Received: 6 January 2024 | Accepted: 8 January 2024

DOI: 10.1002/jha2.857

eJHaem

British Society for Haematology

RESEARCH ARTICLE

Haemoglobin Ottawa, sickle cell trait and vaso-occlusive crises

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Abstract

A girl with a sickle cell trait had severe VOCs (vaso-occlusive crises), her father also had a sickle cell trait but mild VOCs, and her mother had no symptoms. Electrophoresis on agarose gel under alkaline conditions showed haemoglobin AS (HbAS) in the girl and in her father, with an S band increased more than expected (46.2% and 41.2% respectively), and a band migrating at C (16.8% and 8.9% respectively) in both. There was a band at S (19.6 %) in her mother. The C band was attributed to a hybrid tetramer with haemoglobin S (HbS) and a Hb variant. A homozygous c.46G>C mutation (Hb Ottawa, the Hb variant) was detected by Sanger sequencing in the girl. Heterozygosity for Hb Ottawa by Sanger sequencing was shown in both the father and the mother. The father, with HbAS and heterozygous for Hb Ottawa, had mild VOCs. Heterozygosity only for Hb Ottawa did not produce any abnormality in the mother. A sister and two brothers of the index patient presented a Hb variant, probably Hb Ottawa, migrating to the S zone (all 20%) at electrophoresis, without HbS. These last three were asymptomatic. We conclude that Hb Ottawa, an α-globin variant, contributes along with haemoglobin S (HbS) to VOC symptoms.

KEYWORDS
haemoglobin variants, sickle cell trait, vaso-occlusive crises

1 | INTRODUCTION

Haemoglobin (Hb) Ottawa, also known as Hb Siam, is due to GGT(Gly) > CGT(Arg) mutation in codon 15 of the *HBA1* or *HBA2* gene: c.46G > C [1–8]. If heterozygous, Hb Ottawa is an α variant without causing clinical or haematological manifestations. Besides haemoglobin S (HbS), other Hb variants likely result in vascular occlusion crises (VOCs) [9]. These non-S variants generally accompany the HbS mutation. The clinical manifestations are either absent or not specific but may be characteristic of VOCs. The diagnosis of the initiating deviations requires advanced laboratory techniques, including capillary electrophoresis (CE) and DNA sequencing.

Our report has described the case of a homozygous Hb Ottawa patient, who was additionally heterozygous for HbS (HbAS). She suffered from severe VOCs. Her father presented HbAS, but was heterozygous for Hb Ottawa, with mild VOCs, whereas her mother was heterozygous for Hb Ottawa and asymptomatic, with normal blood values. A band in S at electrophoresis indicated that the patient's sister and two brothers also exhibited signs of a variant, presumably Hb Ottawa. They had no symptoms and a normal blood count.

This is the first patient case featuring homozygosity for the α variant Hb Ottawa, alongside heterozygosity for HbS, with a history of painful VOCs.

2 | METHODS

The father, both spouses and the children of appropriate ages provided their written informed consent for the blood tests. Haemoglobin electrophoresis on agarose gel under alkaline conditions was conducted using the Hydragel (Sebia) kit, while CE was performed using the Minicap Flex Piercing instrument.

The following molecular techniques were utilized:

1. Sanger sequencing of *HBB* from 70,442 to 71,083 and from 71,660 to 72,305 within NG_000007.3, *HBA2* from 33,557 to 34,601 in NG_000006.1 and *HBA1* from 37,361 to 38,409 within NG_000006.1
2. MLPA kit from MRC Holland, specifically for *HBA*-P140-C1 and *HBB*-P102-D.

The employed methods allowed the screening of most α and β mutations described in HbVar, a database containing human Hb variants and thalassemias [10].

3 | PATIENT AND FAMILY

The final diagnosis was established when the patient reached the age of 15 years. She had been suffering from VOCs since early childhood, with pain in the arms, thighs, and legs. The VOCs occurred approximately 10 times/year and lasted for 10–14 days, rendering it impossible for her to attend school during the painful episodes. Paracetamol and non-steroidal anti-inflammatory drugs generally provide some relief. The VOCs were triggered by physical exercise, brisk ambient temperature changes, and fever. There was no chronic pain, and the girl never received any transfusion. The final diagnosis was made late in childhood because of her living in a remote village with difficult access to primary healthcare.

