPN cases

Pedigree	Sample	Diagnosis	*JAK2*/*MPL*/*CALR*	cDNA change	Amino acid change	Polyphen 2 score	Polyphen 2 prediction	In healthy controls
1	MPD214	ET	*MPL*-W515L	c.4706G>T	R1569H	0.766	Possibly damaging	0/715
	MPD219	PMF	*CALR*-Type 1	c.4706G>T	R1569H	—	—	—
	MPD227	PMF	*JAK2*-V617F	c.4706G>T	R1569H	—	—	—
2	f16p1	PMF	*JAK2*-V617F	c.4961A>G	E1654G	0.375	Benign	0/649
3	570	PMF	*JAK2*-V617F	c.4352G>C	R1451T	0.942	Probably damaging	0/642
Sporadic	H_0327	PV	*JAK2*-Ex12del	c.4331C>T	S1444F	0.976	Probably damaging	0/650
Sporadic	H_0580	ET	*JAK2*-V617F	c.4331C>T	S1444F	0.976	Probably damaging	0/650
Sporadic	H_0437	PV	—	c.5018C>T	A1673V	0.010	Benign	0/607

cDNA, complementary DNA; del, deletion; Ex12del, exon 12 deletion E543-D544.

(see supplemental Figure 1, available on the *Blood* Web site), and identified 12 shared genomic regions with a total size of 217.87 Mb among the 3 affected subjects (Figure 1B; supplemental 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 (supplemental Figure 2). We performed Sanger sequencing of all the 18 final candidate variants and confirmed DNA variants segregating with the disease in three genes (*RBBP6*, *C20orf3*, and *ARMC5*) (Figure 1C; supplemental Table 2).

To identify which of the *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, *ARMC5*-P507L was excluded due to 7% frequency in healthy controls, whereas *RBBP6*-R1569H and *C20orf3*-D395N were not found in any of the over 700 healthy controls.

Next, we sequenced the exons carrying the identified *RBBP6* and *C20orf3* mutations in an additional 66 MPN families. This analysis yielded two unique mutations in *RBBP6* (E1654G and R1451T; Figure 1D) and 1 polymorphism in *C20orf3* (P406L) present in 4% of the healthy subjects. Unfortunately, for both familial cases with the *RBBP6* mutation, the DNA sample was available from only 1 affected member; therefore we could not obtain data on the mutation segregation with MPN for these 2 additional families. We used Polyphen 2 and SIFT mutation prediction tools to assess the possible effects of the mutations on protein function. RBBP6-R1569H is predicted to be damaging by both tools, whereas C20orf3-D395N mutation is benign (supplemental Table 3). In conclusion, *C20orf3* is unlikely to be involved in familial MPN, whereas *RBBP6* mutations remain strong candidates for familial predisposition to MPN.

Due to the low penetrance associated with *RBBP6* mutations, establishing the family history of MPN may often be difficult. Therefore, we screened for *RBBP6* mutations in 490 sporadic MPN cases. In this analysis, we identified 2 unique germline mutations (S1444F and A1673V) in 3 apparently unrelated subjects and 1 polymorphism (I1661V) present in 0.5% of healthy controls (Table 1; Figure 1E). Overall, we identified 5 different germline *RBBP6* mutations associated with MPN and not detected in the general population (Table 1; Figure 1F).

Apart from the 3 affected members, 8 additional healthy family members carried *RBBP6*-R1569H in the Australian pedigree, consistent with the expected low penetrance.[16] Because common germline SNPs have been shown to contribute to familial MPN,[19] we checked all members of the family for *JAK2* GGCC haplotype and the *rs2736100_C* risk variant in *TERT* (supplemental Table 4). As expected, from the affected members only the one with *JAK2*-V617F mutation was heterozygous for *JAK2* GGCC haplotype, whereas 2 affected members were heterozygous for *TERT* risk variant and the third one was homozygous for the *TERT* risk allele. Although it is not possible to draw any solid conclusions based on a single family, we noticed a trend in *JAK2* GGCC haplotype and *TERT* SNP distribution similar to the pattern we observed in our previous study.[19] The risk allele frequencies for both *JAK2* GGCC haplotype and *TERT rs2736100* were higher in *RBBP6*-R1569H mutant MPN patients compared with *RBBP6*-R1569H mutant unaffected members (16.67% vs 6.25% for *JAK2* GGCC haplotype, 66.67% vs 50.00% for *TERT* SNP).

We have identified germline *RBBP6* mutations in ~5% of familial MPN cases (3/67) and in ~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 *JAK2* GGCC haplotype and *TERT rs2736100* SNP, seem to have an additive effect on the MPN risk in *RBBP6* mutation carriers.

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.[22] Because the 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 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 to MPN phenotype remains elusive. There are examples of germline mutations in cancer-associated genes causing a specific familial phenotype, eg, retinoblastoma[23] (*RB1*), neurofibromatosis[24] (*NF1*), melanoma[25] (*CDKN2A*), and others. Similarly, germline *RBBP6* mutations may predominantly predispose toward myeloproliferative phenotypes.

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Contribution: A.S.H. and R.K. conceived and designed the experiments; A.S.H., R.G., C.K., R.J., T.K., T.B., J.D.M.F., B.G., A.C.M., K.P., and K.L.B. performed the experiments; A.S.H., R.G., C.K., A.S., R.J., T.K., D.C., J.D.M.F., F.P.B., J.C., K.L.B., and R.K. analyzed the data; A.S.H., A.S., F.P.B., and J.C. performed the statistical analysis; H.G., E.R., F.P., D.P., R.H., J.C., K.L.B., G.S.-F., M.C., E.H., and R.K. contributed reagents/materials/analysis tools; A.S.H. and R.K. wrote the paper; and all authors contributed to the final version of the manuscript.

