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SHORT REPORT

Heterozygosity for bisphosphoglycerate mutase deficiency expressing clinically as congenital erythrocytosis: A case series and literature review

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Summary

Erythrocytosis is associated with increased red blood cell mass and can be either congenital or acquired. Congenital secondary causes are rare and include germline variants increasing haemoglobin (Hb)-oxygen affinity (e.g., Hb or bisphosphoglycerate mutase (*BPGM*) variants) or affecting oxygen-sensing pathway proteins. Here, we describe five adults from three kindreds with erythrocytosis associated with heterozygosity for *BPGM* variants, including one novel. Functional analyses showed partial BPGM deficiency, reduced 2,3-bisphosphoglycerate levels and/or increased Hb-oxygen affinity. We also review currently known *BPGM* variants. This study contributes to raising awareness of *BPGM* variants, and in particular that heterozygosity for BPGM deficiency may already manifest clinically.

INTRODUCTION

Erythrocytosis is characterised by an increased mass of red blood cells (RBCs), reflected by elevated haemoglobin (Hb) and/or haematocrit (Hct) levels. Primary erythrocytosis include intrinsic defects of erythroid progenitors and is associated with a subnormal or undetectable serum erythropoietin (EPO) level. In contrast, secondary erythropoiesis is caused by defects extrinsic to RBCs, and is often associated with inappropriate normal or elevated EPO levels. Both forms can be congenital and acquired. Diagnosing the most common cause of primary erythrocytosis, polycythaemia vera, a myeloproliferative neoplasm due to clonal proliferation of RBC precursors, is greatly simplified by the detection of variants in Janus kinase 2 (*JAK2*; Mendelian Inheritance in Man [MIM] #147796). In the absence of *JAK2* variants, underlying causes of secondary erythrocytosis should be evaluated. Congenital forms of secondary erythrocytosis are rare and include germline variants in the genes encoding oxygen-sensing pathway proteins: von Hippel Lindau (*VHL*; MIM #608537), hypoxia-inducible factor (HIF)-2α (*EPAS1*; MIM #603349), HIF-prolyhydroxylase-2 (*EGLN1*; MIM #606425), and genes encoding Hb (*HBB*, MIM #141900; *HBA1*, MIM #141800; *HBA2*, MIM #141850) and bisphosphoglycerate mutase (*BPGM*; MIM #613896).[1] Here, we describe five adults from three kindreds with erythrocytosis associated with heterozygosity for a variant in *BPGM*, including one novel. Functional analyses were performed to evaluate their pathogenic nature. We also review currently known *BPGM* variants. Our study expands the knowledge of *BPGM* variants in patients with erythrocytosis and the associated haematological, functional and clinical phenotype, and raises awareness that only heterozygosity for BPGM-deficiency may already clinically manifest itself.

The five cases described were referred to our tertiary care centre because of suspected congenital erythrocytosis. Acquired causes for secondary erythrocytosis, such as smoking, were excluded in all patients. Informed consent was obtained, and all procedures were conducted in agreement with the principles of the Declaration of Helsinki. DNA sequence analysis of relevant coding exons, including flanking splice-site consensus sequences, of eight genes most commonly involved in congenital erythrocytosis was performed [*EPOR* (NM_000121), *HBB* (NM_000518), *HBA1* (NM_000558), *HBA2* (NM_000517), *VHL* (NM_000551), *EPAS1* (NM_001430), *EGLN1* (NM_022051), *BPGM* (NM_001293085)]. The only detected variants in *BPGM* were evaluated using Ensembl, Genome Aggregation Database (gnomAD), Polymorphism Phenotyping version 2 (PolyPhen-2), Sorting Intolerant From Tolerant (SIFT) and Human Gene Mutation Database (HGMD®) and classified using the American College of Medical Genetics and Genomics (ACMG) criteria.[2]