The Hb electrophoresis on agarose carried out in February 2021 showed HbAS with a band migrating in zone C (HbA 37.8%, HbS 46.2%, and HbX 16.8%, Figure 1 and Table 1). The patient was examined at the outpatient clinic in January 2022. Jaundice, pallor of mucosae, or hepatosplenomegaly were absent. Heart and lung examinations were normal, as were growth and maturation. Because of the VOC symptoms, 30 mg/kg/day hydroxycarbamide (HU) was prescribed [11]. CE was performed in March 2022. Besides heterozygosity for HbS, it identified the Hb variant (HbA 34.3%, HbF 4.9%, HbS+HbX 42.1%, HbA2+S/X hybrid 2.8%, S/X hybrid 14.7% and A2/X hybrid 1.2%, Figure 2). VOCs were less painful following the HU treatment initiation. Upon follow-up in May 2023, the patient reported less intense VOCs. Her laboratory data were as follows (Table 1): Hb 114 g/L (N: 110–160 g/L), haematocrit (Hct) 34.8% (N: 37%–47%), red blood cell count (RBC) 3.51×10^{12}/L (N: 3.5–5.5 $\times 10^{12}$/L), mean cell volume (MCV) 96 μm^3 (N: 80–100 μm^3), mean corpuscular Hb (MCH) 32.6 pg (N: 27–34 pg), mean corpuscular Hb concentration (MCHC) 329 g/L (N: 320–360 g/L), white blood cell count (WBC) 3.850×10^9/L (neutrophils 1.920×10^9/L, lymphocytes 1.650×10^9/L, monocytes 200×10^9/L, eosinophils 90×10^9/L), platelet count 146×10^9 /L (N: 100–300 $\times 10^9$/L), reticulocytes 137×10^9/L (N < 100×10^9/L), SpO_2 98%, total bilirubin 9.6 mg/L (N: 2–14 mg/L), direct bilirubin 2.6 mg/L (N: 0–20 mg/L) and indirect bilirubin 7 mg/L (N: 2–7 mg/L).

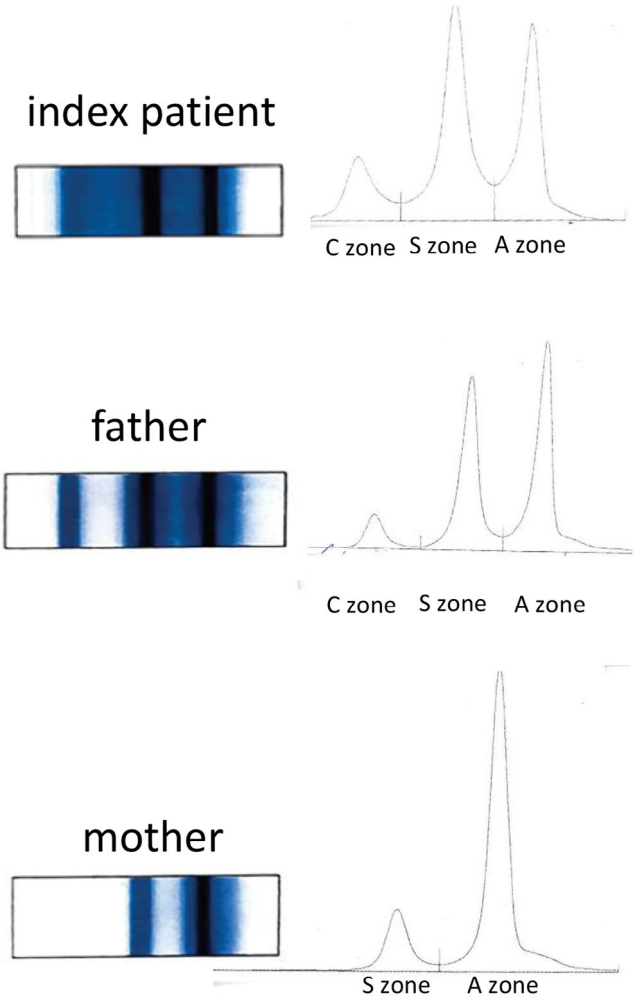

FIGURE 1 Haemoglobin electrophoreses on agarose at alkaline pH of the index patient, of her father and of her mother. The quantitative data are in Table 1.

