RAPID COMMUNICATION

Primary Familial Polycythemia: A Frameshift Mutation in the Erythropoietin Receptor Gene and Increased Sensitivity of Erythroid Progenitors to Erythropoietin

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Primary familial and congenital polycythemia (PFCP) is characterized by erythrocytosis with normal arterial Po_2, blood P_{50}, and serum erythropoietin (EPO) levels. In two PFCP families EPO receptor (EPOR) polymorphisms cosegregated with PFCP. A heterozygous insertion of G at *EPOR* nucleotide 5975 was identified in genomic DNA from polycythemic members of family no. 2. 5974insG shifts the reading frame at codon 430, predicting amino acid substitutions and truncation of the last 64 amino acids. Wild-type and mutant *EPOR* transcripts were detected in erythroid progenitors from affected individuals. Burst-forming units-erythroid from patients exhibited increased colony size and sensitivity to EPO. Transfected Ba/F3 cells expressing *EPOR* 5974insG exhibited increased EPO sensitivity compared with cells expressing wild-type *EPOR*. The functional effect of this *EPOR* mutation was directly compared with the other C-terminal mutations reported in unrelated PFCP families by expression in Ba/F3 cells. The transfected cells with another primary polycythemia associated *EPOR* mutant construct (G6002A) also exhibited increased sensitivity to EPO.

THE TERMS POLYCYTHEMIA and erythrocytosis are used interchangeably to indicate an increase in the number of circulating red blood cells. Primary polycythemias are caused by a defect of hematopoietic progenitor cells in contrast to secondary polycythemias, which are driven by factors extrinsic to erythroid progenitor cells such as increased levels of erythropoietin (EPO).[1-3] In primary polycythemias, the altered regulation of hematopoietic progenitor cells results in an autonomous or enhanced response of erythroid progenitors to normal growth factors mediating cellular proliferation and survival. The most common primary polycythemia is polycythemia vera, which may result from somatic mutation of a hematopoietic stem cell resulting in exaggerated myeloid proliferation, although the molecular events leading to this disease are not understood.[1,3] In contrast, the increased RBC mass in secondary polycythemia is mediated by increased EPO stimulation caused by either hypoxemia or ectopic secretion of EPO by tumors. Familial secondary polycythemias result in some cases from tissue hypoxia to which the body's homeostatic control mechanism react appropriately (such as high oxygen affinity hemoglobins [Hbs] or defects in 2,3-biphosphoglycerate production), whereas in other families increased EPO levels may be caused by a defect in the oxygen-sensing mechanism.[3-5] Primary familial and congenital polycythemias (PFCP) results from mutations affecting growth of hematopoietic/erythroid progenitor cells. We and others have reported several families and sporadic cases with PFCP.[2,6,7] Although it often appears to be a benign disease characterized by pure accumulation of erythrocytes, the clinical course of this condition is incompletely characterized and may be variable in its severity. The data from these studies led us to hypothesize that an altered EPO receptor (EPOR) may be responsible for the disease phenotype in at least some families.

MATERIALS AND METHODS

Case report and clinical studies. A 24-year-old white man (III:1, see pedigree in Fig 1A) was noted to have an Hb concentration of 22.5 g/dL and a hematocrit (Hct) 70% of unknown duration. His serum EPO level at age 24 years was 4 mU/mL (normal range 10 to 26 mU/mL). Studies of Hb structure and Hb-oxygen dissociation were normal. After diagnosis he was treated by phlebotomy approximately every 3 months when he became symptomatic ("being flushed in the face" and "feeling tired, lazy"). At 29 years of age, he suffered an occipital lobe hemorrhage. His first daughter (IV:1, see Fig 1A), now 8 years old, was found to have an Hb concentration of 23.1 g/dL, Hct of 65%, and serum EPO of 2 mU/mL at age 4 years, and has remained asymptomatic without treatment. His second daughter (IV:2), now 4 years old, was thought not to be affected at birth, but has subsequently developed polycythemia (Hb 17.5 g/dL, Hct 49.6%) without symptoms. The propositus' mother (II:2) was also found to be also polycythemic (Hct 68%) at age 48 years. She reported headaches and feeling "sluggish," symptoms that were relieved by phlebotomy every 3 to 4 months. She has had no cerebral or coronary vascular accidents. There was no other history of polycythemia or other hematologic disease in the family. No hematologic data were available for deceased family member I-2, but he was not plethoric in appearance.

