


CASE REPORT

Hemoglobin I-Toulouse: A rare hemoglobinopathy presenting with low oxygen saturations

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Abstract

We report a child with persistently low oxygen saturations (SpO₂ 90%–92%) [normal SpO₂ > 98%], with delayed diagnosis due to the co-existing congenital pulmonary airway malformation with possible arterio-venous malformation. The diagnosis was only achieved after low oxygen saturations incidentally discovered from the child's father. The eventual cause was Hemoglobin I-Toulouse, making both patients the first reported cases with low oxygen saturations.

KEYWORDS

genetics, hematology, pediatrics and adolescent medicine, respiratory medicine

1 | INTRODUCTION

Hemoglobin is a polypeptide tetramer composed of two alpha and two non-alpha-globin chains. Each hemoglobin has four heme groups binding up to four oxygen molecules. Once beyond infancy, the majority of hemoglobin (Hb) is Hb A, with small percentages of other variants such as Hb F and Hb A2 (1). Hemoglobinopathies, a group of conditions due to globin gene mutations, result in altered production, stability, and oxygen-carrying ability of hemoglobin.

Hb I-Toulouse is a hemoglobin variant produced from lysine to glutamic acid substitution at position 67 in beta globin. This leads to a change in the ionic bond between lysine in position 67 of beta-globin and the propionic chain of heme (2). The alteration causes instability of the hemoglobin associated with mild hemolytic anemia and increased methemoglobin levels (2), through association with low oxygen affinity and low oxygen saturations (SpO₂) has never been previously reported. We report a child presenting with persistently low SpO₂, which was

attributed to the low-affinity hemoglobin Hb I-Toulouse. Consent was acquired from the patient's father for the report. Cases of Hemoglobin I-Toulouse are particularly rare, with only five cases previously reported in the literature, none though with low SpO₂. This is therefore the first reported case of this hemoglobin variant associated with low oxygen affinity.

2 | CASE HISTORY

An 8-year-old male who presented with shortness of breath was referred to respiratory medicine for persistently low oxygen saturations of 90%–92%. These low saturations were unresponsive to supplementary oxygen and persisted beyond the resolution of illness. There were no features of cardiac failure, no digital clubbing, and no change in the oxygen saturations with exercise. The child was overweight (BMI = 27 kg/m²) and had reported daily night-time snoring. History revealed recurrent cough and wheeze over the preceding 3 years. The patient's father

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was a Solomon Islander and mother was Samoan. The parents recalled a possible antenatally diagnosed congenital lung lesion with no further imaging postnatally. However, the patient remained asymptomatic throughout childhood, continued to participate in school sports, had no exercise limitation, and had good musculoskeletal development. Repeated examinations showed no significant findings other than the low SpO₂. When the child was 15 years of age (7 years after his initial presentation), his father was also noted to have low SpO₂ unresponsive to supplementary oxygen during a surgical procedure.

3 | INVESTIGATIONS AND DIFFERENTIAL DIAGNOSIS

Echocardiography showed a structurally normal heart with no evidence of pulmonary hypertension and polysomnography was not suggestive of obstructive sleep apnoea. Spirometry was normal. Initial investigations demonstrated eosinophilia ($0.64 \times 10^9/L$, reference range $<0.6 \times 10^9/L$) and reticulocytosis (2.90%, $123.0 \times 10^9/L$, reference range $10\text{--}100 \times 10^9/L$), with a normal methemoglobin proportion of 1.2% (reference range $<3\%$) on a venous blood gas. Chest CT with angiography demonstrated a right-sided paramedian Congenital pulmonary airway malformation (CPAM) (Figure 1) supplied by a single pulmonary artery branch, which drained into a pulmonary vein with subsequent drainage to the left atrium. Few direct connections between the artery and the vein (AVM) were also reported, which were considered to be creating a right to left shunt. At that time, this shunting was thought to be the cause of the low oxygen saturations and the child was managed conservatively. Subsequent echocardiograms failed to demonstrate a definite AVM and bubble echo for AVM was also negative. Two repeat CT scans of the chest over the next 5 years demonstrated the right lower lobe CPAM, but no clear AVM was demonstrated. Cardiac catheterisation was not performed.