Analysis of the β-globin cluster showed the heterozygous c.20A > T mutation in exon 1 of the *HBB* β-globin gene (HbS mutation), while the α-globin cluster analysis revealed a homozygous mutation c.46G > C in exon 1 of the α1-globin gene *HBA1* (Hb Ottawa). Therefore, HbX turns out to be Hb Ottawa.

The index patient's father was 46 years old. He was first consulted in 2019 for pain in his arms, thighs, and legs occurring upon intense physical activities, especially during hot weather. His clinical examination was normal, except for mild arterial hypertension. There was neither jaundice, pallor of mucosae, nor splenomegaly. His laboratory data were as follows (Table 1): Hb 147 g/L, Hct 44.7%, RBC 5.290×10^{12}/L, MCV 84.5 μm^3, MCH 27.7 pg, MCHC 328 g/L, WBC 4.280×10^9/L (neutrophils 2.450×10^9/L, lymphocytes 1.328×10^9/L, eosinophils 300×10^9/L, monocytes 210×10^9/L), and platelet count 222×10^9/L. Agarose gel Hb electrophoresis identified HbA 49.9%, HbS 41.2%, and

TABLE 1 Clinical data, haemoglobin electrophoreses on agarose, haemoglobin capillary electrophoreses, haematological values, gene sequencing analyses of the index patient, of her father and her mother.

	Index patient	Father	Mother
VOCs	**Severe**	**Mild**	**Absent**
Hb electrophoresis on agarose			
A zone (%)	37.8	49.9	80.4
S zone (%)	46.2	41.2	19.6
C zone (%)	16.8	8.9	
Capillary electrophoresis			
HbA (%)	34.3	46.8	78
HbF (%)	4.9	0.5	
HbS + HbX (%)	42.1	42.4	
HbA2 + S/X hybrid (%)	2.8		
S/X hybrid (%)	14.7	7.3	
HbA2/Hb X hybrid (%)	1.2	0.7	1.4
HbA2 (%)		2.3	1
HbX (%)			19
Haematological values			
Hb (g/L)	114	147	141
MCV (μm^3)	96	84.5	89.5
Molecular techniques			
α globin cluster sequencing	homozygous Hb Ottawa	heterozygous Hb Ottawa	heterozygous Hb Ottawa
β globin cluster sequencing	heterozygous HbS mutation	heterozygous HbS mutation	no HbS mutation

Abbreviations: Hb, haemoglobin (N: 110–160 g/L); MCV, mean cell volume (N: 80–100 μm^3); VOCs, vaso-occlusive crises.
HbA2, (N: 2%–3,3%).
HbF, (N: 0.2%–1%).
With sickle cell trait—HbAS, N: S = 35%–38%, A = 62%–65%.