Proband 1 concerned a *JAK2*-negative 65-year-old female patient from Belgium. She had lifelong high Hb levels and started with phlebotomy (target Hct <50%) and low-dose acetylsalicylic acid (ASA) upon an Hb increase to 188 g/l, associated with recurrent headaches and hypertension. Phlebotomy improved these symptoms. At referral, her Hb level was 168 g/l and the Hct level was 49% (Table 1). She had a 39-year-old son with a medical history of retinal vein occlusion and lifelong erythrocytosis. Their EPO levels were normal. Both were heterozygous for a novel missense variant in *BPGM*: c.535C > T p.(Arg179Cys). The population frequency for this variant is very low (3.98^{-5} in gnomAD version 2.1.1, variant identification [ID] 7-134 346 794-C-T), which corresponds with PM2_moderate according to the ACMG 2015 criteria.[2] The mild clinical phenotype may explain the small number of individuals in the control population who carry this variant. The variant segregates in two affected family members (PP1_supporting) and in silico analysis of structural data shows a possible effect of the amino acid change to the stability of the α-helix, though further studies need to be performed (PP3_supporting). Finally, functional analyses support a loss of function or a hypomorphic function of BPGM (see below; PP4_strong). Therefore, although we should be cautious about interpreting the significance of variants in rare diseases, based on the current observations, we would classify the c.535C > T p.(Arg179Cys) variant as likely pathogenic.

Proband 2 was a 56-year-old, *JAK2*-negative Dutch man with a medical history of nephrolithiasis. He had asymptomatic erythrocytosis, discovered by chance in 2006 because of non-specific abdominal complaints. He underwent phlebotomy once every 1–4 months (target Hct <55%), and low-dose ASA was started. The patient moved and the current haematologist prompted further investigations into the increased Hb levels (Table 1). There was no splenomegaly, and no pulmonary or kidney abnormalities on imaging studies (chest X-ray and abdominal ultrasonography) and function tests. Phlebotomy was temporarily halted but initiated again when Hct exceeded 60% (target Hct <60%). He remained asymptomatic during phlebotomies. His 60-year-old brother also had a diagnosis of *JAK2*-negative erythrocytosis with mild clinical symptoms (fatigue, headache) from the age of 29 years. After a normal bone marrow biopsy in 2001, this brother underwent phlebotomy (target Hct <55%) once every 2 months until 2019, and he started on low-dose ASA. Phlebotomy slightly improved his clinical symptoms. In both patients, EPO levels were increased. DNA sequence analysis of proband 2 and his brother revealed heterozygosity for a missense variant in *BPGM*: c.269G > A p.(Arg90His). The allele frequency in the total population is 1.06^{-5} (gnomAD version 2.1.1, variant ID 7-134 346 528-G-A). Their sister, who did not have erythrocytosis, did not show this variant.

Proband 3 was a 32-year-old French male patient who presented with headache and tinnitus for 3 years. He had lifelong high Hb levels and low EPO at referral (Table 1). He was *JAK2*-negative and bone marrow biopsy was normal. He was treated with weekly phlebotomies (target Hct <50%). However, phlebotomies were poorly tolerated due to severe fatigue. However, they did improve the hyperviscosity-related symptoms at that time. Proband 3 was also heterozygous for the c.269G > A p.(Arg90His) missense variant in

TABLE 1 Clinical and functional laboratory test results of the families with congenital erythrocytosis

