Benefit of phlebotomy and low-dose aspirin in the prevention of vascular events in patients with *EPOR* primary familial polycythemia on the island of New Caledonia

Hereditary or congenital erythrocytosis is a group of rare, inherited disorders, including congenital erythrocytosis associated with a germline mutation in erythropoietin receptor (*EPOR*). Of the 130 cases reported in the literature to date, only 25 provide information on the presence or absence of vascular events, and only 18 with information on treatment, mainly phlebotomy but without a clear benefit on vascular events, and without aspirin in most cases. Our data from a homogeneous (both genetically and in terms of medical management) cohort of 33 affected subjects from the island of New Caledonia, a French territory in the South Pacific, suggest the possible benefit of phlebotomy and low-dose aspirin in the prevention of vascular events in patients with erythrocytosis due to an *EPOR* mutation.

In 1985, Prchal and collaborators reported an autosomal dominant polycythemia associated with a hypersensitivity of colony-forming unit erythroblast colony growth to erythropoietin (EPO) and low serum EPO levels. This entity was called primary familial and congenital polycythemia or familial erythrocytosis type 1.[1] A few years later, Juvonen *et al*. described similar patterns in a large family in which the proband was an Olympic gold medalist.[2] The molecular signature at the origin of the phenotype was then identified by de La Chapelle *et al.* as a mutation in the *EPOR* gene leading to a truncated protein.[3] Since then, it has been noted that the majority of reported pathological variants are nonsense or frameshift mutations, and almost all of them reside in exon 8 of the *EPOR* gene, which encodes the negative feedback regulatory domains of the receptor. The lack of EPOR cytoplasmic suppressors results in overexpression of the receptor and thereby prolonged phosphorylation of JAK2 with excessive activation of STAT5 and the RAS-MAPK signal transduction pathway.[4] Recently, Pasquier *et al.* reported another mechanism from the consequences of *EPOR* mutations: distal truncations induced by frameshift mutants confer EPO hypersensitivity that depends on the appearance of a new C-terminal tail which causes pre-activation of EPOR and JAK2, constitutive signaling and hypersensitivity to EPO by increasing EPOR dimerization and stability at the cell surface.[5] According to guidelines from the American College of Medical Genetics and Genomics and the Association of Molecular Pathologists, among the 35 *EPOR* variants reported to be associated with primary familial and congenital polycythemia to date, four are classified as benign or likely benign, three are variants of uncertain significance, and 28 are pathogenic or likely pathogenic.[6] Mutations in *EPOR* are relatively rare among patients with *JAK2*-negative erythrocytosis, and are found in only approximately 1% of cases.[7]

We recently reported a new *EPOR* mutation (c.1293del, p.Ser432Alafs*21) in patients from different unrelated families. Interestingly, this mutation has been exclusively observed in patients from the island of New Caledonia, strongly supporting a founder effect.[7] In order to further explore the patients with erythrocytosis related to this *EPOR* mutation, a family survey was carried out in New Caledonia and in France where some patients currently live. In particular, we aimed to assess the vascular risk and to evaluate the possible impact of treatments.

A total of 33 patients (14 males, 19 females), mean age 43 years old (from 2 months to 82 years old), from four unrelated families from New Caledonia with the germline *EPOR* mutation (c.1293del, p.Ser432Alafs*21) were included (Figure 1, Table 1). All patients signed an informed consent form. Comprehensive clinical and biological data were available for 26 of them. The mean hemoglobin concentration and hematocrit at diagnosis were 185 g/L and 56.3% in males and 179 g/L and 54.9% in females, respectively. When available, the serum EPO level at diagnosis was low in all cases (<5 mU/mL). Four of the cases were children (from 2 months to 15 years old) and had no treatment or history of thrombosis.