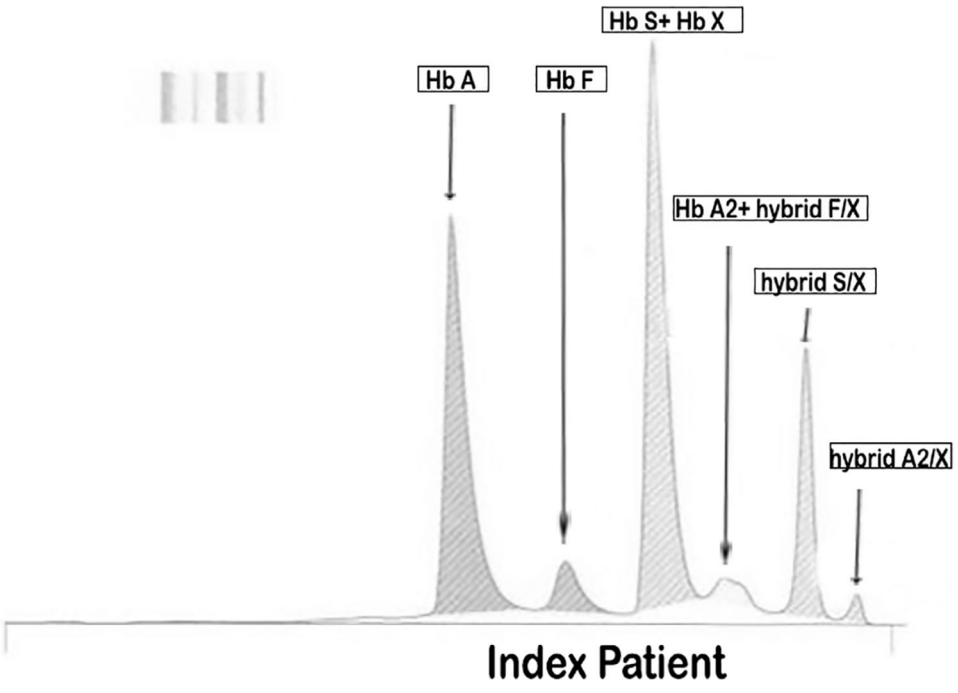

FIGURE 2 Haemoglobin capillary electrophoresis of the index patient.

