

Case Report

The Role of Advanced Genetic Analyses to Determine a Complex Hemoglobinopathy

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Abstract

We describe a two-year-old with mild anemia and marked microcytosis of unclear etiology. Hematologic evaluation suggests an apparent HbCC disease or HbC β^0 thalassemia. Parental testing and routine genetic analysis could not provide a definitive diagnosis. Advanced genetic analyses with SOMA and qPCR were required to uncover a large *de novo* deletion of the beta globin locus revealing HbC $\epsilon\gamma\delta\beta^0$ thalassemia. This case exemplifies the importance of advanced genetic technology in hemoglobinopathies that are inconsistent with routine molecular and laboratory testing. Accurate molecular analyses are necessary to facilitate precise determination of genotype-phenotype correlations and, in some cases, are critical to documenting *de novo* variants.

ABBREVIATIONS

HPLC: high performance liquid chromatography; **Hb:** Hemoglobin; **MCV:** mean corpuscular volume; **SOMA:** single nucleotide polymorphism oligonucleotide microarray analysis; **SNP:** single nucleotide polymorphism; **qPCR:** quantitative PCR; **aCGH:** array comparative genomic hybridization

INTRODUCTION

Hemoglobin gene variants are commonly diagnosed via hemoglobin electrophoresis or high performance liquid chromatography (HPLC). These methods are limited in determining novel or complex globin gene mutations, specifically those associated with thalassemia phenotypes and/or *de novo* features. Advanced genetic analyses and sequencing bridge the gaps in diagnostic ability allowing for more accurate genotype-phenotype correlation. This technology has identified rare cases of spontaneous or inherited deletions in the beta globin locus including $\epsilon\gamma\delta\beta$ globin mutations [1-4]. However, the resultant phenotype of these mutations in conjunction with common hemoglobin variants remains to be well described.

CASE PRESENTATION

A 2 year old Latino male was seen in consultation for severe microcytosis without anemia. He was born full term with an uncomplicated pregnancy and delivery. His hemoglobin (Hb) was 13.7 and mean corpuscular volume (MCV) 66 at 25 days of

life (normal Hb 12 MCV 96). Newborn screen showed HbF, HbC, and no HbA. Analysis by HPLC and hemoglobin electrophoresis at 30 days of life revealed: HbF 71.4%, HbC 29.1%, HbA and HbA2 both 0%, suggesting homozygous C disease, HbCC.

He remained asymptomatic with normal growth and development. Over the next two years, his Hb decreased to a baseline of 10 with a MCV of ~50. Two months prior to presentation, he began to develop symptoms of exertional fatigue, as his mother noticed that he would often have to take breaks when running and playing with friends. There was no pallor, jaundice, palpitations, dark urine, fevers, headache, or other clinically significant illnesses. Blood counts at this time were significant for Hb 9.7 and MCV 52, with normal iron studies, prompting referral for hematologic evaluation.

Upon presentation to our Pediatric Hematology clinic, his physical examination was normal without tachycardia, jaundice, pallor, or hepatosplenomegaly. His blood counts showed a normal Hb 11, MCV 51.3, RBC 6.24, RDW 21, reticulocyte count 1.4, platelet 247, and WBC 10. He had not been prescribed or administered any iron supplementation or other medications. Peripheral smear revealed a mixed erythrocyte population of target cells, spherocytes, and anisocytosis with microcytic cells; normal platelets of varying sizes, normal granulation patterns; normal size and distribution of leukocytes (Figure 1). A repeat HPLC showed HbC 93.4%, HbF 3.2%, and A2 3.1%, again consistent with HbCC disease or compound heterozygosity

for HbC and beta zero (β^0) thalassemia (Figure 2). Marked microcytosis also supported a concomitant thalassemia. Initial thalassemia gene testing by an outside commercial laboratory indicated a one alpha globin 3.7 gene deletion and homozygous HbCC, with a caveat that a large deletion may invalidate their findings.

Family history was significant only for maternal anemia of pregnancy (Hb 9.3, MCV 91), and she was known to have HbAA by prenatal HPLC. This raised concern for HbC homozygosity secondary to uniparental disomy, and testing of both parents was performed. Results confirmed maternal studies and demonstrated paternal microcytosis (Hb 15.8, MCV 77.4) associated with HbC trait and the same α -thalassemia trait mutation (Table 1).