Among the 22 adults, 16 were being treated with a combination of aspirin and phlebotomy, two with a combination of anticoagulant therapy (for atrial fibrillation) and phlebotomy, one with phlebotomy alone, and three were not receiving any treatment. Of note, no thrombosis was observed in healthy relatives, but a vascular event was reported in three patients (3/22=13%) who were not receiving any treatment: no recurrence of vascular events was noted in these three patients after treatment had been initiated. On the other hand, no hemorrhagic events have been reported in this cohort to date. Remarkably, over 50% of patients treated with phlebotomies (9/17) reported improvements in headaches, dizziness, tinnitus and visual disturbance. The hematocrit threshold for phlebotomies was guided by the onset of symptoms or, in asymptomatic patients, essentially varied from 51% to 55%.

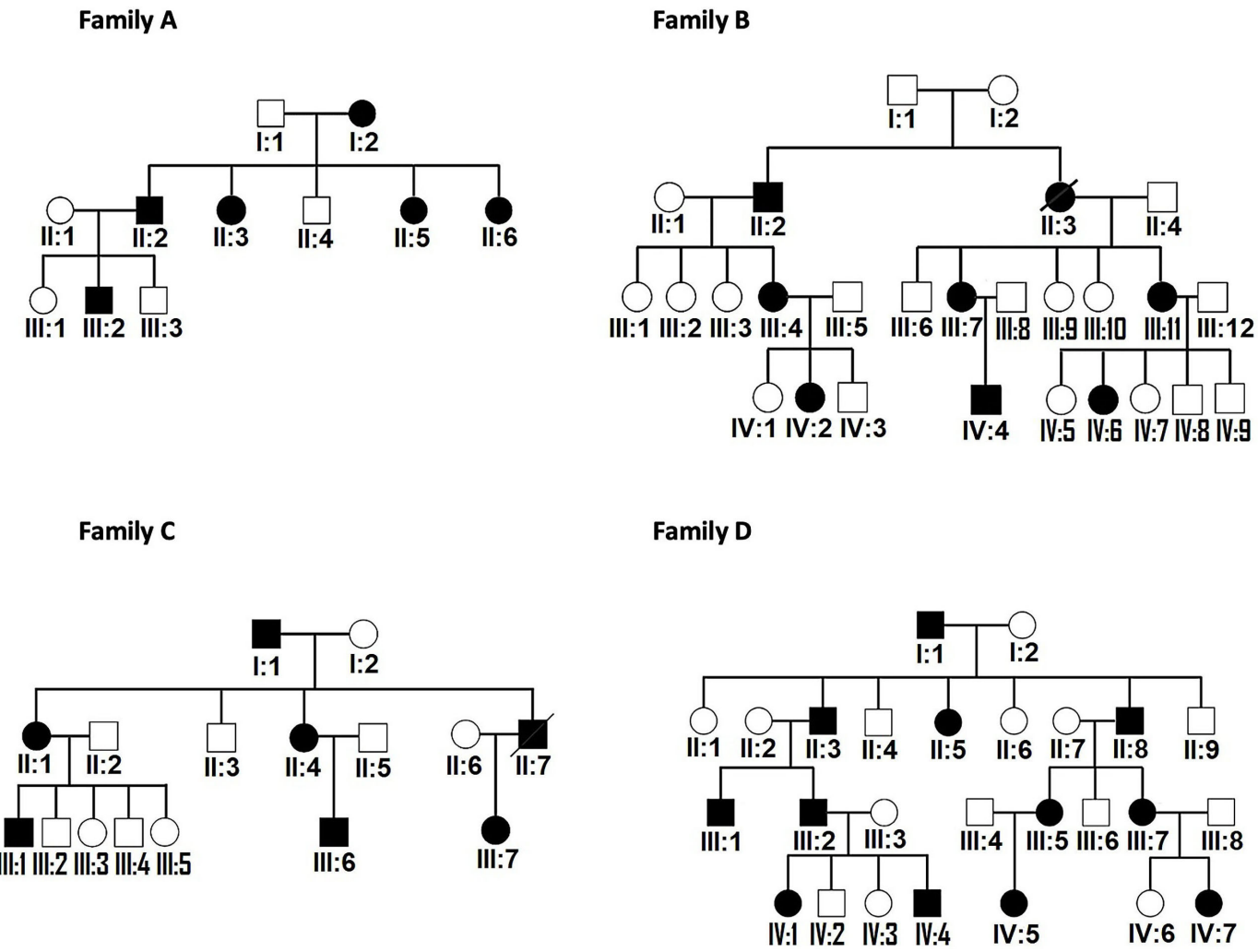

Figure 1. Family trees of New Caledonian patients with *EPOR* mutations. The black circles and squares represent people in the family with erythrocytosis (crossed-out: deceased). Patients C II:1, C II:4, C II:7, C III:6 and D III:1 have been reported by Filser *et al.*[7] Patient #11 (Family B IV:6) is a 29-year-old woman living in Eastern France but originally from New Caledonia, with a history of three spontaneous early miscarriages. Her hemoglobin concentration and hematocrit were 177 g/L and 53%, respectively. Biological investigations for thrombophilia were negative, but she was an active smoker and obese. Later on, after introduction of low-dose aspirin, she had two successful full-term pregnancies in 2015 and 2019. Finally, in 2023, an *EPOR* mutation similar to that in the other New Caledonian patients (c.1293del p.Ser432Alafs*21) was detected. Patient #18 (Family D II:5) is a 50-year-old woman who presented with spontaneous deep vein thrombosis of the lower limb in 2016 in New Caledonia. She had no risk factors for venous thrombosis but was treated for high blood pressure. She had no treatment at the time of the deep vein thrombosis. Three years later, a mutation of the *EPOR* gene (c.1293del p.Ser432Alafs*21) was detected. Her hemoglobin concentration was 187 g/L, her hematocorit was 59%, and her leukocyte and platelet counts were $5x10^9$/L and $1,865x10^9$/L, respectively. Phlebotomies were initiated in 2019 associated with low-dose aspirin. Serum