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Case Report

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"The Long Journey of Unexplained Erythrocytosis": Erythrocytosis due to High-Oxygen Affinity Hemoglobinopathy – Hemoglobin Variant Little Rock (HBB: c.432C>A) – A Report of a Swiss Family and Review of the Literature

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Erythrocytosis · Polycythemia · High-oxygen affinity hemoglobinopathies · Next-generation sequencing · Hb Little Rock

Abstract
The differential diagnosis of erythrocytosis is complex, involving a tailored algorithm. Congenital causes are rare and such patients commonly face a long journey looking for diagnosis. This diagnosis requires expertise and accessibility to modern diagnostic tools. We present the case of a young Swiss man with long-standing erythrocytosis of unknown origin and his family. The patient had an episode of malaise as he went skiing above 2,000 m altitude. In the blood gas analysis, p50 was low (16 mm Hg) and erythropoietin was normal. Using next-generation sequencing, a mutation in the hemoglobin subunit beta gene was found, a pathogenic variant known as hemoglobin Little Rock causing high oxygen affinity. Some family members also had unexplained erythrocytosis, therefore the mutational status of the family was analyzed, the grandmother and mother showed the presence of the same mutation. The use of modern technology finally offered a diagnosis to this family.

Introduction

Erythrocytosis is an abnormal increase in hemoglobin (Hb) and/or hematocrit (Hct) values above the reference ranges considered physiological for a healthy person. For the diagnosis and management, it is important to understand the potentially different underlying mechanisms involved. Thus, there is erythrocytosis in which the red blood cell (RBC) production is independent of the mechanisms that normally regulate the erythropoiesis; they are considered as primary erythrocytosis and can be acquired or inherited. A classic example of an acquired form of primary erythrocytosis is polycythemia vera (PV). PV is a chronic myeloproliferative neoplasm that carries a somatic *JAK2* V617F mutation or another mutation functionally similar to *JAK2* [1]. Thanks to this

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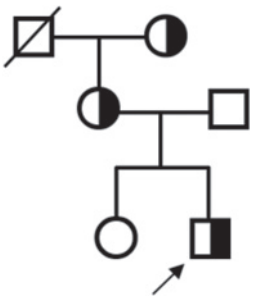

Proband is indicated by arrow. Hb Little Rock (mutation in *HBB* gene, Variant c. 432C>A p.(His144Gln)) is shown in black.

Fig. 1. Pedigree of the family.

molecular marker and clear diagnostic criteria, the diagnosis is straightforward [2]. In contrast, congenital primary erythrocytosis is rare and is caused by germline mutations of erythropoietin (EPO) receptor mutations [3, 4].

Secondary erythrocytosis can also be congenital or acquired. Acquired forms are most frequently seen in clinical practice. They occur as a consequence of excess serum EPO, either as physiological compensation for insufficient oxygen supply to peripheral tissues (lung conditions, sleep apnea, right-to left cardiac shunts, smoking, high altitude, renal artery stenosis, and polycystic kidney disease) or as autonomous production of EPO by tumors, or the use of anabolic drugs [5]. Congenital secondary erythrocytosis is rare and mostly caused by germline mutations including mutations in genes involved in the oxygen-sensing pathway and Hb with abnormal oxygen affinity among others [6, 7]. The World Health Organization (WHO) recommends clear diagnostic criteria for the diagnosis of PV: Hb >16.5 g/dL in men and Hb >16 g/dL in women, or Hct >49% in men and Hct >48% in women, or RBC mass >25% above predicted mean normal value [2]. These Hb and Hct values are conventionally applied in the evaluation of all forms of erythrocytosis. The diagnostic approach of erythrocytosis may be complex and quite challenging; a tailored algorithm is recommended. Before proceeding with any investigation, studying many relevant aspects like patient's medical history, careful analysis of prior blood values, patient's habits, toxic exposure, medication, comorbidities, and information about family history for erythrocytosis is recommended. Using this approach, most likely causes might be identified [5]. Ultimately, performing a *JAK2* molecular test, the diagnosis of PV will be assessed. Various diagnostic algorithms proposing the steps to follow in JAK2-negative erythrocytosis have been published [5, 6, 8]. We describe here a Swiss family in which congenital erythrocytosis was initially diagnosed in the youngest of its members. This patient has faced a long journey due to his erythrocytosis, until we finally diagnosed him with a high-oxygen affinity hemoglobinopathy due to a variant known as Little Rock Hb. As a result of his diagnosis, the investigation was extended to the entire family. In fact, excess of RBC has been described as common in his mother's family. Therefore, clinical history and laboratory data were collected, as well as molecular investigations performed on all family members (shown in Fig. 1; Table 1). Three generations were affected by the same mutation and phenotype.