In vitro assays of erythroid progenitors. Peripheral blood (PB) samples (two 10-mL tubes of heparinized blood) from the propositus, his daughter, and an unrelated normal subject were shipped by courier mail to Birmingham, AL. The mononuclear cells were separated by centrifugation through an Isopaque (Sigma, St Louis, MO) density gradient (1.077 g/mL). Erythropoiesis was examined by in vitro cultures as described[2,7-9] with the following modifications: for the suspension liquid cultures, the mononuclear cells were further en-

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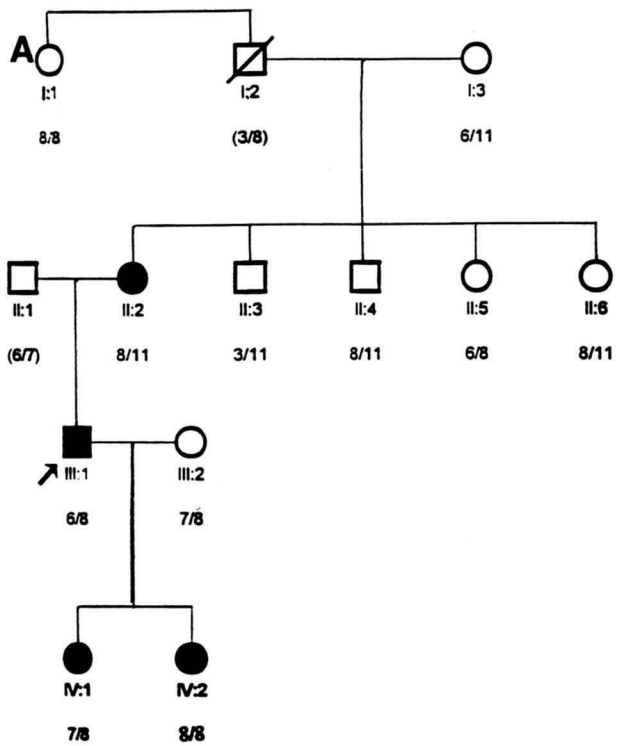
riched for $CD34^+$ cells by magnetic activated cell sorting (MACS; Miltenyi Biotec, Sunnyvale, CA). A single passage of labeled mononuclear PB cells through the magnetic column resulted in 60% purity of $CD34^+$ cells.[10] Erythroid progenitors were expanded 100 times more efficiently in the serum-free liquid culture (GIBCO SC medium [GIBCO BRL, Gaithersburg, MD], supplemented by PIXY321 and SCF,[11] with a half media changes every 3 days) than in serum-containing media.[11] The expanded progenitor cells (obtained on day 10) were cultured at a final concentration of 1×10^5 cells/mL in a complete, methylcellulose serum containing medium (MethoCult H-4531; StemCell Technology Inc, Vancouver, BC, Canada) in duplicate 35-mm Petri dishes. The following EPO concentrations were used: 0.00, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.0, and 3.0 U/mL. Cultures were maintained in a humidified atmosphere of 5% carbon dioxide in air at 37°C. Large erythroid colonies (BFU-E) were scored at 14 days using standard criteria.[8,9]

DNA studies. Genomic DNA was isolated from PB leukocytes according to standard procedures.[12] Selected regions of the *EPOR* gene (see Fig 3) were amplified by polymerase chain reaction (PCR)[13] in a total volume of 50 μL containing 100 ng of genomic DNA, 25 pmol/L of forward and reverse primers (see Fig 3), 200 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 20 mmol/L Tris-HCl, pH 8.3, 50 mmol/L KCl, 1 U of Taq polymerase (Promega Corp, Madison, WI). Forward primers were: 1A, GCCTCTATGACTGGGAGTGG (nucleotides no. 5336 through 5355, see ref 13); 2A, GAGGACCCACCTGCTTCC (nucleotides no. 5659 through 5677); and 3A, TCCTGCTCATCTGCTTTGG (nucleotides no. 5896 through 5914). Reverse primers were: 1B, GCGCTCTGAGAGGACTTCC (nucleotides no. 5697 through 5679); 2B, CAAAGCTGGCAGCAGAGG (nucleotides no. 5959 through 5942); and 3B, CATCTGCAGCCTGGTGTCC (nucleotides no. 6231 through 6213). Initially, 40 cycles of amplification were performed in a