erythropoietin was not tested in this patient. Patient #19 (Family D II:8) is a 54-year-old man, followed in New Caledonia for congenital erythrocytosis. He had no history of thrombosis and was treated with phlebotomy without antiplatelet agents. The only known cardiovascular risk factor was active smoking. In February 2022, he discontinued phlebotomies, and 4 months later, in May 2022, he presented with non-ST-elevated myocardial infarction for which he was subsequently phlebotomized and treated using low-dose aspirin. At the time of this thrombotic event, he had a hemoglobin of 204 g/L, hematocrit of 67%, leukocyte count of $115x10^9$/L and platelet count of $1,695x10^9$/L. No recurrence has been observed since then.

It is worth noting that the distribution of cardiovascular risk factors was similar in the three groups, i.e., *EPOR*-mutated patients with vascular events, EPOR-mutated patients without vascular events and healthy relatives (Table 1). Overall, the use of aspirin (or oral anticoagulant in 2 patients) and phlebotomy was associated with a decreased risk of vascular events (P<0.001, Fisher exact test).

The occurrence of vascular events in more than 10% of the cohort raises the question of the seriousness of this complication, which was initially considered to have little effect. The occurrence of a myocardial infarction when phlebotomy was discontinued in one patient, and the cessation of miscarriages followed by two successful full-term pregnancies after the introduction of low-dose aspirin in another patient suggest the beneficial effect of these treatments in people with *EPOR*-related congenital erythrocytosis. In this regard, as far as obstetric complications of congenital erythrocytosis are concerned, one could imagine an analogy with the antiphospholipid syndrome, even if the pathophysiology is very different. In the antiphospholipid syndrome, the clinical criteria used to make the diagnosis may be either vascular thrombosis, or pregnancy-related morbidity including (i) one or more unexplained deaths of a morphologically normal fetus at or beyond 10 weeks of gestation, (ii) one or more premature births before 34 weeks of gestation, or (iii) three or more unexplained consecutive spontaneous abortions before 10 weeks of gestation.[8] Similarly, in our study, the recurrence

Table 1. Demographic data of New Caledonian patients with *EPOR* mutations.

