

When is Apparent Hemoglobin S Trait Something More Complex?

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CASE DESCRIPTION

A hemoglobinopathy investigation was completed for a 7-year-old male of unknown ethnicity and no significant past medical history or transfusion history. A complete blood count (Sysmex XN10-4 Automated Hematology Analyzer, Sysmex) showed unremarkable hemoglobin (Hb) at 126 g/L [reference interval (RI): 110–157 g/L], red blood cell count [$4.86 \times 10^{12}/L$ (RI: $3.80\text{--}5.60 \times 10^{12}/L$)], mean corpuscular volume [80 fL (RI: 75–95 fL)], and mean corpuscular hemoglobin [26 pg (no RI available)]. Ferritin and iron studies were also unremarkable. Capillary electrophoresis (Capillarys 2 Flex Piercing, Sebia) analyzed at Alberta Precision Labs demonstrated the presence of HbA (80.7%), HbA₂ (3.3%), and a variant hemoglobin in the HbS zone (Fig. 1A). Of note, the variant hemoglobin percentage was 16.0% of total hemoglobin, which is substantially lower than would be expected for sickle cell trait alone (approximately 40%, Fig. 1B).

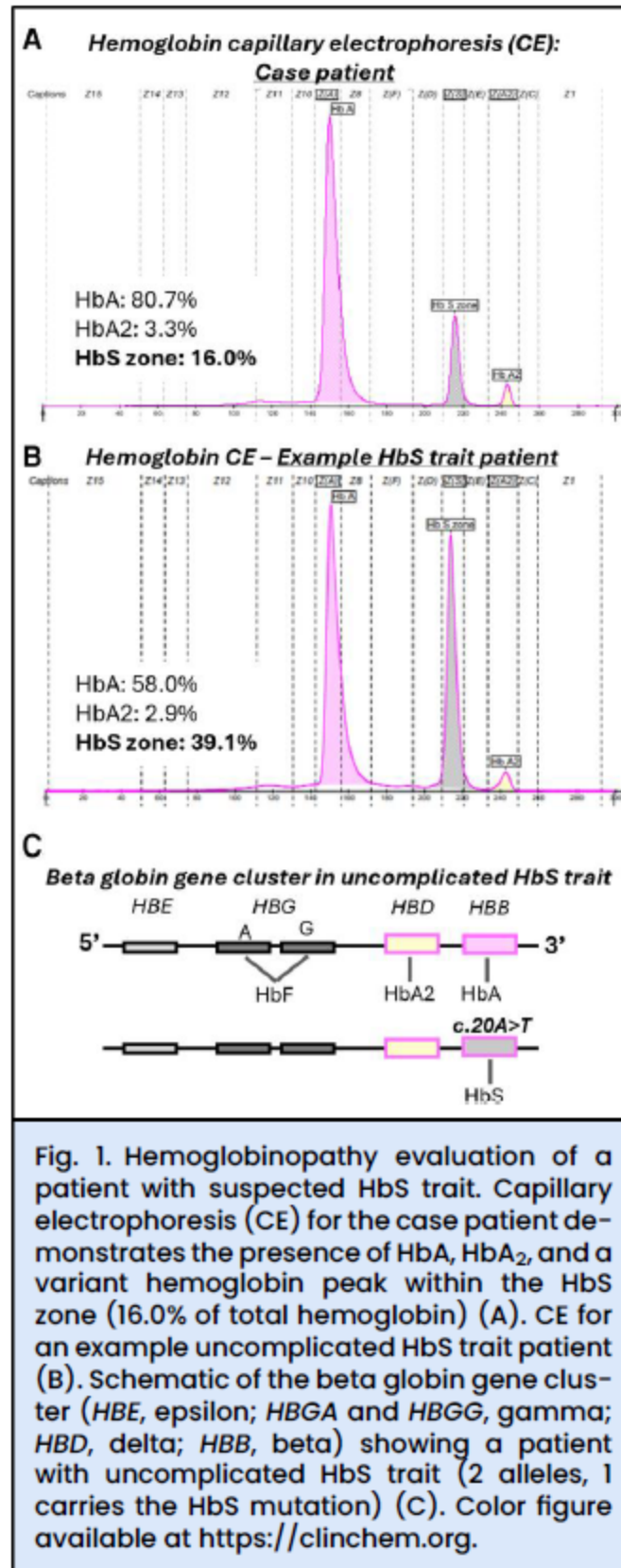
The presence of a variant hemoglobin peak in the HbS zone, combined with a lower than expected proportion of the variant hemoglobin, raised several distinct diagnostic possibilities. Initially, the possibility of the HbS zone peak being a variant other than HbS was considered. Based on the HbS zone peak percentage alone, an alpha chain variant was considered, as they are typically present at 15%–25% of total hemoglobin (1). However, this type of variant is characterized by an additional minor peak (<1%) representing the variant HbA₂ formed by the alpha chain variant with delta globin chains, which was not observed in this case. A single peak in the HbS zone of <20% raised suspicion for Hb Kenya, a gamma-beta gene fusion variant. However, Hb Kenya is typically associated with mild microcytic anemia and is considered a type of hereditary persistence of fetal hemoglobin (1); neither microcytosis nor an increased HbF percentage was observed here. Depending on the laboratory algorithm, analysis with a second method such as a gel electrophoresis or a sickle solubility test may distinguish between the presence of HbS and another variant (although the HbS percentage in this case is very close to the limit of detection for the sickle solubility test). In this case, for identification of the variant hemoglobin, Sanger sequencing of the *HBB* gene from promoter to polyadenylation site was performed at the Alberta Children's Hospital Molecular Genetics Laboratory using DNA extracted from whole blood and analyzed on a 3500 Genetic Analyzer (ThermoFisher Scientific). Sequence analysis confirmed a heterozygous c.20A>T (p.Glu6Val) pathogenic variant consistent with HbS, and no other variants were detected (Fig. 1C).

In sickle cell trait, the HbS percentage is most affected by the number of alpha globin genes, with loss of each alpha globin gene dropping the HbS by approximately 5% (i.e., 40% in aa/aa, 35% in -a/aa, 30% in -/aa, and 25% in -/-a) (1, 2). The presence of iron deficiency can also repress alpha globin production preferentially over beta globin production (2), reducing HbS in sickle cell trait patients (1). In the case patient, PCR analysis of the *HBA1* and *HBA2* genes was performed using nested primers designed to identify 7 common deletions that result in alpha-thalassemia (deletions: 3.7 kb rightward, 4.2 kb leftward, 20.5 kb, SEA, THAI, FIL, and MED) and no deletions were detected. Subsequent sequence analysis of the *HBA1* and *HBA2* genes did not detect any reportable variants. Based on these results, combined with the

absence of microcytosis, the possibility of concurrent alpha-thalassemia or iron deficiency was considered extremely unlikely.

QUESTIONS TO CONSIDER	
1.	Why does the presence of thalassemia impact the expected percentage of a variant hemoglobin?
2.	What other mechanisms have been recognized to modify the expected percentage of a variant hemoglobin?
3.	How can molecular genetic testing play a role in complex and/or unusual hemoglobinopathy investigations?
4.	Why is it important to have a correct and complete diagnostic conclusion to a hemoglobinopathy investigation?

Continued below....



REFERENCES

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Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the August 2026 issue of *Clinical Chemistry*. To view the case and comments online, go to <https://academic.oup.com/clinchem/issue/72/8> and follow the link to the Clinical Case Study and Commentaries.

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