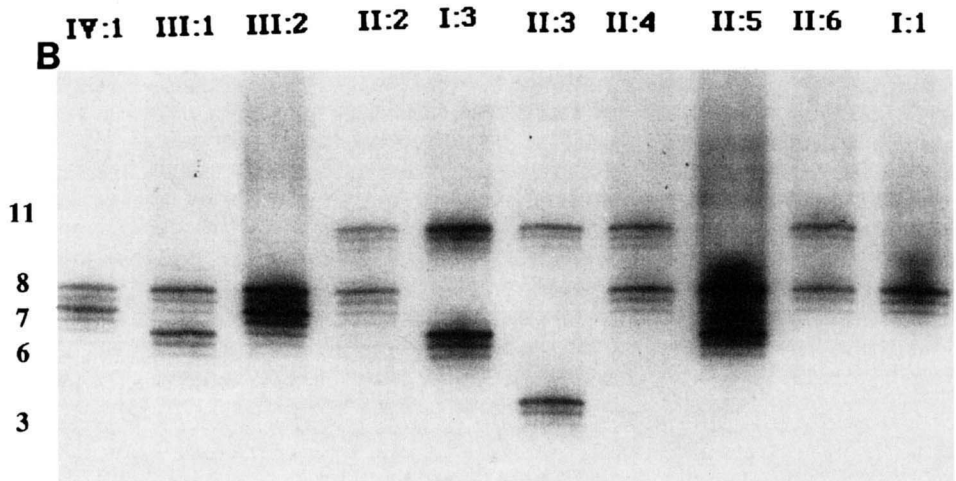


Fig 1. Linkage of PFCP to *EPOR* locus. (A) Pedigree of PFCP family no. 2. Affected individuals are indicated by (●) and (○), respectively. *EPOR* genotypes based on GGAA repeat polymorphism are shown. The arrow indicates the propositus. The genotypes of I:2 and II:2 are inferred from the genotypes of their offspring. (B) Analysis of *EPOR* polymorphism. Two microsatellite polymorphic markers (GGAA)n and (GA)n located in the 5′ untranslated region of *EPOR* were amplified by PCR using genomic DNA isolated from PFCP family no. 2 members and separated by 6% polyacrylamide denaturing gel electrophoresis.[17] The designation of GGAA alleles at the left margin has been previously published.[17] The polycythemic phenotype segregated in affected subjects (IV:1, III:1, II:2, and also IV:2—data not shown) with GGAA allele 8.

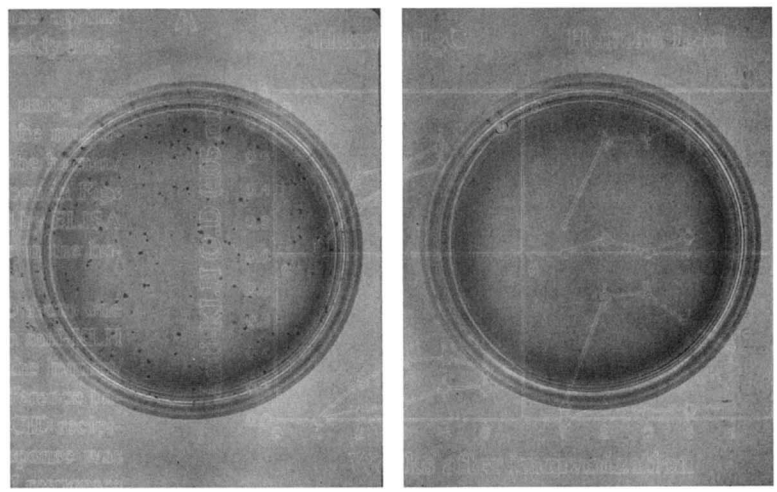


Fig 2. Effect of PFCP mutation on size of BFU-E colonies in 0.8% MethoCult. BFU-E colonies from the propositus (left dish, 35 × 10 mm) had an extremely large diameter (1 to 2.5 mm) which allowed them to be counted without the use of microscope. In contrast, the control BFU-E colonies (right dish) were not visible. The BFU-E colonies were scored on day 14 of culture in media containing 1 U/mL of EPO.

Perkin-Elmer Model 480 thermocycler (Perkin-Elmer, Norwalk, CT) using the primers 1A and 3B. Each cycle consisted of 1 minute at 94°C, 2 minutes at 58°C, and 3 minutes at 72°C. The final elongation step was extended to 7 minutes. For the second round of amplification, 1 μL of the first PCR reaction mix was used as template, and the extension was performed with various combination of the above-nested primers under the same reaction conditions.

Subcloning of PCR products. The 0.3-kb PCR fragments (using primers 3A and 3B) containing the negative regulatory region of *EPOR* were first sequenced directly. When a mutated as well as a normal sequence appeared to be present, the PCR amplified products were subcloned into pCR II vector (Invitrogen Corp, San Diego, CA). Nucleotide sequences were determined for eight clones of each PCR product using a Sequenase 2.0 kit (USB, Cleveland, OH) according to the manufacturer's specifications.

Search for mutation in the family members, and polycythemic and normal controls. Genomic DNAs were amplified as above (using primers 3A and 3B). PCR products were digested with BsmF I (New England Biolabs, Beverly, MA) and restriction fragments were visualized after electrophoresis on a 3% NuSieve (FMC BioProducts, Rockland, ME) 1% agarose gel.

Allelic transcripts of EPOR in erythroid progenitors. Erythroid progenitors were prepared from PB. Mononuclear cells were prepared by Ficol/Hypaque methods enriched for $CD34^+$ cells by magnetic sorting using MACS, and expanded in serum-free liquid culture. Serial samples from these cultures were sequentially obtained and processed. RNA from cultured erythroid progenitors was prepared by acid guanidium thiocyanate extraction.[14] cDNA was synthesized from poly(A)$^+$ RNA with recombinant Moloney murine leukemia virus reverse transcriptase (SuperScript; GIBCO BRL). One microgram of RNA was dissolved in 31.5 μL of DEPC-treated water, heated to 65°C for 3 minutes, and kept at room temperature for 3 minutes after adding the final reaction mixture containing 2.5 μL of 10 mmol/L dNTPs, 2.5 μL of 0.1 mol/L DTT, 10 μL of 5× SuperScript buffer (GIBCO BRL), and 2.5 μg of random hexamers (Pharmacia, Piscataway, NJ). Then 200 U of reverse transcriptase (GIBCO BRL) was added and incubated at 42°C for 1 hour. Primers 3A and 3B were used for PCR amplification of cDNA. The PCR was performed as described above except that the primer 3A was first 5′ end-labeled with [γ-P^{32}] adenosine triphosphate in the presence of T4 polynucleotide kinase (New England Biolabs). The PCR products were separated on a 6% denaturing polyacrylamide gel, autoradiographed, and the intensity of the signal was determined by using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Functional analysis of the human EPOR mutation. Site-directed mutagenesis[15] was performed using the pCEP-4 expression vector

