"hypoxia sensing" since hypoxia and iron deprivation leads to a posttranslational increase in both HIF-1α and HIF-2α proteins and increased transcription of an array of their target genes.[2]

The recent discovery of an iron-responsive element (IRE) in the 5′ untranslated region (UTR) of *HIF2A* reveals a novel regulatory link between iron availability and *HIF2A* expression.[3] In iron deficiency, the iron regulatory proteins (IRPs) repress the translation of HIF-2α protein by binding to its 5′ IRE.[3] Recent data implicates HIF-2α in the control of renal, hepatic, and brain Epo production in vivo.[4-7] Altogether, these data indicate new molecular connections between *HIF2A* expression, erythropoiesis, and iron availability.

Polycythemia/erythrocytosis can be associated with raised Epo levels, which are indicative of a deregulated oxygen-sensing pathway. Indeed, defects in *VHL* and *PHD2* have been described in a minority of patients.[8-10] We hypothesized that germ-line mutations in the 5′ IRE of the *HIF2A* gene would uncouple HIF-2α synthesis from negative translational control, predicting polycythemia/erythrocytosis with increased Epo levels (Figure 1). Consequently, a group of 147 such individuals referred to Belfast or Salt Lake City was screened for defects in the 5′ IRE by PCR-direct sequencing (Figure 1). The sequence in all 294 alleles was in full concordance with the published genomic sequence of *HIF2A* (EPAS1, RefSeq; AC016696, nucleotide 81298–81330).[11] However, 4 single-nucleotide polymorphisms (SNPs) were detected (Figure 1B). According to the Entrez SNP database,[12] the T allele for the rs17039192 SNP (nucleotide 81261; Figure 1) is present in the Japanese population at a frequency of 0.14. Within the group of 120 patients referred to Belfast, 3 T alleles were detected in individuals from China and Vietnam. Of the 27 patients with polycythemia studied in Salt Lake City, this T allele was not detected. However, we found 3 previously unreported SNPs at 3′ of the IRE at nucleotides 81405, 81411, and 81424; their allele frequencies in 27 patients with polycythemia were 0.85, 0.69, and 0.37, respectively; these did not meaningfully differ from 25 control participants, whose frequencies were 0.58, 0.20, and 0.28. We conclude that the loss of iron-regulated control of HIF-2α translation by the IRP/IRE regulatory system is not the cause of polycythemia/erythrocytosis in a large collection of patients collected from the United Kingdom, Europe, and North and Central America.

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References

1. Percy MJ. Genetically heterogeneous origins of idiopathic erythrocytosis. Hematology. 2007;12:131-139.
2. Hirota K, Semenza GL. Regulation of angiogenesis by hypoxia-inducible-factor-1. Crit Rev Oncol Hematol. 2006;59:15-26.
3. Sanchez M, Galy B, Muckenthaler MU, Hentze MW. Iron-regulatory proteins limit hypoxia-inducible factor 2 alpha expression in iron deficiency. Nat Struc Mol Biol. 2007;14:420-426.
4. Rosenberger C, Mandriota S, Jurgensen JS, et al. Expression of hypoxia-inducible factor-1alpha and -2alpha in hypoxic and ischemic rat kidneys. J Am Soc Nephrol. 2002;13:1721-1732.
5. Rankin EB, Biju MP, Liu Q, et al. Hypoxia-inducible factor-2 (HIF-2) regulates hepatic erythropoietin in vivo. J Clin Invest. 2007;117:1068-77.
6. Gruber M, Hu CJ, Johnson RS, Brown EJ, Keith B, Simon MC. Acute postnatal ablation of Hif-2alpha results in anemia. Proc Natl Acad Sci U S A. 2007;104:2301-6.
7. Chavez JC, Baranova O, Lin J, Pichiule P. The transcriptional activator hypoxia inducible factor 2 (HIF-2/EPAS-1) regulates the oxygen-dependent expression of erythropoietin in cortical astrocytes. J Neurosci. 2006;26:9471-9481.
8. Ang SO, Chen H, Hirota K, et al. Disruption of oxygen homeostasis underlies congenital Chuvash polycythemia. Nat Genet. 2000;232:614-21.
9. Gordeuk V, Stockton DW, Prchal JT. Congenital polycythemias/erythrocytoses. Haematologica. 2005;90:109-16.
10. Percy MJ, Zhao Q, Flores A, et al. A family with erythrocytosis establishes a role for prolyl hydroxylase domain protein 2 in oxygen homeostasis. Proc Natl Acad Sci U S A. 2006;103:654-659.
11. RefSeq: AC016696. http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=16756330. Accessed July 25, 2007.
12. Entrez SNP database. http://www.ncbi.nlm.nih.gov/sites/entrez. Accessed July 25, 2007.

