investigated for BCR-ABL transcripts with 2 different polymerase chain reaction (PCR) methods as previously described in detail.\textsuperscript{1,2} Forty-six (1.8\%) cases showed an ambiguous result and were not considered for further analysis. Of the remaining 2498 cases, 904 (36.2\%) were BCR-ABL\textsuperscript{+} (599 = 24\% minor breakpoint region [m-bcr] and 282 = 11.3\% major breakpoint region [M-bcr]), 15 = 0.6\% both, M-bcr and m-bcr, and 8 atypical transcripts), and 1594 were BCR-ABL\textsuperscript{−}. Atypical transcripts were not systematically detected before 2000\textsuperscript{1} and thus had to be excluded from further analysis. We grouped patients into age cohorts at 10-year intervals according to their age at diagnosis, each comprising between 277 and 481 patients and found a remarkable increase of BCR-ABL frequency in adolescents and young adults (Figure 1). It increased from 12.7\% in adolescents (15-24 years) to 30.6\% and 43.7\% in patients aged 25 to 34 and 35 to 44 years, respectively. In patients older than 44 years, the BCR-ABL frequency showed no further increment and ranged between 42\% and 44\%. The increase of BCR-ABL frequency was paralleled by a relative increase of m-bcr transcripts. These transcripts accounted for 16.4\% of all BCR-ABL-positive cases in adolescents (15-24 years). Their relative frequency increased to 22.5\% in 25- to 34-year-olds and to 36.8\% in 35- to 44-year-olds and remained between 33\% and 36.2\% from then on.

The reason for this age dependency is not obvious. The relative frequencies of immunologic subtypes (78.2\% common, 19.9\% pre-B, 1.9\% pro-B) of BCR-ABL\textsuperscript{+} patients did not differ significantly across the age groups. Moreover, the frequency of BCR-ABL was also not significantly different in woman compared with men. Our study thus had to be excluded from further analysis. We grouped patients into age cohorts at 10-year intervals according to their age at diagnosis, each comprising between 277 and 481 patients and found a remarkable increase of BCR-ABL frequency in adolescents and young adults (Figure 1). It increased from 12.7\% in adolescents (15-24 years) to 30.6\% and 43.7\% in patients aged 25 to 34 and 35 to 44 years, respectively. In patients older than 44 years, the BCR-ABL frequency showed no further increment and ranged between 42\% and 44\%. The increase of BCR-ABL frequency was paralleled by a relative increase of m-bcr transcripts. These transcripts accounted for 16.4\% of all BCR-ABL-positive cases in adolescents (15-24 years). Their relative frequency increased to 22.5\% in 25- to 34-year-olds and to 36.8\% in 35- to 44-year-olds and remained between 33\% and 36.2\% from then on.

The reason for this age dependency is not obvious. The relative frequencies of immunologic subtypes (78.2\% common, 19.9\% pre-B, 1.9\% pro-B) of BCR-ABL\textsuperscript{+} patients did not differ significantly across the age groups. Moreover, the frequency of BCR-ABL was also not significantly different in woman compared with men. Our study excluded lymphatic blast crises in patients with known chronic myeloid leukemia (CML). Previous work has indicated that M-bcr– and m-bcr–positive ALL may arise from different sets of hematopoietic progenitor cells,\textsuperscript{3} but this does not explain the age dependency. A number of genetic markers in ALL show a marked age dependency (reviewed in Armstrong and Look\textsuperscript{4}), eg, TEL-AML1, TLX1, and TLX3, MLL aberrations (especially in infant ALL). Our data provide additional information on the biology of BCR-ABL-positive ALL and substantiate evidence of age-dependent variation in the genetic background of ALL.

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References


To the editor:

Autosomal dominant erythrocytosis and pulmonary arterial hypertension associated with an activating HIF2α mutation

Erythropoietin production is regulated by the transcription factor hypoxia-inducible factor (HIF). Erythrocytosis with raised erythropoietin levels due to dysregulated HIF activity is recognized as a consequence of a hypomorphic HIFα E3 ubiquitin ligase (VHL) allele (Chuvash polycythemia), inactivating mutations of the HIFα hydroxylase PHD2 and, very recently, activating mutations of HIF2α.\textsuperscript{1-3} We report that erythrocytosis in a large kindred originally reported in 1979\textsuperscript{4} is due to an activating HIF2α mutation. Two affected individuals developed severe pulmonary hypertension, a hitherto unrecognized consequence of mutations in the pathway.

This study was approved by the Hammersmith and Queen Charlotte’s and Chelsea Hospitals local research ethics committee. Informed consent was obtained in accordance with the Declaration of Helsinki. At presentation, affected family members (Figure 1A) exhibited erythrocytosis (hemoglobin up to 228 g/L) with elevated total red cell volume and reduced plasma volume (Figure 1B). Other hematological parameters, serum biochemistry, arterial
oxygenation, and oxygen-hemoglobin dissociation were normal. Serum erythropoietin was elevated, rising to more than 2000 milli-IU/mL. II-1 and II-3 both had documented pulmonary arterial (PA) hypertension in their sixth decade without any evidence of thromboembolism. A genome-wide single nucleotide polymorphism screen was compatible with linkage to HIF2α/H9251 (Logarithm or odds ratio [LOD] 1.81, Figure 1C,D) and resequencing disclosed heterozygosity for a G3A substitution at position 2097 (Figure 1E), predicting a glycine-to-arginine change at residue 537 of the protein (HIF2α/H9251 Arg537), present in all affected and no unaffected individuals. Expression of HIF2α/H9251 Arg537 in HepG2 (a hepatoma cell line) cells revealed increased activation of a hypoxia response element-luciferase reporter construct compared with wild-type (Figure 1F). HIF2α/H9251 Arg537 was more active than HIF2α/H9251 Trp537, consistent with a more severe phenotype in this family.