Table 1. Numbers of BFU-E Colonies at Different EPO Levels

	EPO	Levels	mU/mL	
Subject	**0.00**	**0.03**	**0.06**	**0.12**
Propositus	**0**	**15**	**33**	**68**
	(0, 0)	(13, 17)	(31, 36)	(67, 69)
NC	**0**	**0**	**3**	**8**
	(0, 0)	(0, 0)	(2, 3)	(7, 9)
PV	**18**	**24**	**28**	**34**
	(19, 17)	(25, 22)	(28, 27)	(35, 33)

Each bold figure represents the mean BFU-E score per 1 × 10^5 of bone marrow cells plated. Individual scores for each of two 1-mL cultures are given in parentheses.

Abbreviations: NC, normal control; PV = polycythemia vera.

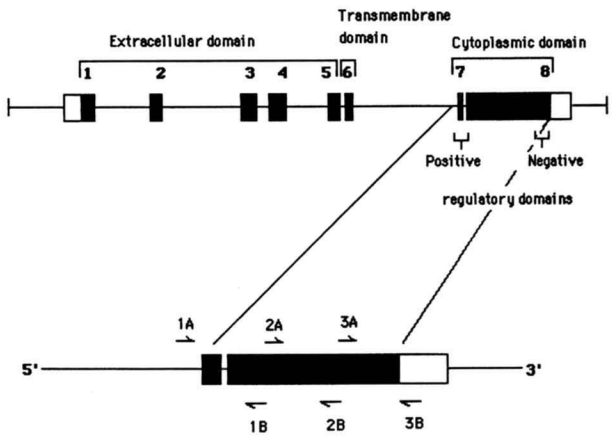


Fig 3. Amplification of DNA encoding positive and negative regulatory domains of *EPOR*. Exons 7 and 8 were amplified by PCR from genomic DNA using 3 pairs of primers (1A, 1B, 2A, 2B, 3A, 3B) encompassing sequences encoding the positive and negative regulatory domains of *EPOR*. Nucleotide sequences of forward primers and reverse primers are described in Materials and Methods.