To the editor:

Antithrombin Cambridge II (A384S): prevalence in patients of the Paris Thrombosis Study (PATHROS)

The antithrombin (AT) Cambridge II (A384S), which results from a nucleotide (nt) G > T substitution at position 13268 of the gene (numbering system from Olds et al[1]), is a variant associated with borderline or mildly reduced antithrombin heparin cofactor activity, but normal immunologic level. This AT variant was initially reported in 4 unrelated heterozygotes, 3 of them being asymptomatic.[2] Later, it was found in a cohort of 9669 blood donors in West Scotland in 10 nonrelated individuals (prevalence, 1.14 per 1000); despite former exposure of most of these donors to at-risk situations, only 1 of them had a history of venous thrombosis (VT),[3] suggesting that the mutation may be a mild risk factor for VT; haplotype analysis performed in 18 families (including 12 families of blood donors) suggested that the mutation had at most 4 independent origins in the United Kingdom.

Recently, Corral et al studied the prevalence of the AT A384Smutation in Spain.[4] In this large case-control study of venous thromboembolism (1018 patients/1018 healthy volunteers), the AT Cambridge II was found in 0.2% of volunteers and 1.7% of patients (odds ratio [OR] 9.75; 95% confidence interval [95% CI], 2.2-42.5). The results suggested that the Cambridge II variant may be a prevalent genetic risk factor for VT and the most frequent cause of AT deficiency in European populations.

We have investigated the prevalence of this mutation in patients from the case-control Paris Thrombosis Study (PATHROS). Recruitment criteria and the characteristics of this study were previously reported.[5] The study was approved by local ethics committee, and all participants gave their informed consent. Briefly, consecutive patients with at least 1 established episode of deep VT or pulmonary embolism were recruited from a university hospital in Paris. A total of 88% of patients were born in Europe. Thrombophilia screening included AT activity measurement (Stachrom ATIII; Diagnostica Stago, Asnières, France).

Genotyping for the AT A384S mutation was performed on genomic DNA by amplification-digestion; the exon 6 of the AT

gene was amplified by polymerase chain reaction (PCR) using the primers 5′CTGCAGGTAAATGAAGAAGG3′ and 5′GGAAGAGGTGCAAAGAATAAG3′; digestion by *Pvu*II (New England Biolabs, Hitchin, United Kingdom) led to changes in size in the amplified wild-type DNA only. Confirmation of the presence of the 384s allele was performed by sequencing.

A total of 2 (0.4%) of the 473 patients (95% CI, 0%-1%) were heterozygous for the AT Cambridge II variant, which was therefore significantly less frequent in PATHROS than in the Spanish patients (1.7%; 95% CI, 0.9%-2.4%: $P = .02$). Both were women who developed only 1 VT, at ages 32 and 75, respectively. Both had normal AT activity.

These results suggest a possible heterogeneity in the geographic distribution of the Cambridge II variant, possibly due to a founder effect. Moreover, AT A384S was present in only 10 of 192 probands with AT deficiency who had AT gene sequencing in our laboratory since the year 2000. Altogether, these data do not argue for the AT Cambridge II being a prevalent genetic risk factor for thrombosis or a very frequent cause of AT deficiency in France.

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The participating members of the Paris Thrombosis Study are as follows: M. Alhenc-Gelas, J. Emmerich, E. Arnaud, J. N. Fiessinger, M. Aiach, AP-HP Hôpital Broussais and INSERM U428, Paris, France; V. Nicaud, INSERM U258, Paris, France.

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References

1. Olds RJ, Lane DA, Chowdhury V, De Stefano V, Leone G, Thein SL. Complete nucleotide sequence of the antithrombin gene: evidence for homologous recombination causing thrombophilia. Biochemistry. 1993;32:4216-24.
2. Perry DJ, Daly M, Harper PL, et al. Antithrombin Cambridge II, 384 Ala to Ser: further evidence of the reactive center loop in the inhibitory function of the serpins. FEBS Lett. 1991;285:248-250.
3. Tait RC, Walker ID, Perry DJ, et al. Prevalence of antithrombin deficiency in the healthy population. Br J Haematol. 1994;87:106-112.
4. Corral J, Hernandez-Espinosa D, Soria JM, et al. Antithrombin Cambridge II (A384S): an underestimated genetic risk factor for venous thrombosis. Blood. 2007;109:4258-4263.
5. Alhenc-Gelas M, Arnaud E, Nicaud V, et al. Venous thromboembolic disease and the prothrombin, methylene tetra hydrofolate reductase and factor V genes. Thromb Haemost. 1999;81:506-510.