	Family 1		Family 2			Family 3	
	Proband 1 (F)	**Son**	**Proband 2 (M)**	**Brother**	**Sister**	**Proband 3 (M)**	
Heterozygous BPGM variant Variable	c.535C>T p.(Arg179Cys)	c.535C>T p.(Arg179Cys)	c.269G>A p.(Arg90His)	c.269G>A p.(Arg90His)	None	c.269G>A p.(Arg90His)	Normal range
Hb, g/l	168	200	182	163	156	193	M: 139–172 F: 119–155
Hct, %	49	57	59	51	48	57	M: 41–50 F: 36–46
RBC, $\times 10^{12}$/l	5.5	6.5	6.7	6.0	4.9	6.3	M: 4.2–5.5 F: 3.7–5.0
MCV, fl	90	88	88	86	96	91	80–97
MCH, fmol	1.90	1.92	1.69	1.69	1.96	1.89	1.75–2.25
MCHC, g/l	340	350	309	317	329	337	306–371
ARC, $\times 10^{9}$/l	208	221	110	77	134	64	25–120
WBC, $\times 10^{9}$/l	9.9	9.1	9.3	7.7	9.5	3.8	4.0–10.0
Platelets, $\times 10^{9}$/l	259	220	189	205	331	201	150–450
EPO, u/l	7	7	46[a,b]	56[b]	NA	2	3–32 4–20[a]
BPGM activity, u/g Hb	5.7	5.5	4.0	4.5	5.8	3.8	5.4–8.3
2,3-BPG, µmol/g Hb	25.6	21.7	11.8	14.7	23.1	14.4	28.8 ± 1.7[c]
P_{50}, mmHg	20.0	20.1	18.9	19.1	22.8	18.5	24.3 ± 1.4[c]

Abbreviations: 2,3-BPG, 2,3-bisphosphoglycerate; ARC, absolute reticulocyte count; BPGM, bisphosphoglycerate mutase; EPO, erythropoietin; F, female; Hb, haemoglobin; Hct, haematocrit; M, male; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NA, not available; P_{50}, oxygen pressure at an oxygen saturation of 50% during deoxygenation; RBC, red blood cells; WBC, white blood cells.

[a]Normal ranges of EPO level differ based on local laboratory reference ranges.

[b]EPO levels of Family 2 were based on prior measurements at the referring hospital.

[c]Local laboratory normal ranges of 2,3-BPG and P_{50} are presented as mean ± standard deviation based on healthy control samples simultaneously analysed for quality control of the assay ($n = 6$; in duplo for P_{50}).

TABLE 2 Reported variants of the human *BPGM* gene associated with erythrocytosis