As the child's father was also noted to have low SpO₂ 7 years after the initial workup, a familial low oxygen affinity hemoglobin variant was then suspected. The arterial blood gas of the father revealed a SaO₂ of 90% with a PaO₂ of 54 mmHg, which was concordant with the pulse oximetry measured oxygen saturations. The methemoglobin was mildly elevated at 2.3%. Elevated p50 at 42.1 mmHg in the absence of acidosis was consistent with a low oxygen affinity hemoglobin.

Capillary electrophoresis (Sebia Minicap™) performed on the child revealed a beta chain variant with very fast alkaline (pH 9.2) electrophoretic migration consisting of 40.8% of total hemoglobin (Z15 zone), with 56.6% Hb A, 2.6% Hb A2 and less than 0.5% Hb F (Figure 2). Isopropanol precipitation test was positive demonstrating the reduced stability of the variant. Cation exchange (pH 7) high-performance liquid chromatograms (HPLC) (Biorad Variant II Turbo™) revealed the variant as an “unknown” peak with a retention time of 1.94 min (Table 2). The raised hemoglobin F peak of 1.5% was likely artefactual due to co-elution of variant hemoglobin with glycosylated beta chain N terminal valine (Hb I-Toulouse1c). The HPLC chromatograms also showed a small unquantitated peak at a retention time of 1.24 min, which likely represents a partially oxidized species of hemoglobin resulting from the structural instability of the variant. The results were reproduced in the patient's father (Table 3). Genetic testing revealed both the patient and his father were heterozygous for the pathogenic variant HBBc.199A > G p (Lys67Glu), also known as beta-globin variant I-Toulouse. The hemoglobin variant was then established as the cause of patient's low oxygen saturations.

4 | OUTCOME AND FOLLOW-UP

Both the child and the father were followed up routinely by the respiratory physician and hematologist after the diagnosis of Hb I-Toulouse. Both patients remained

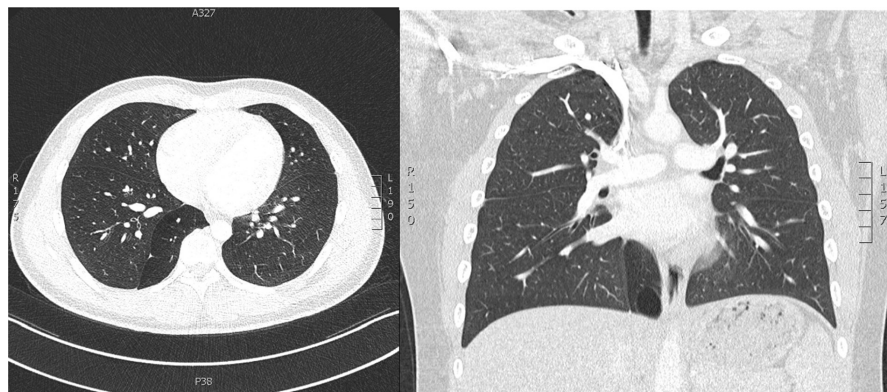


FIGURE 1 CT chest showing right lower lobe CPAM

persistently low oxygen saturations. Patients remain asymptomatic and no active treatments were required.

5 | DISCUSSION

Low oxygen affinity hemoglobin as a cause of low SpO₂ on pulse oximetry has been previously described, but the diagnosis can often be delayed due to the rarity of these

hemoglobin variants. Cases of Hemoglobin I-Toulouse are particularly rare, with only five cases previously reported in the literature, none though with low SpO₂ (Table 1). This is therefore the first reported case of this hemoglobin variant associated with low oxygen affinity. The diagnosis in our case was further delayed due to the co-existing CPAM with a possible reported AVM, which confounded the diagnosis and was only excluded as a cause for the low SpO₂ once the patient's father's history was discovered.