These findings confirm that an activating HIF2α mutation dysregulates erythropoietin production in humans. That HIF2α plays a central role in regulating erythropoietin is supported by several observations. First, in hepatoma and neuroblastoma cell lines, erythropoietin mRNA was suppressed by siRNA-mediated silencing of HIF2α (Logarithm or odds ratio [LOD] 1.81, Figure 1C,D) and resequencing disclosed heterozygosity for a G → A substitution at position 2097 (Figure 1E), predicting a glycine-to-arginine change at residue 537 of the protein (HIF2α/H9251 Arg537), present in all affected and no unaffected individuals. Expression of HIF2α/H9251 Arg537 in HepG2 (a hepatoma cell line) cells revealed increased activation of a hypoxia response element-luciferase reporter construct compared with wild-type (Figure 1F). HIF2α/H9251 Arg537 was more active than HIF2α/H9251 Trp537, consistent with a more severe phenotype in this family.

These findings have several implications. First, there should be a high index of suspicion for pulmonary hypertension in other kindreds with activation of the HIF pathway. Second, inhibitors of PHD enzymes, which are in late stage clinical trials for treatment of anemia, may cause pulmonary hypertension. Third, it raises the possibility that polymorphic variation in HIF2α contributes to the marked differential susceptibility to erythrocytosis, reduced plasma volume, and pulmonary hypertension in humans at high altitude.


The authors are grateful to the family and the family's physicians, and thank Dr C. Mein for performing the SNP genotyping and Mr T. Bhattacharyya for technical assistance.

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Contribution: D.P.G. and P.H.M. designed research, analyzed data, and wrote the paper. D.P.G. and S.K.H. carried out research. E.G.D.T. and C.D.L.R. contributed, looked after the patients, and read and approved the paper.
In 1998 a female patient received an allogeneic stem cell (SC) transplant from her sister 9 months after she was diagnosed with a high-risk myelodysplastic syndrome (RAEB2) and was treated with chemotherapy aiming to achieve complete remission. A myeloablative conditioning regime was followed by infusion of peripheral blood stem cells (PBSC) from the donor. Two years later, a second myeloablative SC transplantation (SCT) with PBSC from the same donor was performed because of relapse. Seven years after the second SCT, the patient continues to be in complete remission with persistent complete donor chimerism.

The sister of the above-mentioned patient was found to be the only HLA-matched sibling donor. Blood examination 5 months before the first PBSC collection revealed an unexplained leukocytosis (19 × 10⁹/L) and thrombocytosis (750 × 10⁹/L). Both resolved spontaneously after 2 months. Three months before the first PBSC collection the donor also presented with a portal venous thrombosis (PVT). Additional analyses, including coagulation studies, bone marrow biopsy, and human androgen receptor (HUMARA) assay revealed no evidence of an underlying thrombophilia or clonal myeloproliferative disorder (MPD). Both donor and recipient have normal blood counts after more than 7 years’ follow-up and have not developed overt MPD.

To the editor:

The JAK2V617F mutation can occur in a hematopoietic stem cell that exhibits no proliferative advantage: a case of human allogeneic transplantation

In 1998 a female patient received an allogeneic stem cell (SC) transplant from her sister 9 months after she was diagnosed with a high-risk myelodysplastic syndrome (RAEB2) and was treated with chemotherapy aiming to achieve complete remission. A myeloablative conditioning regime was followed by infusion of peripheral blood stem cells (PBSC) from the donor. Two years later, a second myeloablative SC transplantation (SCT) with PBSC from the same donor was performed because of relapse. Seven years after the second SCT, the patient continues to be in complete remission with persistent complete donor chimerism.

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After the report of JAK2V617F mutations in the majority of Philadelphia− MPDs, donor and recipient samples were analyzed (method kindly provided by H. El Housni, Department of Medical Genetics, Free University of Brussels, Brussels, Belgium). Informed consent was obtained in accordance with the Declaration of Helsinki. The assay showed no background amplification on DNA of healthy blood donors. Moreover, JAK2V617F positivity was confirmed by M. Girardot (laboratory of S.N.C.).

The donor was found to be JAK2V617F⁺ in all samples tested (Figure 1), which may explain the PVT and the episode of transient leukocytosis and thrombocytosis observed before the first PBSC collection. In the recipient, the mutation was confirmed to be absent before the first transplantation. Immediately after the second transplantation a steep increase in the JAK2V617F mutational burden was seen, which decreased significantly over the course of 1 year to approximately 1% and still continues to be detectable at this level. At least 2 hypotheses may explain this observation. First, the heterogeneity of the transplanted stem cell pool might be different with respect to the original donor stem cell pool. Second, the transplanted JAK2V617F⁺ hematopoietic stem cells (HSCs) might have exhibited an intrinsic homing or proliferative disadvantage compared with the JAK2 wild type HSCs, possibly due to a different bone marrow microenvironment. Interestingly, specific genetic variations were recently found to be associated with the different clinical phenotypes of JAK2V617F⁺ MPDs. These or other genetic loci could be responsible for a different composition of the bone marrow microenvironment.

In conclusion, the fact that 7 years after transplantation a low number of hematopoietic cells still harbors the JAK2V617F mutation definitively confirms that progenitor cells with long-term repopulating capacities are targets for this mutation. This case also strongly suggests that the JAK2V617F mutation does not necessarily result in a proliferative advantage at the stem...
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