				Haematological and functional phenotype							Clinical phenotype
Reference	*BPGM* variant	Allele frequency[a]	No. of subjects (Inheritance)	Hb, g/l	Hct, %	RBC, $\times 10^{12}$/l	EPO, u/l	BPGM activity, u/g Hb	2,3-BPG, µmol/g Hb	P_{50}, mmHg	Clinical data
Normal range[b]	NA	NA	NA	M:139–172 F:119–155	M:41–50 F:36–46	M:4.2–5.5 F:3.7–5.0	3–32	NA[b]	NA[b]	24.3 ± 1.4	
Oliveira et al. (2018)[9]	c.127A > C p.(Lys43Gln)[c]	NA	2 (Heterozygote)	143–183 (normal: 120–160)	NA	NA	NA	NA	NA	NA	Asymptomatic patients, relationship unknown
Oliveira et al. (2018)[9]	c.184C > T p.(Arg62Trp)	3.98^{-6}	1 (Heterozygote)	200 (normal: 120–160)	NA	NA	21.6 (normal: 2.6–18.5)	NA	NA	31 (normal: 24–30)	Patient with syncope, headaches, and fatigue responsive to phlebotomy and ASA therapy
Hoyer et al. (2004)[10]	c.185G > A p.(Arg62Gln)	1.06^{-5}	1 (Homozygote);	192	58.9	6.22	8 (normal: 4–24)	0.16 (~3% of normal)	0.3 (~2% of normal)	19 (normal: 25–30)	Iranian Jewish (Meshadi), consanguine family: 28-year-old man with plethora
	c.185G > A p.(Arg62Gln)		3 (Heterozygote)	134–164	40.5–46.1	4.56–5.72	NA	2.52–4.79 (50%–100% of normal)	8.7–14.4 (50%–100% of normal)	27–28 (normal: 25–30)	The two asymptomatic parents and male sibling of the family mentioned above
Lazana et al. (2021)[11]	c.260T > C p.(Leu87Pro)	NA	1 (Homozygote, uniparental disomy);	165–184	52–55	NA	9.7	NA	NA	18 (normal: 27–33)	60-year-old female who became lethargic, breathless on exertion and experienced headaches during regular phlebotomy therapy
	c.260T > C p.(Leu87Pro)		1 (Heterozygote)	NA	NA	NA	NA	NA	NA	NA	Her son with a normal haematological and clinical phenotype
Rosa et al. (1978)[12]; Galacteros et al. (1984)[13]; Lemarchandel et al. (1992)[14]	c.268C > T p.(Arg90Cys) and c.61delC p.(Arg21Valfs*28)	7.97^{-6} NA	4 (Compound heterozygote);	168–190	52–60	5.0–5.9	NA	Undetectable	0.3–0.4 (~3% of normal)	17.3–22	French family: 42-year-old man with headaches and ruddy (red) complexion who died of brain cancer, and three sisters with the same phenotype. Their parents died of vascular events aged 75 and 77 years
	c.268C > T p.(Arg90Cys)	7.97^{-6}	3 (Heterozygote)	143–180	43–56	4.9–5.6	NA	2.33–2.53 (40%–50% of normal)	9.2–11.3 (~60% of normal)	19.5–24	Three offspring (one woman and two men) of the family mentioned above with intermediate phenotype
Petousi et al. (2014)[3];	c.269G > A p.(Arg90His)	1.06^{-5}	2 (Heterozygote);	155–193 (normal: M: 130–180; F: 115–155)	58.6[d]	5.2–6.5 (normal: M: 4.5–6.5; F: 3.9–5.6)	7.5–15.9 (normal: 2.5–10.5)	3.27–3.62 (65%–85% of normal)	11.3–14.5 (40%–80% of normal)	23.9[e] (normal 27–33)	Three unrelated Caucasian families: – 27-year-old man with fatigue and his asymptomatic mother
This study	c.269G > A p.(Arg90His)		2 (Heterozygote);	163–182	51–59	6.0–6.7	46[b] – 56 ([b]normal: 4–20)	4.0–4.5 (55%–70% of normal)	11.8–14.7 (40%–50% of normal)	18.9–19.1	– 56-year-old and 60-year-old asymptomatic male siblings

TABLE 2 (Continued)

Reference	*BPGM* variant	Allele frequency[a]	No. of subjects (Inheritance)	Haematological and functional phenotype							Clinical phenotype
				Hb, g/l	Hct, %	RBC, $\times 10^{12}$/l	EPO, u/l	BPGM activity, u/g Hb	2,3-BPG, µmol/g Hb	P_{50}, mmHg	Clinical data
	c.269G>A p.(Arg90His)		1 (Heterozygote)	193	57	6.3	2	3.8 (55% of normal)	14.4 (50% of normal)	18.5	– 32-year-old man with headaches and tinnitus
Camps et al. (2016)[15]	c.304C>A p.(Gln102Lys)	NA	1 (Heterozygote)	186 (normal: 130–180)	52.5 (normal: 45–52)	NA	Normal	NA	NA	NA	A 52-year-old man with a medical history of myocardial infarction
Oliveira et al. (2018)[9]	c.344G>A p.(Trp115*)	NA	1 (Homozygote)	193 (normal: 120–160)	NA	NA	10 (normal: 2.6–18.5)	NA	NA	27 (normal: 24–30)	Asymptomatic patient with thrombocytopenia
Oliveira et al. (2018)[9]	c.506G>A p.(Trp169*)	NA	1 (Heterozygote)	155 (normal: 120–160)	NA	NA	5.7–19.2 (normal: 2.6–18.5)	NA	NA	29 (normal: 24–30)	Asymptomatic patient
Present study	c.535C>T p.(Arg179Cys)	3.98^{-5}	2 (Heterozygote)	168–200	49–57	5.5–6.5	7	5.5–5.7 (80%–90% of normal)	21.7–25.6 (75%–90% of normal)	20.0–20.1	A 65-year-old woman with headaches with phlebotomy and ASA therapy, and her 39-year-old son with a medical history of retinal vein occlusion
Oliveira et al. (2018)[9]	c.-409_-398del12[c]	NA	1 (Heterozygote)	NA	NA	NA	NA	NA	NA	NA	Besides erythrocytosis: unknown
Oliveira et al. (2018)[9]	c.-403C>T[c]	NA	1 (Heterozygote)	167 (normal: 120–160)	NA	NA	3.4 (normal: 2.6–18.5)	NA	NA	23 (normal: 24–30)	Positive family history and symptoms, not otherwise specified, were reported
Oliveira et al. (2018)[9]	c.-382-35G>C[c]	NA	3 (Heterozygote)	185 (normal: 120–160)	NA	NA	13.4 (normal: 2.6–18.5)	NA	NA	19 (normal: 24–30)	Positive family history, fatigue and splenomegaly were reported by at least one person with the variant