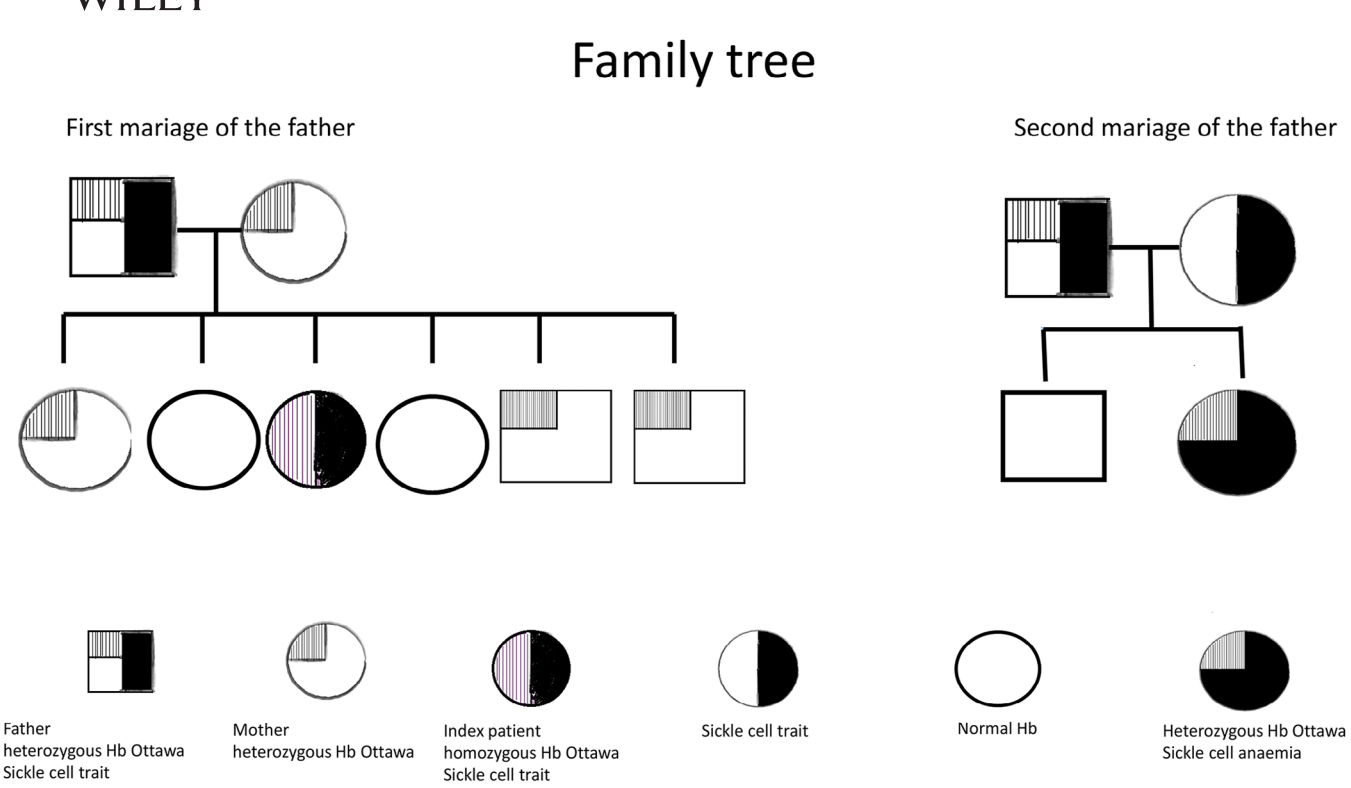

FIGURE 3 Family tree, squares are males, circles are females.

HbX 8.9% (Figure 1). March 2022 CE results displayed HbA 46.8%, HbF 0.5%, HbS+HbX 42.4%, HbA2 2.3%, S/X hybrid 7.3%, and A2/X hybrid 0.7%.

Sequencing of the β-globin gene highlighted the heterozygous mutation c.20A > T in exon 1 of the β-globin *HBB* gene (HbS), whereas that of the α-globin gene showed the heterozygous c.46G > C mutation in exon 1 of the α-globin *HBA1* gene (Hb Ottawa). Hence, we concluded HbX to be Hb Ottawa.

The mother was 39 years old, without any complaints. Moreover, her physical examination was normal. She had an Hb of 141 g/L and an MCV of 89.5 μm^3. Agarose electrophoresis results displayed HbA at 80.4% and HbX at 19.6% (Figure 1). March 2022 CE results identified HbA 78%, HbX 19.6%, HbA2 1% and X/A2 hybrid 1.4%. We concluded that a Hb variant migrated to the S-band.

Sequencing of the β-globin gene showed the heterozygous change c.316-70C > G in exon 3 of the β-globin gene *HBB*. This polymorphism is often associated with a δ variant. On the other hand, the α-globin gene sequencing revealed a heterozygous c.46G > C mutation in exon 1 of the α-globin gene *HBA1*. These results led us to conclude that HbX is Hb Ottawa.

The parents of the index patient had six children (Figure 3). Besides the index patient, a girl and two boys presented electrophoresis results displaying an HbX migrating to the S zone. The sickling test in whole blood was negative in the two boys. No HbS was detected on CE, and all three lived normal lives, without symptoms. In addition, their physical examinations were normal.

The father of the index patient contracted a second marriage (Figure 3). The present spouse of 30 years old carried HbAS and was healthy. At that time, the first child was 3 years old, with normal and uneventful growth. Furthermore, his Hb electrophoresis exhibited standard results. Nevertheless, the second child was admitted at 4 months of age with dactylitis, and her electrophoresis results showed a homozygous HbS. Laboratory analysis identified HbF 31.7%, HbS 48.9% and HbX 19%. CE results identified HbF 32.9%, HbS+HbX 47.4%, HbA2+F/X hybrid 6.4% and S/X hybrid 13.3%. She likely presented the Hb variant. When treated with HU therapy, there were no longer any VOCs.

4 | DISCUSSION

Hb variant Ottawa caused by the c.46G > C mutation in one of the α-globin genes *HBA2* or *HBA1* is uncommon. To date, 22 individuals, including the three mentioned in this report, have been reported. This mutation occurs independently of ethnicity, but it had never been reported in Africa before [12, 13]. Nevertheless, children of African origin bearing the mutation were discovered in Brazil [7]. There are no clinical or haematological anomalies if the Hb mutation is heterozygous and in the absence of another Hb mutation, as demonstrated by the index patient's mother. On electrophoresis, in alkaline conditions, it shows up as a small band migrating in zone S. The health implications of having homozygous Hb Ottawa remain uncertain. The index patient's homozygosity resulted from distant consanguinity in her parents.