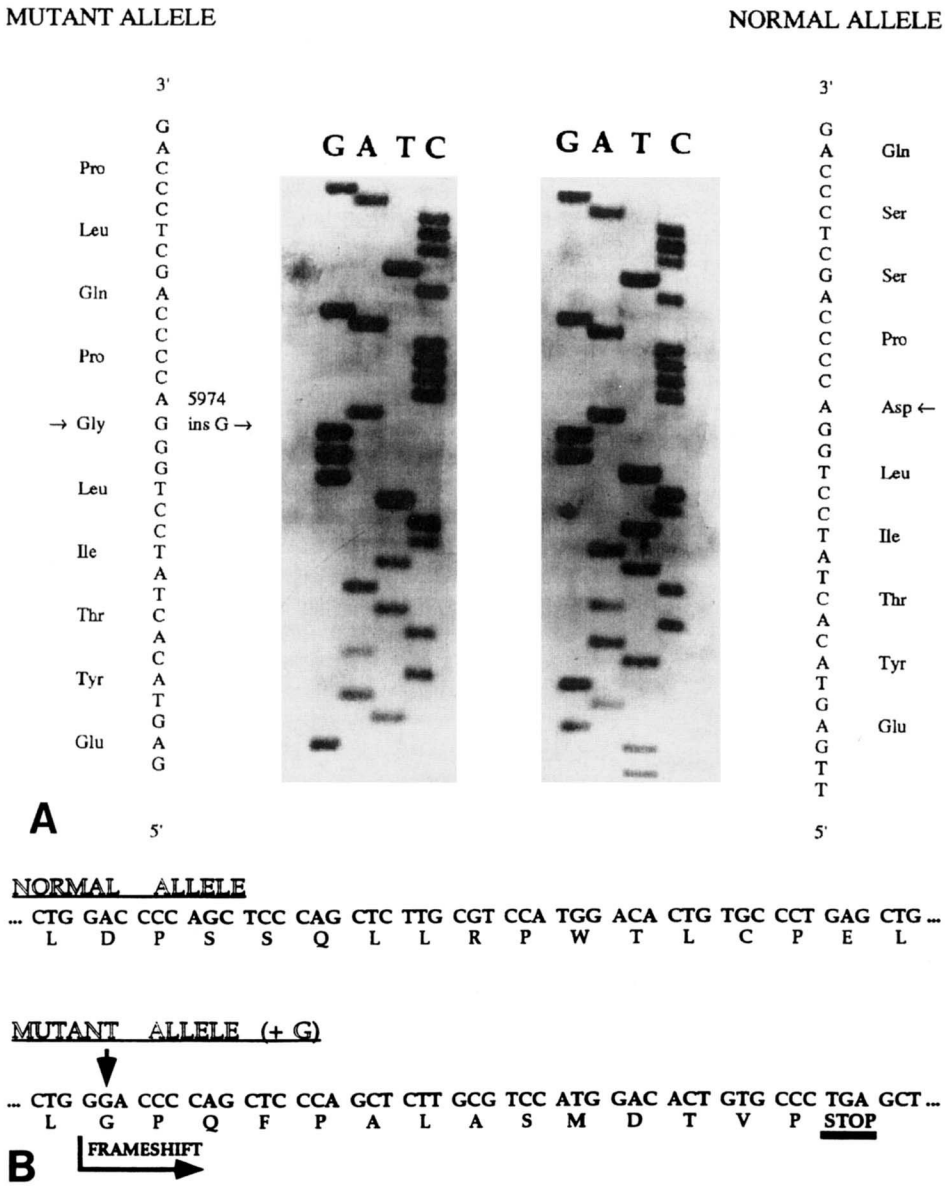


Fig 4. Identification of frameshift mutation in *EPOR*. (A) Nucleotide sequence analysis of wild-type and mutant *EPOR* genes. The normal (right panel) and mutant (left panel) alleles were amplified by PCR from genomic DNA of the propositus and analyzed by nucleotide sequence analysis. Insertion of a G residue at nucleotide 5974 was detected in the mutant *EPOR* allele (arrow), which results in a translational frameshift. (B) The mutant allele encodes a truncated EPOR. The 5974insG frameshift mutation leads to a premature termination codon located 15 codons downstream, predicting an EPOR peptide that is truncated by 64 amino acids. The sequence shown begins at codon 429.

(Invitrogen, Corp, San Diego, CA) containing *EPOR* cDNA insert,[12,16] kind gifts of Drs J.C. Winkelmann (Department of Medicine and Institute of Human Genetics, University of Minnesota Medical School, Minneapolis) and A.D. D'Andrea (Dana-Farber Cancer Institute, Boston, MA). The G insertion at codon 430 was introduced into the full-length human *EPOR* cDNA. The presence of the mutation was verified by nucleotide sequence analysis. Ba/F3 cells were electroporated with mutated and wild-type human *EPOR* cDNA cloned into pCEP-4. Growth characteristics of cells in medium under varying levels of EPO and in the presence of interleukin-3 (IL-3) (WEHI-3 conditioned media) were measured by means of the MTT assay described elsewhere.[16] Dose-response curves were generated for cells grown in EPO or in IL-3.

RESULTS

In vitro assays of erythroid progenitors. Significant numbers of BFU-E erythroid colonies were seen in clonogenic cultures of PB from the propositus and his daughter (IV:2) at very low EPO concentrations (0.01 U/mL), although no BFU-E colonies formed in cultures without EPO. This type of EPO dose response is seen in all PCFP patients studied in our laboratory and is different from that seen in normal control subjects and polycythemia vera individuals, respectively.[3,7] In addition, these colonies were unusually large (many contained 20,000 to 100,000 cells) and could be seen by the naked eye (Fig 2). These colonies were much larger than those seen in over 500 individual cultures from normal subjects, patients with polycythemia vera, other subjects with PFCP, and other unclassified polycythemic disorders, performed under similar culture conditions. The PB BFU-E culture from the propositus was repeated over an extended range of low EPO concentrations (Table 1), and these data confirmed the hypersensitivity demonstrated at low EPO levels.

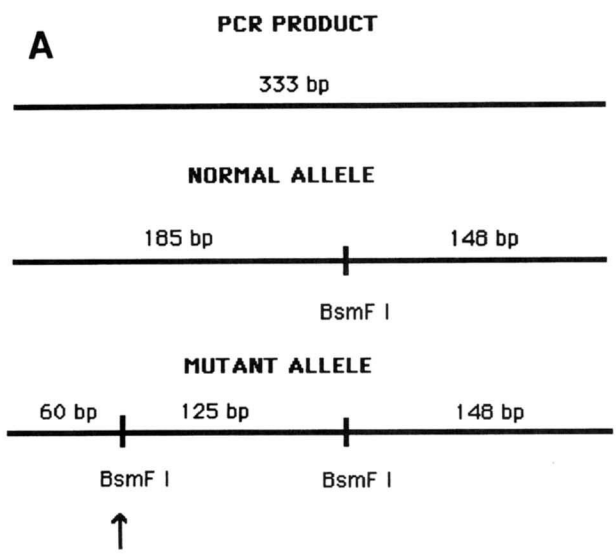
Linkage of PFCP phenotype with EPOR *polymorphism.* We used two microsatellite polymorphic markers, GGAA and GA repeats, located in 5′ untranslated region of *EPOR,*[17] to investigate genetic linkage between the polycythemic phenotype and *EPOR* locus in nine PFCP families. In two of these families our data were consistent with genetic linkage of *EPOR* polymorphism with polycythemic phenotype. In PFCP family no. 2 (Fig 1A) the polycythemic phenotype segregated with the GGAA polymorphic repeat allele no. 8 (Fig 1B) in the three generations studied starting with the mother (II:2) of the propositus. The segregation of allele no. 8 in the pedigree was consistent with genetic linkage (under the assumption of a new mutation in II:2), and we thus performed nucleotide sequence analysis of *EPOR* in genomic DNA from affected and nonaffected family members.