Abbreviations: ASA, acetylsalicylic acid; 2,3-BPG, 2,3-bisphosphoglycerate; BPGM, bisphosphoglycerate mutase; EPO, erythropoietin; F, female; Hb, haemoglobin; Hct, haematocrit; M, male; NA, not available; P_{50}, oxygen pressure at an oxygen saturation of 50% during deoxygenation; RBC, red blood cells.

[a]Allele frequency data of the total population are based on the gnomAD version2.1.1.

[b]Normal ranges based on our local laboratory reference range; if other normal ranges were mentioned in the referred paper, these were reported separately in the table. For BPGM activity and 2,3-BPG levels, data were normalised to 100% for healthy control samples.

[c]Classified as variants of unknown significance.

[d]Hct only reported of the proband.

[e]Calculated P_{50} value based on arterial blood gas analysis reported instead of a p50 value based on automatic measurement of the oxygen dissociation curve.

BPGM. To our knowledge, the c.269G > A p.(Arg90His) variant was only reported once before (HGMD CM149154). That case was also heterozygous.[3]

We subsequently performed functional analyses to assess the impact of the identified variants. BPGM modulates the synthesis of 2,3-BPG through the Rapoport–Luebering shunt of the glycolytic pathway in RBCs (Supplementary Figure S1). The 2,3-BPG modulates oxygen release by binding to deoxyhaemoglobin, thereby reducing Hb-oxygen affinity.[4] Thus, reduced BPGM activity would result in decreased 2,3-BPG levels, ultimately reducing oxygen release in tissues. Consequently, hypoxia-induced EPO production stimulates erythrocytosis to compensate for decreased tissue oxygenation.

The BPGM activity measurements were performed as described by Beutler.[5] Quantitative analysis of 2,3-BPG was based on previously described methods using liquid chromatography tandem mass spectrometry after snap freezing whole blood collected in EDTA tubes in liquid nitrogen within 1–2 h after collection.[6] Cryovials were stored in −80°C until analysing. Oxygen equilibrium curves were obtained from whole blood samples collected in heparin tubes using a Hemox Analyser (TCS Scientific Corp).[7] The P_{50}, the oxygen tension when Hb is 50% saturated with oxygen, was calculated using TCS Hemox OEC program software (v2.00.14).

The results are summarised in Table 1. BPGM activity was decreased in all cases heterozygous for the c.269G > A p.(Arg90His) variant (proband 2, his brother, and proband 3). BPGM activity was low–normal in the two cases with the c.535C > T p.(Arg179Cys) variant. In all individuals, 2,3-BPG levels were reduced and most pronounced in the ones with the lowest BPGM enzymatic activity. In addition, decreased P_{50} levels were measured in all affected individuals, indicating a left-shift of the oxygen equilibrium curve and, hence, increased Hb-oxygen affinity. EPO levels were only increased in proband 2 and his brother, possibly due to their phlebotomies. EPO levels were (low–)normal in the other cases.