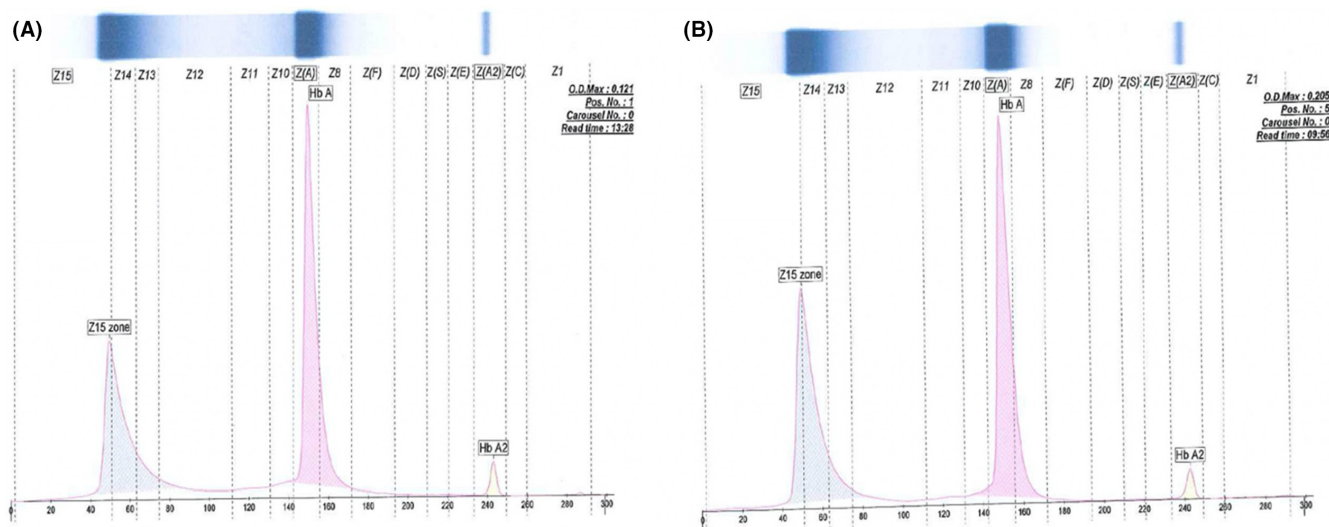


FIGURE 2 Capillary electrophoretogram of the child (A) and father (B)

TABLE 1 Reported cases of Hb I-Toulouse

Study	Presentation	Investigations
Rosa et al ³	A 39-year-old French Caucasian man, who was discovered to be anemic after an acute attack of gout	The investigations showed MCV of 120, and electrophoresis showed a fast-moving abnormal hemoglobin
Tejuca et al ⁴	Nicaraguan girl, who was admitted to hospital for joint pain and hemolytic anemia	Investigations indicated macrocytic anemia, and the electrophoresis showed a compound heterozygote carrying Hb S and another β chain variant, ⁴ indicating an association between Hb S and Hb Toulouse
Hendy & Cauchi ⁵	A 25-year-old pregnant Solomon islander and her 10-year-old paternal cousin presented with symptoms of α thalassemia	Serum iron was reduced in both, and hemoglobin was found to be moderately unstable. Cellulose acetate revealed an abnormal band migrating to Hb H, which was isolated and mapped to be consistent Hb I-Toulouse. The microcytosis in these two was likely due to concomitant α -thalassemia. Genotypes of two patients revealed the difference in α globin, as the 25-year-old genotype was $-\alpha^{3.7}/-\alpha^{3.7}$, whereas the 10-year-old cousin's genotype was $\alpha\alpha/-\alpha^{3.7}$
Pullon ⁶	A 50-year-old Solomon Island woman, who presented to the hospital with thrombocytosis	Hemoglobin electrophoresis on cellulose acetate revealed the presence of two major bands; one with mobility of Hb A and the other fast band running in the Hb I position. DNA mapping result supported the mass spectrometry findings with the variant previously reported as Hb I-Toulouse. α analysis was undertaken and a normal (α) 2 gen was not detected, indicating homozygosity for 3.7 kb α gene deletion and $-\alpha^{3.7}/-\alpha^{3.7}$ genotype

TABLE 2 HPLC chromatogram of the child

Peak name	Calibrated area %	Area %	Retention time (min)	Peak area
P1	–	0.2	0.91	3255
F	1.5 ^a	–	1.02	20,951
Unknown	–	0.7	1.24	9773
P2	–	3.6	1.36	51,200
Unknown	–	42.2	1.94	595,427
Ao	–	49.9	2.47	698,332
A2	2.4	–	3.62	33,326

Note: Total area: 1,412,254. F Concentration = 1.5%. A2 Concentration = 2.4%.

^aValues outside of expected range.