Case Report

Proband

A healthy 33-year-old Swiss male patient was referred to the hematology clinic for further diagnostic evaluation of an isolated erythrocytosis described since 1999. The patient was asymptomatic until January 2021, when he began to experience some general symptoms as chest pain that motivated an emergency consultation. An echocardiography did not reveal any pathology. Occasionally, the patient experienced dizziness and headache. He associates these symptoms with situations in which he has had low fluid intake volume during the day. He also described an episode of malaise as he went skiing above 2,000 m altitude. He did not have any history of thrombosis and neither did anyone else in the family. Apart from a mild nicotine use (<5 pack-years), no other risk factors that contribute to the erythrocytosis could be identified. He denied ever having used anabolic substances. He also reported a family history of known excess erythrocytes in his mother and maternal grandmother. The physical examination was unremarkable.

The laboratory findings confirmed the erythrocytosis showing a Hb of 196 g/L and Hct 57%; the RBC indices and RBC morphology were normal (shown in Table 1). The white blood cells and the platelet count were normal. A molecular investigation was performed which showed that the patient did not carry any *JAK2*

Table 1. Summary of laboratory findings of the proband and family members

Parameters (units)	Reference range	Proband	Mother	Grandmother	Father	Sister
Hb, g/L	121–154 (f) 140–168 (m)	196	174	175	158	144
Hct, %	34–47 (f) 42–50 (m)	57	52	52	46	41
MCV, fL	80–98	87	89	92	88	84
MCHC, g/L	320–360	347	339	341	341	346
WBC, G/L	4.5–11	5.2	3.9	5.1	2.9	6.7
Platelets, G/L	150–450	193	205	175	172	224
Creatinine, µmol/L	44–97 (f) 66–115 (m)	73	59	101	79	80
Ferritin, µg/L	20–250	90	97	23	–	102
ALAT, U/L	10–40	21	26	22	110	10
EPO, mU/mL	4–26	19.5	–	–	–	–
P50a, mm Hg	24–28	16	–	–	–	–

f, female; m, male; MCV, mean corpuscular volume; MCHC, mean corpuscular hemoglobin concentration; ALAT, alanine aminotransferase; EPO, erythropoietin; P50a, oxygen tension at which Hb is 50% saturated; –, data not available.

mutation (neither V617 nor exon 12). In the blood gas analysis, p50 was low (16 mm Hg), methemoglobin 0.7% (normal), COHb 3% (normal), and EPO was within normal levels. The combination of an isolated erythrocytosis with low p50 and family history was highly suspicious for a congenital erythrocytosis, especially a high-oxygen affinity hemoglobinopathy. After obtaining the consent of the patient, a genetic analysis to investigate germinal line mutations was undertaken. For this investigation, we used an "in house" NGS panel for erythrocytosis; the genes contained in this panel were published elsewhere [9]. The molecular results showed a heterozygous mutation in the *HBB* gene (hemoglobin subunit beta gene), variant c. 432C>A p.(His144Gln), corresponding to the Hb variant Little Rock. After receiving the diagnosis, the patient felt relieved. His long journey facing the repeated questions concerning the use of anabolic substances or other habits represented an additional burden for him. Furthermore, clinical and genetic counseling was performed in reference to the diagnosis. We drew his attention to those aspects that can lead to a physiological increase of the number of erythrocytes and therefore may represent an additional risk for this type of patients, due to increased blood viscosity. For example, when going to the mountains (something common in Switzerland due to the country's geography) the patient needs to ensure a good hydration. Furthermore, as the patient experienced an episode of intense malaise as he was above 2,000 m altitude, we prescribed prophylactic aspirin in case of a longer stay (>5 days) in high altitude. The patient had had a number of symptoms that could be associated with hyperviscosity, but the relationship is not clear.

Family History and Analysis

Case 1: Mother

The mother of the patient is now 68 years old. The laboratory findings show an isolated erythrocytosis without any other abnormality (shown in Table 1). She is asymptomatic, only reporting some episode of dizziness as she was in her thirties. She has never suffered any thrombosis. The molecular results show the presence of the same heterozygous mutation in the *HBB* gene as our patient.

Case 2: Father

The laboratory findings of the father of our patient show that the Hb and Hct are in the normal range (shown in Table 1). As expected, the molecular results did not show the presence of the *HBB* gene mutation described in our proband.

Case 3: Grandmother

The laboratory findings of the maternal grandmother of our patient show an isolated erythrocytosis (shown in Table 1). This motivated a hospitalization in 1976 for further diagnostic workup. In the discharge report, the physician described general symptoms with fatigue, headaches, and chest pain when exercising. The physical examination was unremarkable. In the patient history, no findings pointed to a secondary etiology. Further diagnostic, namely, an extended blood analysis, head, thorax, and abdomen X-rays, intravenous urography, and an ECG, did not reveal any abnormality. A bone marrow biopsy performed in 1976 showed increased erythropoiesis with a left shift without any other abnormality. Ultimately, the erythrocytosis was labelled as of unknown origin. She did not receive any specific treatment. She is now 90 years old and has never suffered

any thrombosis. The molecular results show the presence of the same heterozygous mutation in the *HBB* gene as our proband.