Variants in the *BPGM* gene are very rare. For example, no *BPGM* variants were identified when sequencing 70 cases with idiopathic erythrocytosis and elevated EPO.[8] To the best of our knowledge, only 12 other variants (15 families) associated with erythrocytosis have been described to date (Table 2).[3,9–15] The first family with complete BPGM deficiency was reported in 1978 by Rosa et al.[12] Four family members were compound heterozygous for the c.268C > T (p.Arg90Cys) and the c.61delC (p.Arg21Valfs*28) variants.[14] Since then, only a limited number of other variants in *BPGM* have been described and characterised. Most of them concerned missense and nonsense variants. Interestingly, arginine at either codon 62 or codon 90 is the most frequently mutated residue (Arg62Trp, Arg62Gln, Arg90Cys, Arg90His). Both these catalytic site residues are involved in substrate binding, with Arg62 located at the bottom of the active site pocket, whereas Arg90 plays a key role in stabilising and functioning of BPGM.[16] We report here the second and third family with an arginine–histidine substitution at residue 90 of *BPGM*. In 2014, Petousi et al.[3] first identified this variant by whole-genome sequencing in a 27-year-old man and his mother. Similar to our cases, they had decreased levels of 2,3-BPG and BPGM activity. The reported P_{50} was decreased, but this was calculated from arterial blood gas analysis and not actually measured with the Hemox Analyser as in the present study.[7] The precise structural consequences of the novel p.(Arg179Cys) variant require further investigation. Arg179 constitutes the last residue of α-helix 7 (residues 158–179) and terminal arginines are considered important for helix stability.[17] In addition, the possible interaction between the side chain of Arg179 and the sidechain of Glu176 may be important. Disruption of this interaction by the Arg179Cys mutation could disrupt helix formation, leading to alteration of the downstream substrate binding site within the catalytic domain of *BPGM*.

Historically, BPGM deficiency is considered an autosomal recessive disorder (https://omim.org/entry/613896). However, from the growing number of cases of heterozygous *BPGM* variants associated with erythrocytosis, an autosomal dominant inheritance pattern with variable penetrance or expression also appears to emerge (Table 2). In several but not all heterozygous cases, functional effects including higher Hb-oxygen affinity were found, sometimes more pronounced than effects on Hb and Hct levels. On the other hand, some homozygous patients remained asymptomatic despite extremely low BPGM activity. This shows that the pathophysiology of congenital erythrocytosis is complex and that description of cases like the ones presented here may contribute to a better understanding of this particular rare cause of erythrocytosis. Ultimately, this may answer the question whether an intervention would be necessary and effective in all cases, keeping the risk of thrombosis versus tissue hypoxia in mind.

In conclusion, two rare heterozygous variants in the *BPGM* gene, a novel c.535C > T p.(Arg179Cys) and the previously once reported 269G > A p.(Arg90His) variant were found to be associated with secondary congenital erythrocytosis. Of these, the latter variant had a more severe functional effect, but not directly a more severe clinical effect. Our case series also clearly illustrates that EPO levels are not reliable to identify patients with *BPGM* variants. Awareness of cases with *BPGM* variants could help to provide more insight towards an earlier diagnosis, the inheritance pattern, genotype–phenotype relationship, and management of patients with *BPGM* variants.

AUTHOR CONTRIBUTIONS

Myrthe J. van Dijk co-ordinated the project, collected data, performed experiments, performed analyses, and wrote the manuscript; Brigitte A. van Oirschot and Judith J. M. Jans performed experiments and analyses; Manon C. Stam-Slob, Esmé Waanders, Bert van der Zwaag, Eduard J. van Beers and François Girodon collected data and performed analyses; Peter Willem van der Linden, Jose M. Torregrosa Diaz, François Girodon, Rik Schots, and Noortje Thielen provided patient samples and patient data; Eduard J. van Beers reviewed the

manuscript; Richard van Wijk co-ordinated the project, supervised the project and reviewed the manuscript.

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CONFLICT OF INTEREST

No relevant conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

Original data and protocols can be obtained by contacting r.vanwijk@umcutrecht.nl. Individual participant data will not be shared.

PATIENT CONSENT STATEMENT

Verbal consent was obtained.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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