Pt#	Place in family tree	Sex	Age in years	CV risk factor	Treatment	Thrombosis	Ferritin	Hemorrhage while on LDA	Effects of Ptm on symptoms
1	Family A I:2	F	82	NA	Ptm and LDA	No	NA	NA	NA
2	Family A II:2	M	45	No	Ptm	No	129 μg/L	NA	Asymptomatic/Ptm if Hct >47%
3	Family A II:3	F	57	No	Ptm and LDA	No	NA	No	Asymptomatic/Ptm if Hct >51% or HS
4	Family A II:5	F	43	High BP	Ptm and LDA	No	NA	No	Yes on headaches
5	Family A III:2	M	21	Obesity	Ptm and LDA	No	22 μg/L	No	Asymptomatic/Ptm if Hct >55% or HS
6	Family B II:2	M	82	High BP	Ptm and DOAC	No	132.7 μg/L	NA	Asymptomatic/Ptm if Hct >51% or HS
7	Family B III:4	F	58	High BP	Ptm and LDA	No	43 μg/L	No	Yes on headaches
8	Family B III:7	F	52	No	Ptm and LDA	No	13 μg/L	No	Yes on headaches
9	Family B III:11	F	61	High BP	Ptm and LDA	No	NA	No	Yes on tinnitus
10	Family B IV:4	M	32	Obesity	Ptm and LDA	No	NA	No	Yes on headaches, dizziness, ophthalmic flashes
11	Family B IV:6	F	29	Obesity	No	Yes	NA	NA	No Ptm
12	Family C II:1	F	64	High BP, obesity	Ptm and LDA	No	NA	No	Asymptomatic/Ptm if Hct >55% or HS
13	Family C II:4	F	60	High BP, obesity	Ptm and DOAC	No	41.4 μg/L	NA	Asymptomatic/Ptm if Hct >52% or HS
14	Family C III:1	M	40	Obesity	Ptm and LDA	No	NA	No	Yes on headaches
15	Family C III:6	M	15	NA	No (child)	No	NA	NA	NA
16	Family C III:7	F	24	No	Ptm and LDA	No	31.6 μg/L	No	Yes on headaches
17	Family D II:3	M	59	High BP, smoking, obesity, diabetes	Ptm and LDA	No	NA	NA	NA
18	Family D II:5	F	50	High BP	No	Yes	52.3 μg/L	NA	No Ptm
19	Family D II:8	M	54	Smoking	No	Yes	25 μg/L	NA	Ptm 2 per month (Asymptomatic, when Hct >51%), and LDA
20	Family D III:1	M	36	Smoking	Ptm and LDA	No	NA	No	Asymptomatic/Ptm if Hct >54% or HS
21	Family D III:2	M	33	No	Ptm and LDA	No	NA	No	Asymptomatic/Ptm if Hct >54% or HS
22	Family D III:5	F	35	Obesity	Ptm and LDA	No	NA	No	Yes on headaches and dizziness
23	Family D III:7	F	36	Smoking	Ptm and LDA	No	NA	No	Yes on headaches
24	Family D IV:4	M	14	NA	No (child)	No	NA	NA	NA
25	Family D IV:5	F	<1	NA	No (child)	No	NA	NA	NA
26	Family D IV:7	F	10	NA	No (child)	No	NA	NA	NA

Pt: patient number; M: male; F: female; CV: cardiovascular; Ptm: phlebotomy; BP: blood pressure; LDA: low-dose aspirin; DOAC: direct oral anticoagulant; Hct: hematocrit; NA: not available; HS: hyperviscosity symptoms.

of three miscarriages in the young woman with the *EPOR* mutation was considered to be a vascular event.