These results seemed to confirm diagnosis of HbCC disease via paternal uniparental disomy. Due to diagnostic uncertainty, single nucleotide polymorphism (SNP) oligonucleotide microarray (SOMA) was performed. Molecular cytogenetic studies identified a *de novo* heterozygous 60kB interstitial deletion in the short arm of chromosome 11. Specifically, SOMA indicated a loss on chromosome 11 from positions 5,248,426-5,308,253 (Hg19). This region contains 5 of the genes (*HBD*, *HBBP1*, *HBG1*, *HBG2*, *HBE1*) that comprise the beta globin locus on chromosome 11 (Figure 3). Given the absent probe coverage of the SNP microarray for the *HBB* gene, the deletion was believed to be slightly larger than indicated by SOMA, and quantitative PCR (qPCR) probes were designed to cover regions within the beta globin gene cluster including the *HBB* gene. The qPCR

analysis revealed that the *de novo* deletion was slightly larger than that indicated by SOMA, and in fact extended to include the *HBB* gene (Figure 3). These results confer a diagnosis of HbC- $\epsilon\gamma\delta\beta$ thalassemia, or clinically described HbC β^0 thalassemia due to a paternally inherited HbC trait and an extensive, *de novo* beta globin locus deletion.

At presentation, the patient was started on folic acid supplementation, and serial clinical evaluations to present age 5 reveal his baseline at Hb 9. He also developed splenomegaly on exam confirmed on abdominal ultrasound measuring ~13cm. He continues to be followed regularly for surveillance for other phenotypic manifestations.

DISCUSSION

This case report identifies inconsistencies in diagnosis of hemoglobinopathies using routine parental HPLC testing and standard genetic analyses. This case demonstrates the benefits of advanced genetic testing to identify complex hemoglobinopathies and provide an accurate diagnosis that may have otherwise been obscured. Chromosomal microarray analysis, either by array comparative genomic hybridization (aCGH) or SOMA technology, offers significant advantages over traditional molecular technologies such as fluorescence in situ hybridization, Southern blot and Gap-PCR. The primary benefit is the ability to perform a high resolution scan of the entire genome for submicroscopic imbalances and to more specifically map the breakpoints of regions identified with microdeletions and microduplications. Phylipsen *et al.* recently reported the use of