With HbAS, the migration of Hb Ottawa to the S-band renders it difficult to detect using conventional biological approaches. Nevertheless,

a sickle cell trait with an abnormally high HbS concentration and a C band on electrophoresis conducted in alkaline conditions should raise suspicion of a Hb variant. CE effectively illustrated the migrations of Hb Ottawa towards zone S and showed that the band at zone C on agarose electrophoresis was the result of a hybrid HbS/Hb Ottawa tetramer.

The presence of both HbS and Hb Ottawa in a case has suggested a possible slight haemolysis [14]. However, there were no documented blood anomalies for the child bearing the same mutations, as described in Brazil [7].

The father of the index patient displayed a heterozygous genotype for both Hb Ottawa and HbS. At that time, his VOCs were mild. However, his daughter, who was homozygous for Hb Ottawa and heterozygous for HbS, suffered from serious VOCs. Therefore, the pronounced symptoms in the index patient were due to the homozygous mutation and increased Hb Ottawa.

As for the index patient, she was not anaemic, and there had been no signs of organ damage detected until then. Oxygen transport was unlikely to be hindered by the association of Hb Ottawa and HbS. The patient did not complain of breath shortness and had a normal pulse oximetry. In the absence of VOCs, there was no evidence of sickling in her red blood cells upon examination of the blood film.

The Hb Ottawa heterozygous mother of the index patient should be tested for in vitro Hb sickling, instability, or insolubility [15].

To understand how vaso-occlusion occurs in this environment, further biological tests must be performed to explore the interaction between Hb Ottawa and HbS, in addition to investigating the possible role of the hybrids.

An interesting observation is that so far, non-S sickling variants are β-globins, which occur with an S mutation [9]. This is the first α Hb variant that, in combination with HbS, facilitates the occurrence of VOCs.

The decrease in VOC severity in the index patient is encouraging, presumably due to the HbF increase induced by HU. This demonstrates that the interaction between Hb Ottawa and HbS does not hinder HbF formation.

The family has been informed and premarital genetic counselling will be provided to all concerned parties [16].

Finally, particular attention is requested if an unusual band migrating in S or C is observed at Hb electrophoresis, especially with HbAS and VOCs. Further investigations, such as CE and DNA sequencing, are needed to determine if the migration is caused by a Hb variant, and if so, to further characterize the variant. Unfortunately, the advanced techniques involved are less commonly used in low-income countries.

AUTHOR CONTRIBUTIONS

Cécile Bobillier noticed the abnormal electrophoresis, contacted the family, and organised the blood tests. Isabelle Derclaye performed the Sanger sequencings. Augustin Ferrant examined the patient and family members and wrote the manuscript. Diane Maisin supervised and interpreted the molecular biology data. All authors reviewed and approved the final version of the manuscript.

ACKNOWLEDGEMENTS

We thank Marie-Claude Piffaut and Prof. Frédéric Galactéros for helpful advice. Dr. Wemboo Halatoko, Institut National d'Hygiène, Lomé, Togo interpreted the capillary electrophoreses. The whole family kindly and willingly collaborated.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

FUNDING INFORMATION

This study has not received any funding

DATA AVAILABILITY STATEMENT

The data used are available upon request to the corresponding author.

ETHICS STATEMENT

The authors have confirmed ethical approval statement is not needed for this submission.

PATIENT CONSENT STATEMENT

The father, both spouses and the children of appropriate ages provided their written informed consent for the blood tests

CLINICAL TRIAL REGISTRATION

The authors have confirmed clinical trial registration is not needed for this submission.

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How to cite this article: Bobillier C, Derclaye I, Ferrant A, Maisin D. Haemoglobin Ottawa, sickle cell trait and vaso-occlusive crises. eJHaem. 2024;5:33–38. https://doi.org/10.1002/jha2.857