EPOR *nucleotide sequence analysis.* The positive and negative regulatory regions contained in *EPOR* exons 7 and 8 were amplified by PCR and sequenced (see Methodology and Fig 3). Figure 4A demonstrates insertion of a G residue at nucleotide 5974 in the *EPOR* mutant allele (left) compared with the sequence of the wild-type allele (right) amplified from genomic DNA of the propositus. The 5974insG mutation causes a frameshift such that the translated *EPOR* sequence downstream of the mutation consists of 14 missense codons followed by a premature termination codon (Fig 4B), which would result in synthesis of a truncated protein lacking the last 64 amino acids of the C-terminal domain.[18]

Mutation analysis of relatives and unrelated subjects. The mutation created a new BsmF I restriction site (Fig 5A) that allowed us to screen all other family members (Fig 5B); 20 unrelated subjects with PFCP, polycythemia vera, and other uncharacterized polycythemias; and 20 normal control subjects (data not shown). The restriction fragment diagnostic of the mutant allele was seen only in PCR-amplified genomic DNA from the three affected family members, indicating that *EPOR* 5974insG segregates with the PFCP phenotype and that among the subjects studied it is unique to this family.

EPOR *transcripts in erythroid progenitors.* To determine whether RNA containing the frameshift mutation is transcribed and stable, enriched PB progenitors were expanded in serum-free liquid culture and cell aliquots were collected on days 1, 3, 6, etc for RNA analysis. Using reverse transcription-PCR (RT-PCR) we were able to detect both *EPOR* allelic transcripts (in equal quantities) in erythroid progenitors from the two polycythemic subjects tested (Fig 6 and data not shown).

Functional significance of EPOR *frameshift and nonsense mutations.* The 5974insG mutation is located in the negative regulatory domain of *EPOR.*[16,18,19] The functional effect of this mutation was examined by the MTT proliferation assay in human *EPOR*-transfected Ba/F3 cells. Ba/F3 are derived from mouse hematopoietic cells, are dependent on



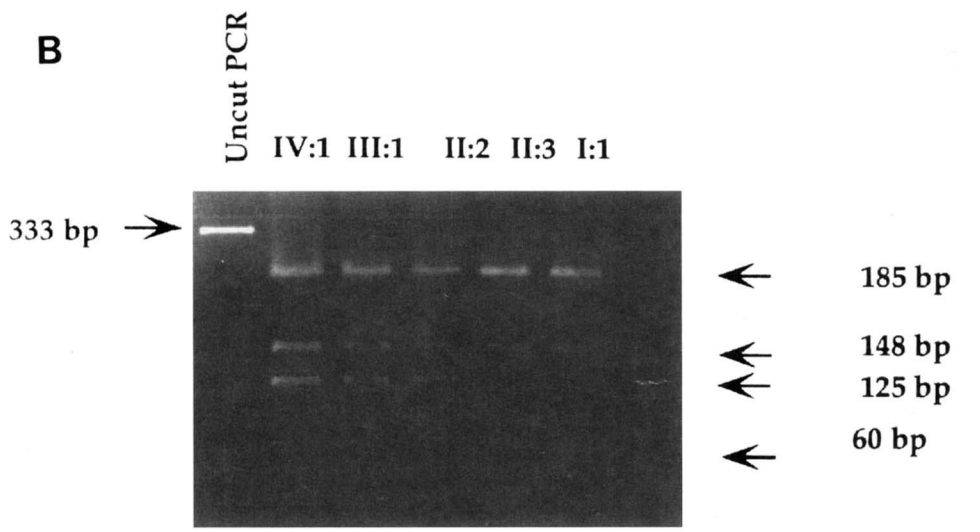


Fig 5. Mutation detection by BsmF I digestion. (A) BsmF I restriction sites in DNA amplified from normal and mutant alleles. Two fragments (185 and 148 bp) result from digestion of the normal allele, whereas three fragments (148, 125, 60 bp) are generated by digestion of mutant allele. (B) Analysis of PFCP family members. The PCR fragment of 333 bp was amplified from genomic DNA and digested with BsmF I. Two bands (185 and 148 bp) were detected on a 4% NuSieve Agarose gel with ethidium bromide in unaffected family members (II:3, I:1) and three bands (185, 148, and 125 bp) were visible in the polycythemic family members (II:2, III:1, IV:1). The 60-bp band was visualized only with PCR primer radioactive labeling (data not shown).