TABLE 3 HPLC chromatogram patient's father

Peak name	Calibrated area %	Area %	Retention time (min)	Peak area
P1	–	0.2	0.89	3857
F	1.7 ^a	–	1.00	29,840
P2	–	4.4	1.35	77,289
Unknown	–	41.2	1.92	720,916
Ao	–	49.9	2.46	872,041
A2	2.5	–	3.60	44,096

Note: Total area: 1,748,038. F Concentration = 1.7%. A2 Concentration = 2.5%.

^aValues outside of expected range.

There are over 1000 hemoglobin variants and the majority of the variants, including types with low oxygen affinity and SpO₂, are asymptomatic, due to the increased oxygen delivery caused by decreased oxygen affinity.¹ Concordance between SpO₂ and SaO₂, in this case, suggests that the absorbance spectrum of Hb I-Toulouse is not markedly altered from that of wild-type Hb A, supporting the methemoglobin results measured by multi-wavelength co-oximetry.

Hb I-Toulouse has been described to be unstable and sensitive to spontaneous oxidation.² The presentations of the previously reported cases include anemia,³ joint pain,⁴ and symptoms of thalassemia⁵ and thrombocytosis,⁶ though it is likely that some of these features were due to compound heterozygosity with Hb S and possibly alpha thalassemia in some of these cases.^{4,5}

Interestingly, two of the previously reported cases^{5,6} were also from Solomon Islands, possibly suggesting clustering of this Hb variant in people of Solomon Island descent. Hb I-Toulouse has been previously reported to cause methemoglobinemia,^{2,6} which is consistent with findings in our case. Although the association between Hb I-Toulouse and alpha thalassemia has been seen in the previous case,⁵ one case of compound heterozygosity

of Hb S- and Hb I-Toulouse presenting with possible vaso-occlusive episodes has been reported,⁴ there is insufficient evidence to define the clinical implications of interactions between Hb I-Toulouse and other beta-globin variants such as Hb S or beta thalassemia. Significant medical issues, such as chronic hemolysis or sickle cell disease phenotype, could occur if the Hb I-Toulouse variant is co-inherited with other beta-globin variants, as reported previously.⁵ Hence, partner testing and prenatal genetic counseling should be considered in couples where Hb I-Toulouse and another beta-globin variant are discovered, since a compound heterozygous state may be associated with an increased risk of hemolysis.

In our case, both the patient and his father presented with low SpO₂, a concordantly low SaO₂, and high p50, making these the first two reported cases of Hb I-Toulouse with low oxygen affinity. The complexity was increased due to the antenatally detected CPAM and a reported AVM. The absence of polycythemia, digital clubbing, a negative bubble echo, and absence of a clear AVM on repeat CTs make a true mixing disorder such as an AVM highly unlikely. It also highlights the importance of family history and SpO₂ monitoring of parents in infants and children with unexplained hypoxia.

6 | CONCLUSION

We report the first case of Hemoglobin I-Toulouse associated with low SpO₂ due to the low affinity to oxygen in this hemoglobin variant. The asymptomatic nature of the variant and the confounding finding of a CPAM in our case significantly delayed the diagnosis. Hemoglobin variants should be included as a differential diagnosis for unexplained low oxygen saturations. Family history and testing should be considered for suspected hemoglobinopathy.

AUTHOR CONTRIBUTIONS

Ziheng Xu was responsible for drafting and formatting of the initial article. Ziheng Xu also revised the article critically, modified the article based on the comments from other authors, and finalized the article. Professor Ian Brent Masters was responsible for the critical revision of the manuscript for important intellectual content and provided important comments. Dr Pasquale Barbaro was responsible for the critical revision of the manuscript for important intellectual content and provided important comments. Dr Stephen Miller was responsible for the acquisition of the hematological data of the article and offered an interpretation of the data. Professor Nitin Kapur was responsible for the design and final approval of the article. Professor Nitin Kapur also modified the article,

provided critical comments, and was the main supervisor of the article.

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None.

CONFLICT OF INTEREST

No conflicts of interest to disclose.

DATA AVAILABILITY STATEMENT

The data that supports the findings of the study can be seen in the attached tables and images.

CONSENT

Informed consent was acquired from the patient's father, and the study was approved by the chair of the Human Research Committee at Queensland Children's Hospital.

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