Interestingly, in a series of pregnant women with congenital erythrocytosis (including those with *EPAS1*, *EGLN1* and *EPO* mutations) we previously found a possible benefit of aspirin or heparin associated with phlebotomy in the management of these high-risk pregnancies, thus strengthening the appeal of this therapeutic approach in pregnant patients with congenital erythrocytosis.[9]

In fact, the discovery of an *EPOR* mutation in a three-time Olympic ski champion could initially have given rise to optimism. Indeed, in the initial report, life span was considered to be unaffected by this mutation.[2] However, severe vascular events were reported in the original proband, who suffered several cardiovascular events and died in his 50s of a stroke, and his affected son had a myocardial infarction at the age of 40, highlighting the potential serious thrombotic complications associated with *EPOR* mutations.[10] Surprisingly, to date, no study has been carried out to investigate possible complications, particularly vascular ones, in patients with an *EPOR* mutation: at most, some case reports described possible complications, but these reports involved very few patients, and data on treatment were not always provided. To the best of our knowledge, 130 cases had been reported in the literature so far, 25 with information on the presence or absence of thrombotic events, and 18 with information on treatment, mainly phlebotomy but without a clear benefit on thrombosis.[5-7] Most of the reported cases had not been treated using low-dose aspirin, mainly because *EPOR*-related polycythemia was considered benign, as opposed to polycythemia vera, for which low-dose aspirin has long been demonstrated to lower the risk of thrombosis. Only two cases have been reported to have been treated with a combination of phlebotomy and aspirin, with no thrombosis observed[6] (*Online Supplementary Table S1*).

The benefit of phlebotomy in congenital erythrocytosis is debated, particularly because it is ineffective, or even harmful, in Chuvash polycythemia: in a large cohort of such patients, a history of previous phlebotomy was associated with a higher risk of thrombosis.[11,12] Similarly, a high rate of thrombosis was reported in a large family with congenital erythrocytosis due to the *EPAS1* (c.1603A>G, p.M535V) mutation, without any obvious benefit of phlebotomy, raising the question of whether phlebotomy is an effective treatment. It was not mentioned whether these patients were also taking low-dose aspirin. On the other hand, we recently reported two large series of *EPAS1/HIF2*- and *EGLN1/PHD2*-mutated patients with congenital erythrocytosis who had a low rate of thrombotic complications,[13,14] suggesting that the mechanisms of thrombosis in secondary congenital erythrocytosis are complex.

Remarkably, there are very few international recommendations on the management of congenital erythrocytosis. The British Society of Haematology recently suggested the use of phlebotomy in combination with low-dose aspirin (by analogy with the recommendations for polycythemia vera), with a threshold hematocrit of 52% being proposed.[15] In fact, the hematocrit threshold should be guided by the relief of hyperviscosity symptoms. This is the case of erythrocytosis related to high-affinity hemoglobins, for which phlebotomy to relieve hyperviscosity symptoms, associated or not with low-dose aspirin, has been suggested. In the absence of randomized trials, partly due to the very rare nature of *EPOR*-related polycythemia, solid therapeutic evidence is difficult to obtain. Our study, although limited by the size of the cohort and its retrospective nature, has the advantage of focusing on a homogeneous population carrying the same germline mutation, whose management was centralized, limiting recruitment or management biases.

Our results shed new light on the management of primary congenital erythrocytosis related to *EPOR* mutations and suggest that the combination of phlebotomy plus low-dose aspirin has a possible benefit in terms of preventing vascular events, thus providing an additional argument in line with recent recommendations.

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Contributions

MR, MG, FD, and M-AG recruited patients. NM, BA, and BG performed genetic analyses, FG, LB, and BG wrote the manuscript and designed the study. FG directed the study. All authors contributed to the research and approved the final manuscript.

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Data-sharing statement

Data and detailed information related to the study are available from the corresponding author upon request.

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