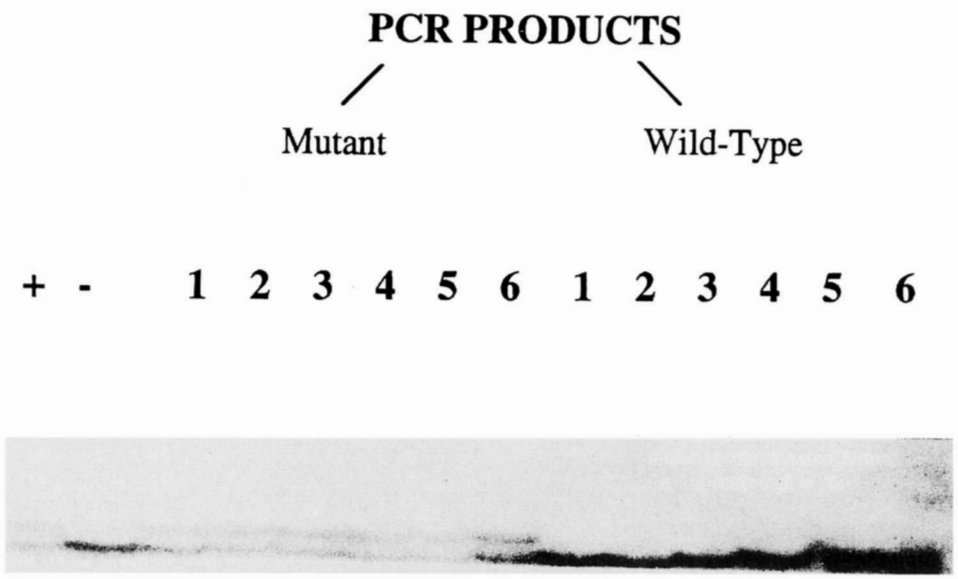


Fig 6. Analysis of *EPOR* transcripts in erythroid progenitor cells from propositus and normal control. CD34$^+$ purified cells from affected subject IV:1 and a normal control were cultured in GIBCO serum-free media. Aliquots of cells were harvested at intervals 1 through 6 (each interval 3 days apart) and analyzed by RT-PCR. Radiolabeled PCR products were separated on a 6% polyacrylamide gel and analyzed by autoradiography. Lane + represents a positive control obtained by PCR amplification of an equal mixture of wild and mutant *EPOR* plasmids, lane − represents a normal control obtained by PCR amplification of plasmid containing wild-type *EPOR* cDNA, "Mutant" lane represents analysis of affected subject' erythroid progenitors cDNA, "Wild-Type" lane represents analysis of normal subject' erythroid progenitors cDNA.

IL-3 for survival, and when transfected with *EPOR*, become EPO dependent.[16,18,19] These properties have been used for studies of *EPOR* function.[16,18,19] A significant hypersensitivity of the *EPOR* 5974insG transfected Ba/F3 cells to EPO, but not to IL-3, was found (Fig 7 and data not shown). In a separate experiment, the functional effect of this *EPOR* mutation was directly compared to the other C-terminal mutation reported in unrelated PFCP families, a substitution of proline to serine at codon 488[12,20] and a nonsense mutation G6002A,[13] by expression in Ba/F3 cells. We found increased sensitivity to EPO in the cells transfected with two of the mutant *EPORs* constructs (5974insG or G6002A) compared to cells transfected with the wild-type *EPOR* (Fig 7).

DISCUSSION

This report presents a new human *EPOR* mutation and provides the first demonstration of the functional significance of this and a previously described *EPOR* mutation that causes polycythemia.

We describe a four-generation family with primary familial polycythemia inherited in autosomal dominant fashion. The normal arterial pO_2, normal $P_{50}O_2$, normal white blood cell and platelet count, as well as normal serum EPO levels in propositus and other affected family members suggested the diagnosis of primary familial polycythemia. Hypersensitivity of propositus' erythroid progenitors was observed at low EPO levels in contrast to normal control; however, no EPO-independent colonies were seen. This type of EPO dose response is observed in all PFCP patients studied in our laboratory and it is different from that seen in normal control subjects and polycythemia vera individuals, respectively.[3,7] In addition, BFU-E colonies were unusually large. These studies suggest that the propositus' erythroid progenitors exhibit not only EPO hypersensitivity, as indicated by BFU-E formation at subcritical EPO concentrations, but also increased EPO-mediated cellular proliferation.