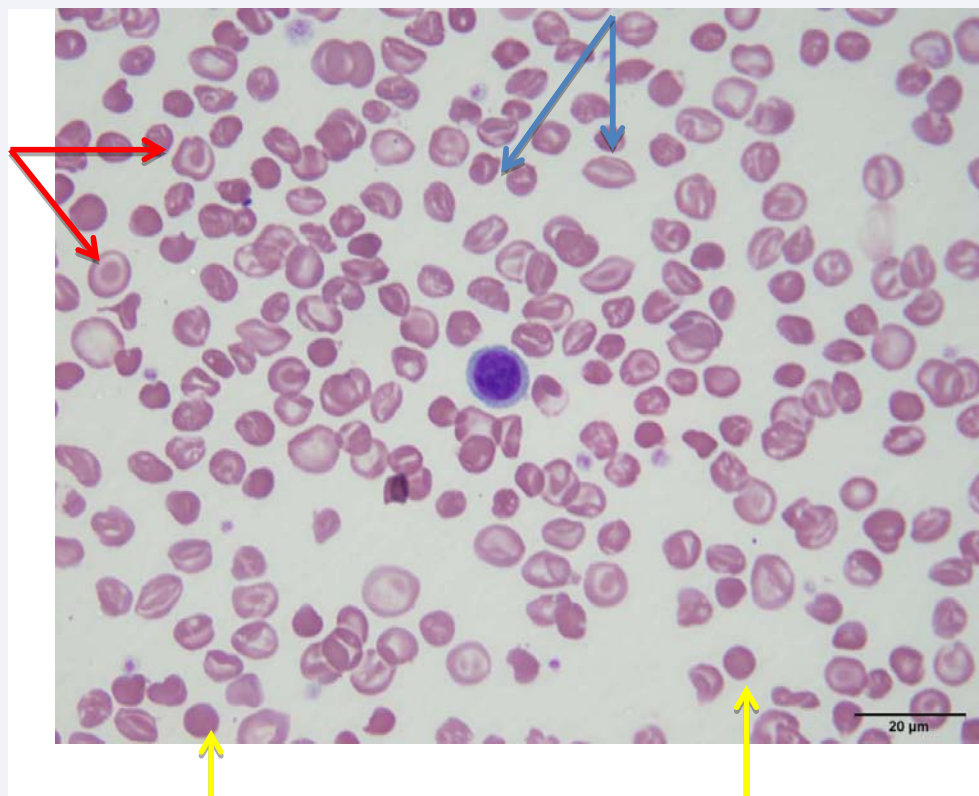


Figure 1 Peripheral blood smear. Red arrow indicates target cells. Yellow arrow indicates spherocytes. Blue arrow indicates microcytic erythrocytes.

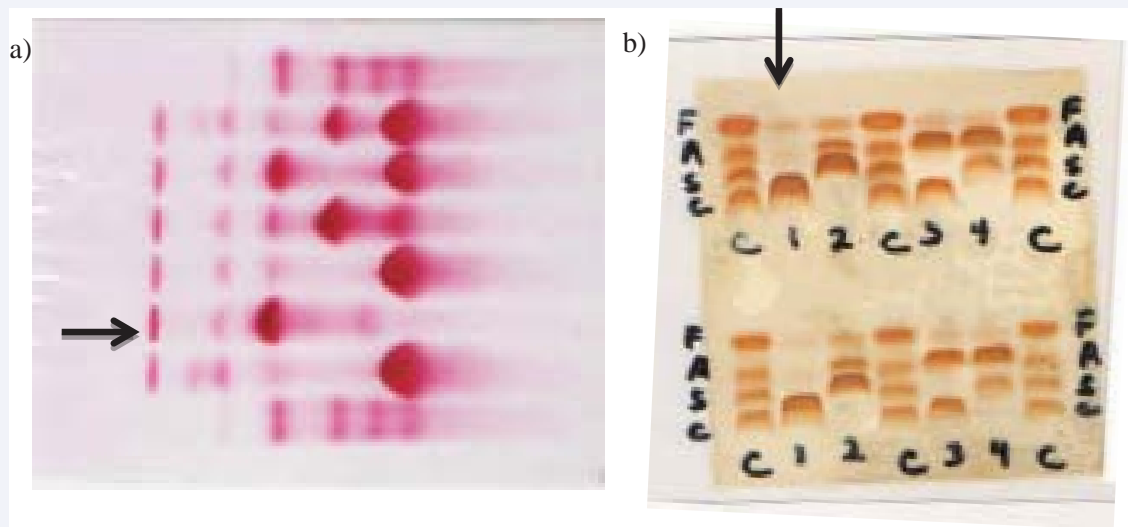


Figure 2 a) High Performance Liquid Chromatography with AFSC controls in uppermost and lowermost lanes. b) Hemoglobin electrophoresis with FASC control far left and far right lanes. Black arrows indicates patient specimen.