To the editor:

Wnt signaling and phosphorylation status of β-catenin: importance of the correct antibody tools

Two recent papers in *Blood* provide the first indications that deregulated Wnt signaling plays a role in the development of acute lymphoblastic leukemias (ALLs).[1,2] Activating mutations in the critical Wnt mediator β-catenin lead to the development of thymic lymphomas, the murine counterpart of T-ALL.[1] The Wnt signaling pathway plays a key role in the development of various cell types and is subject to strict molecular regulation. The localization and consequently the function of β-catenin is regulated by Ser/Thr and possibly Tyr phosphorylation. Nuclear β-catenin serves as a coactivator for Tcf-mediated transcription of target genes that play important roles in leukemic progression.

Given the importance of the phosphorylation status of β-catenin, we have previously generated an antibody specific for β-catenin dephosphorylated at residues Ser37 and Thr41. Wnt signals specifically increase the levels of dephosphorylated β-catenin as detected with this antibody.[3,4] The antibody, named antiactive β-catenin (ABC), is produced by hybridoma clone 8E7. Another antibody claimed to recognize the dephosphorylated form is termed 8E4 (www.upstate.com).

Using 8E4 instead of 8E7, various investigators have studied activation of the Wnt pathway. Gottardi and Gumbiner[5] report that dephosphorylated β-catenin readily interacts with both cytoplasmic cadherins and nuclear Tcf. Derksen et al[6] and Diks et al[7] report very similar patterns of bands in Western blots using 8E4 and a pan–β-catenin antibody, whereas Jamieson et al[8] using 8E7 antibody show that Wnt signaling is activated in blast crises of chronic myelogenous leukemia (CML).

These conclusions critically depend on the specificity of the antibodies. We therefore mapped the antigenic epitope recognized by the 8E4 and 8E7 antibodies using a set of N-terminal deletion clones of β-catenin covering the regulatory region that is the target of the Wnt pathway kinases (Ser33 to Ser45; Figure 1A). Using these constructs, 8E4 was found to recognize all deleted forms (Figure 1B), implying that it does not recognize the regulatory region of β-catenin. These results

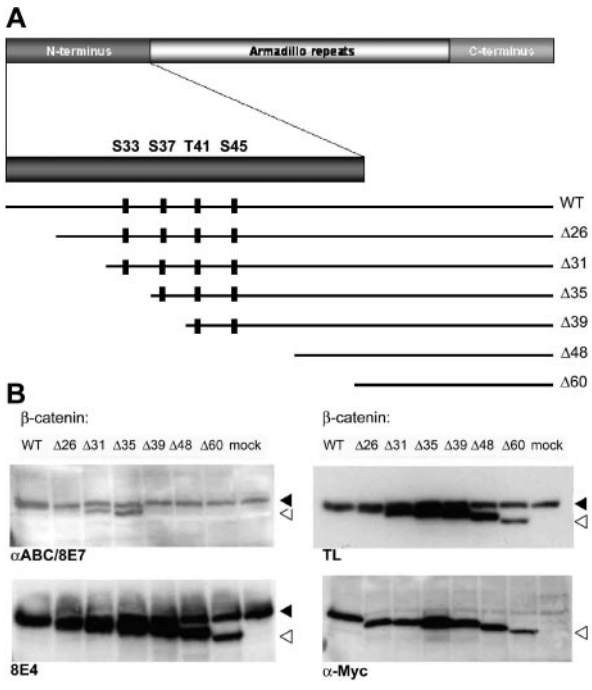

Figure 1. 8E4 recognizes all deleted forms of B-catenin. (A) Schematic overview of the β-catenin molecule, with emphasis on the N-terminus. The 4 well-characterized Ser and Thr amino acids that are regulated by phosphorylation are shown as well as the various deletion constructs used to express myc-tagged truncated forms of β-catenin. (B) The 293 cells were transfected with myc-tagged β-catenin deletion constructs to identify the epitope recognized by 8E7 and 8E4. The endogenous β-catenin band is indicated by a black arrow, the open arrow indicates the transfected, N-terminal deleted form. Similar to the pan–β-catenin antibody (TL), 8E4 recognizes all deletion constructs, whereas 8E7 (αABC) only recognizes deleted β-catenin forms up to Δ35. The following antibodies were used: αABC/8E7 (Upstate Biotech, Lake Placid, NY; catalog no. 05-665), 8E4 (Upstate Biotech; catalog no. 05-601), TL (BD Transduction Laboratories, San Jose, CA; anti–β-catenin; catalog no. 610154), α-Myc (clone 9E10; supernatant of hybridoma used).