Case 4: Sister

The sister of our patient is healthy and does not describe any symptoms. Her Hb and Hct are in the normal range (shown in Table 1). As expected, the molecular results did not show the presence of the *HBB* gene mutation described in our proband.

Discussion

A rare cause of secondary congenital erythrocytosis is high-oxygen affinity hemoglobinopathy. A high oxygen affinity results in lower oxygen delivery in peripheral tissues, including the renal oxygen-sensing mechanism. This triggers a physiological compensation resulting in absolute or relative increase in EPO leading to an activation of erythropoiesis. The measurement of low p50 in the blood gas analysis reflects the high affinity of the Hb for oxygen. The first published case of a Hb variant associated with erythrocytosis was described in 1966 and designated Hb Chesapeake [10]. Since then, more than 200 high-oxygen affinity Hb variants have been identified [9]. High-oxygen affinity hemoglobinopathy is a not an easy diagnosis to make. However, in the presence of isolated erythrocytosis, EPO in normal range, low p50, and family history, high-oxygen affinity hemoglobinopathy should be suspected [9]. In some cases, the abnormal Hb variant can be detected with Hb electrophoresis. However, some abnormal Hb may be silent in the electrophoresis due to Hb instability and therefore undetectable with this method. Due to this restriction and the rapidly evolving use of molecular analysis, the diagnosis nowadays is mostly done using molecular genetic analysis [11]. The use of NGS allows for the rapid and cost-effective examination of multiple gene mutations known as underlying causes of the clinical picture. The NGS panel now in use at the Hematologic Molecular Biology Laboratory at the university hospital Bern analyzes 23 genes for mutations known to be responsible for erythrocytosis (*BHLHE41, BPGM, EGLN1, EGLN2, EGLN3, EPAS1, EPO, EPOR, GFI1B, HBA1, HBA2, HBB, HIF1A, HIF1A(10)N, HIF3A, JAK2, KDM6A, MPL, OS9, SH2B3* [somatic], *SLC30A10, VHL, ZNF197*). The gene variants are then classified according to the American College of Medical Genetics (ACMG) guidelines 2015 [12]. Only variants which are classified as pathogenic, likely pathogenic, or variants of unknown significance are reported. The reported variant was confirmed by Sanger sequencing. In Switzerland, before undertaking this type of investigations, it is necessary to obtain the approval of the patient's health insurance to cover the costs, as well as the informed consent of the patient. The cost coverage policies for this analysis are specific to each country. Using NGS, we detected a mutation in the *HBB* gene corresponding to a rare Hb variant, known as Hb Little Rock. This Hb variant was first described in 1971 [13]. The amino acid substitution of Hb Little Rock is defined as β143 (H21) His → Gln, a site which is important for binding of 2,3-diphosphoglycerate [14]. This variant causes an increased O_2 affinity leading to high-oxygen affinity hemoglobinopathy. This mutation has an autosomal-dominant inheritance [12]. Since 1973, no case report regarding this specific Hb variant has been published. Data of patients presenting with high-oxygen affinity Hb variants are sparse so that such case reports are critical to gain clinical information to choose the best supportive measures and better define the prognosis. Furthermore, in the diagnosis of this type of hemoglobinopathy, there are aspects of cost efficiency to consider. The rapid development of genetic analysis in the last decade allows us nowadays to find mutations resulting in congenital erythrocytosis in a cost-efficient way [11]. Regarding the clinical course of such patients, most are asymptomatic. However, symptoms associated with hyperviscosity such as headaches, vertigo, tinnitus, and paresthesia in the extremities can occur. The laboratory and clinical findings of the three affected members of the presented family are similar to other reported cases of high-oxygen affinity hemoglobinopathy (Hb Linköping, Hb Saint Nazaire) [15, 16]. If patients are symptomatic, a trial of therapeutic phlebotomy is possible with subsequent reassessment of the symptoms. In most cases, the risk of thrombosis is not increased, which fits the clinical profile observed in the reported family. This case report depicts a clear benefit of using NGS to investigate erythrocytosis of unknown origin. The psychological burden from uncertain diagnosis, leading for this patient to repeated questions about the use of anabolic, should not be underestimated. The right diagnosis allows a better understanding of the clinical course, prognosis, and the use of appropriate supportive measures.

Statement of Ethics

Written informed consent was obtained from the patients for publication of the details of their medical case and any accompanying images. Ethical approval is not required for

this type of study in accordance with local and national guidelines.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Camille Perroud: conception of the work; acquisition, analysis, and interpretation of data for the work; drafting the work; and final approval of the version to be submitted. Naomi Porret and Alicia Rovó: analysis and interpretation of data for the work; revising it critically for important intellectual content; and final approval of the version to be submitted.

Data Availability Statement

All data generated or analyzed during the investigation are included in this article. Further inquiries can be directed to the corresponding author.

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