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References

1. Kralovics R, Stockton DW, Prchal JT. Clonal hematopoiesis in familial polycythemia vera suggests the involvement of multiple mutational events in the early pathogenesis of the disease. *Blood.* 2003;102(10):3793-3796.
2. Rumi E, Passamonti F, Pietra D, et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. *Cancer.* 2006;107(9):2206-2211.
3. Bellanné-Chantelot C, Chaumarel I, Labopin M, et al. Genetic and clinical implications of the Val617Phe JAK2 mutation in 72 families with myeloproliferative disorders. *Blood.* 2006;108(1):346-352.
4. Landgren O, Goldin LR, Kristinsson SY, Helgadottir EA, Samuelsson J, Björkholm M. Increased risks of polycythemia vera, essential thrombocythemia, and myelofibrosis among 24,577 first-degree relatives of 11,039 patients with myeloproliferative neoplasms in Sweden. *Blood.* 2008;112(6):2199-2204.
5. James C, Ugo V, Le Couédic JP, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature.* 2005;434(7037):1144-1148.
6. Kralovics R, Passamonti F, Buser AS, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med.* 2005;352(17):1779-1790.
7. Pikman Y, Lee BH, Mercher T, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med.* 2006;3(7):e270.
8. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013;369(25):2379-2390.
9. Nangalia J, Massie CE, Baxter EJ, et al. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med.* 2013;369(25):2391-2405.
10. Lundberg P, Nienhold R, Ambrosetti A, Cervantes F, Pérez-Encinas MM, Skoda RC. Somatic mutations in calreticulin can be found in pedigrees with familial predisposition to myeloproliferative neoplasms. *Blood.* 2014;123(17):2744-2745.
11. Maffioli M, Genoni A, Caramazza D, et al. Looking for CALR mutations in familial myeloproliferative neoplasms. *Leukemia.* 2014;28(6):1357-1360.
12. Rumi E, Harutyunyan AS, Pietra D, et al; Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. CALR exon 9 mutations are somatically acquired events in familial cases of essential thrombocythemia or primary myelofibrosis. *Blood.* 2014;123(15):2416-2419.
13. Delhommeau F, Dupont S, Della Valle V, et al. Mutation in TET2 in myeloid cancers. *N Engl J Med.* 2009;360(22):2289-2301.
14. Ley TJ, Ding L, Walter MJ, et al. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med.* 2010;363(25):2424-2433.
15. Carbuccia N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia.* 2009;23(11):2183-2186.
16. Olcaydu D, Rumi E, Harutyunyan A, et al. The role of the JAK2 GGCC haplotype and the TET2 gene in familial myeloproliferative neoplasms. *Haematologica.* 2011;96(3):367-374.
17. Saint-Martin C, Leroy G, Delhommeau F, et al; French Group of Familial Myeloproliferative Disorders. Analysis of the ten-eleven translocation 2 (TET2) gene in familial myeloproliferative neoplasms. *Blood.* 2009;114(8):1628-1632.
18. Olcaydu D, Harutyunyan A, Jäger R, et al. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet.* 2009;41(4):450-454.
19. Jäger R, Harutyunyan AS, Rumi E, et al. Common germline variation at the TERT locus contributes to familial clustering of myeloproliferative neoplasms. *Am J Hematol.* 2014;89(12):1107-1110.
20. Oddsson A, Kristinsson SY, Helgason H, et al. The germline sequence variant rs2736100_C in TERT associates with myeloproliferative neoplasms. *Leukemia.* 2014;28(6):1371-1374.
21. Tapper W, Jones AV, Kralovics R, et al. Genetic variation at MECOM, TERT, JAK2 and HBS1L-MYB predisposes to myeloproliferative neoplasms. *Nat Commun.* 2015;6:6691.
22. Li L, Deng B, Xing G, et al. PACT is a negative regulator of p53 and essential for cell growth and embryonic development. *Proc Natl Acad Sci USA.* 2007;104(19):7951-7956.
23. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. *Science.* 1987;235(4794):1394-1399.
24. Wallace MR, Marchuk DA, Andersen LB, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. *Science.* 1990;249(4965):181-186.
25. Hussussian CJ, Struewing JP, Goldstein AM, et al. Germline p16 mutations in familial melanoma. *Nat Genet.* 1994;8(1):15-21.

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To the editor:

Complement activation in antiphospholipid syndrome and its inhibition to prevent rethrombosis after arterial surgery

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Deep vein thrombosis accompanied by pulmonary embolism and thrombotic occlusion of the cerebral and coronary arteries are common and severe complications of antiphospholipid syndrome (APS).[1] Antibodies against beta-2 glycoprotein I (β_2GPI) bound to endothelial and other circulating cells, including platelets and monocytes, have been implicated in thrombus formation.[2] Evidence from animal models supports a 2-hit theory with 1 hit represented by the presence of antibodies against the cell-bound β_2GPI and the second hit triggered by mechanical, physical, and/or inflammatory stimuli promoting the deposition of β_2GPI on endothelial cells.[2-4] Infectious agents, arterial hypertension, pregnancy, oral contraceptive pills, and surgical interventions have all been documented as

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