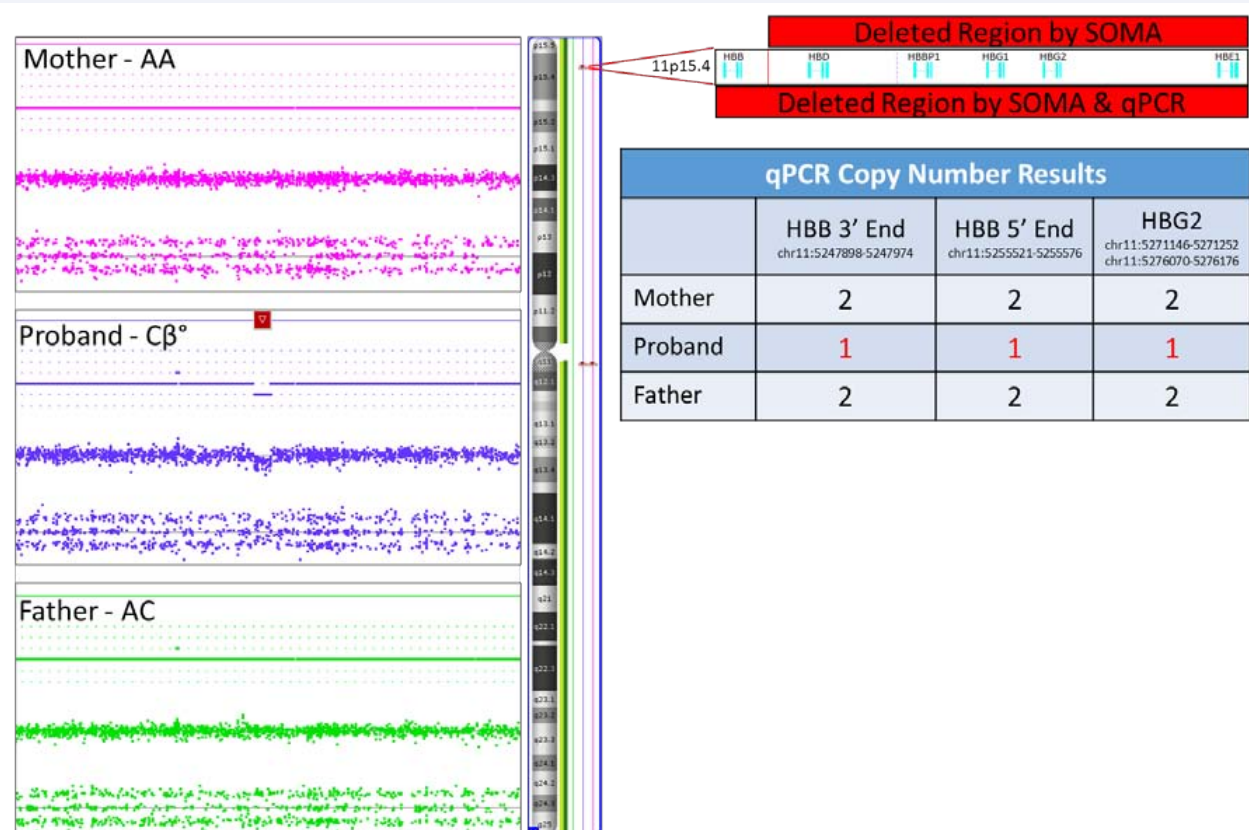


Figure 3 SOMA and qPCR results of patient and both parents. Red box indicates region of de novo deletion of chromosome 11 expanded in the insert with deletion noted in the short arm and the genes located within the deleted region. Note that by SOMA, the *HBB* gene does not appear to be within the deleted region. qPCR analysis indicates that the deletion includes the *HBB* gene. Genomic coordinates are shown using human genome build Hg19.

aCGH for high-resolution breakpoint characterization of small and large rearrangements in patients with α - and β -globin gene cluster rearrangements [5]. The absence of this technology could have clinical ramifications.

Homozygous HbC disease has a fairly benign phenotype characterized by mild hemolytic anemia and splenomegaly. This is in large part due to the increased erythrocyte stability and the presence of large, less osmotically fragile spherocytes

Table 1: Parental laboratory testing.

Maternal history	Paternal history
No β or α thalassemia mutations	Genotype α -/ α ; heterozygous positive for the α .7 α globin deletion
Normal AA	C 36.7%
A 94.9%	A 58.5%
F <1%	F <1%
A2 2.8%	A2 3%
Hb 10.2 MCV 81.9	Hb 15.8 MCV 77.4

Abbreviations: β - beta, α - alpha, Hb - hemoglobin, MCV - mean corpuscular volume

characteristic of HbCC compared to other hemoglobinopathies. However, occasionally older children and adults with HbCC can experience hyperbilirubinemia and cholelithiasis [6].

HbC β^0 thalassemia generally lacks clinically significant sequelae beyond mild anemia, microcytosis, and only rarely splenomegaly [7]. Mild manifestations are generally a result of a compensatory increased HbA2 or HbF. In our patient, lack of a compensatory response may result from his heterozygous deletion of the corresponding globin exons with $\epsilon\gamma\delta\beta$ thalassemia and may lead to a more severe phenotype.

At the same time, comparable genotype-phenotype correlations were described in individuals where the mutation is limited to the beta globin gene and do not have a concomitant α -mutation. Our patient's α -thalassemia mutation may mitigate the concomitant β globin mutations by reducing the number of free α -globin chains [8,9]. This constellation of factors with his newly described genotype may predict a long-term clinically milder phenotype of anemia and splenomegaly.

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