The data obtained from linkage analysis between PFCP phenotype and EPOR polymorphism were consistent with genetic linkage under assumption of a new mutation in subject II:2. Thus, we performed nucleotide sequence analysis of *EPOR* in genomic DNA from affected and nonaffected family members and found the 5974insG G mutation that causes a frameshift predicting truncated protein lacking the last 64 amino acids of the C-terminal domain. This mutation segregates with polycythemic phenotype and it is unique to this family.

By RT-PCR we were able to detect both EPOR allelic transcripts in erythroid progenitors from the two polycythemic subjects tested. Many, but not all, frameshift mutations are associated with reduced or undetectable transcripts. Our data indicate that the frameshifted *EPOR* transcript is present in the same quantity as the normal transcript and is likely to be stable, probably because the mutation is located close to the 3′ end of the coding sequence. This observation is consistent with studies of frameshift and nonsense mutations of human triosephosphate isomerase showing that the stop codon mutations within the first $^3/_4$ of the coding region result in decreased mRNA stability, whereas more distal stop codons do not have this effect.[21]

Furthermore, the functional effect of this mutation was examined by the proliferation assay in human *EPOR*-transfected Ba/F3 cells. A significant hypersensitivity of the *EPOR* 5974insG transfected Ba/F3 cells to EPO complement

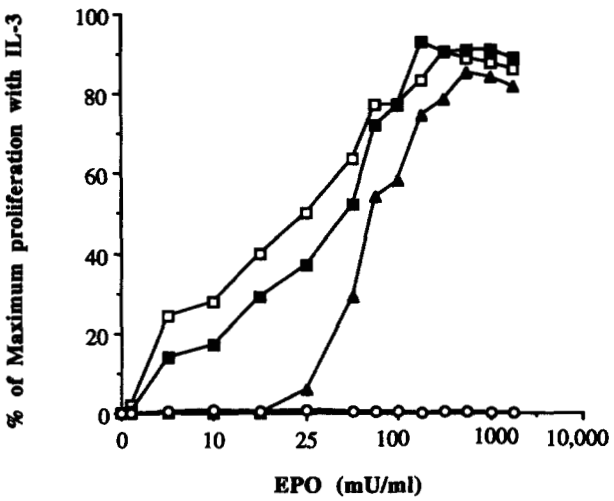


Fig 7. Expression of mutant human EPOR results in hypersensitive EPO-dependent growth of transfected Ba/F3 cells. EPO-dependent growth characteristics were measured by the MTT proliferation assay.[12,16] Ba/F3 cells were transfected with expression vectors encoding wild-type EPOR (▲), mutant EPOR polypeptides (□, 5974insG mutant; ■, G6002A mutant) or no EPOR (○). Growth of transfectants under different EPO concentrations is expressed as percentage of maximal proliferation obtained with IL-3 treatment.

the in vitro assessment of erythroid progenitors and establish a direct role for the *EPOR* mutation in the pathogenesis of PFCP. The observed autosomal dominant inheritance of the polycythemia phenotype is consistent with the "gain-of-function" mutation we describe here.

The *EPOR* gene (Fig 3) was cloned by D'Andrea et al[22] and based on the amino acid sequence that was deduced from the cDNA, a picture of the *EPOR* at the protein level has emerged. EPOR can be divided into extracellular, transmembrane, and cytoplasmic domains. In Friend virus-infected mice, a mutation that changes arginine to cysteine at residue 129 in the extracellular domain was found to render the *EPOR* constitutively active without its ligand,[19] suggesting that the mutated *EPOR* can function as an oncogene in susceptible cells.[18] Deletion of the cytoplasmic portion of the mouse EPO receptor increases EPO-mediated proliferation[16,18] and thus it functions as a negative regulatory domain, whereas the deletion of more proximal sequences localized within exon 7 defined the positive regulatory domain. The studies of the polycythemic family described here directly implicate an altered cytoplasmic domain of *EPOR* as a molecular basis for development of polycythemia. Our data are in agreement with experimental site-directed mutagenesis experiments from two laboratories[16,18] wherein C-terminal deletions of *EPOR* cytoplasmic domain resulted in increased sensitivity of transfected Ba/F3 cells to EPO, thus implicating this portion of the receptor as a negative regulatory domain.

Moreover, we were able to directly compare the effects of two naturally occurring *EPOR* mutations with respect to the minimal EPO concentration required to effect survival and/or proliferation of hematopoietic cells and the magnitude of the growth response at any given EPO concentration. These two mutations have proline as the last amino acid albeit in different locations (5974insG at codon 443 and G6002A at codon 438),[13] compared with wild-type EPOR serine. However, the 5974insG's proline at codon 443 is preceded by 15 different amino acids not found in the wild-type allele or in G6002A mutant. Other EPO-mediated hematopoietic effects, namely EPO-mediated differentiation[23] and prevention of apoptosis,[24] altered by these EPOR mutations are presently under analysis. Future studies to determine the mechanism by which these EPOR mutations affect signal transduction processes such as the interaction with Jak-2 kinase,[25] hematopoietic phosphatase,[26] and other *EPOR* interacting signal mediators[27] may provide important insights into the molecular programs that regulate erythropoiesis.

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