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Case report

# Clinical and molecular characterization of novel deletions causing epsilon gamma delta beta thalassemia: Report of two cases

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ABSTRACT

Epsilon gamma delta beta $(\varepsilon\gamma\delta\beta)^0$ - thalassemia is a very rare disorder that results from large deletions in the β-globin gene cluster which abolish all regional globin chain gene expression from that allele. Since it is an exceedingly rare cause of neonatal anemia and is not detected by routine newborn screening, it is usually not suspected clinically and commonly undiagnosed or misdiagnosed. In this study, we describe two patients diagnosed in our hospital with $(\varepsilon\gamma\delta\beta)^0$-thalassemia based on the results obtained from DNA microarray analysis of their peripheral blood. The first patient of mixed European descent presented as a neonate with microcytic hemolytic anemia, hyperbilirubinemia, hypoglycemia and hypothermia, and was found to have a 2.2 Mb loss that included the entire β-globin gene cluster and the locus control region (LCR). The second patient, also of mixed European descent, presented in the neonatal period with anemia, thrombocytopenia and cutaneous extramedullary hematopoiesis, and was found to have a 59 kb loss that included the β-globin LCR, *HBE1, HBG1,* and *HBG2* genes. Both cases highlight the importance of recognizing the clinical features of $(\varepsilon\gamma\delta\beta)^0$-thalassemia and implementing appropriate testing to clarify the diagnosis and manage the condition.

## 1. Introduction

Epsilon gamma delta beta $(\varepsilon\gamma\delta\beta)^0$-thalassemia is an exceedingly rare cause of neonatal anemia with just over 30 cases reported to date [1,2]. It results from the deletion of the β-globin gene cluster and/or the β-globin locus control region (LCR), a long-range cis-regulatory element that enhances expression of the linked β-globin genes. The condition can have variable phenotypic presentation ranging from normal to severe anemia requiring in utero and/or neonatal transfusions. Without proper testing, the diagnosis may be overlooked, and the patient may be improperly treated. Herein, we describe clinical and molecular characterization of two novel $(\varepsilon\gamma\delta\beta)^0$-thalassemia deletions in two unrelated patients presenting with anemia in the neonatal period. Both patients had high nucleated red blood cell count in addition to anemia, while the second patient also had thrombocytopenia, cutaneous extramedullary hematopoiesis (CEH), hepatosplenomegaly, and seizures. The cutaneous presentation of the second case has been published [3]. Both patients were found to have distinctly sized deletions involving the β-globin gene cluster by single-nucleotide polymorphism (SNP) microarray analysis, consistent with a diagnosis of $(\varepsilon\gamma\delta\beta)^0$- thalassemia. The deletions identified in these two patients are distinct from those reported in the literature.

## 2. Case 1

A female, European Caucasian patient was born at 37 weeks gestation by induced vaginal delivery due to preeclampsia to a 27-year-old G5P3 female with A + type blood. The newborn, whose blood typed as A-, presented with hypothermia, hypoglycemia, and jaundice on the first day of life. Complete blood cell count (CBC) was significant for anemia, a high nucleated red blood cell count, and elevated reticulocyte count; Coombs test was negative. A heel stick showed a glucose level of 35 mg/dL, and additional blood testing found elevated bilirubin: total 9.8 mg/dL, direct 0.7 mg/dL. Blood glucose levels improved with IV dextrose and supplemental feedings, and hyperbilirubinemia improved with phototherapy. Newborn screening was negative for hemoglobinopathy. Multiple additional studies were done at 2 weeks of age. Peripheral blood smear revealed thrombocytosis, microcytic hypochromic red blood cells with anisopoikilocytosis, frequent target cells, some schistocytes and microspherocytes, and no blasts (Fig. 1A). Hemoglobin electrophoresis showed normal hemoglobin electrophoresis pattern, no abnormal hemoglobin variant or evidence of beta

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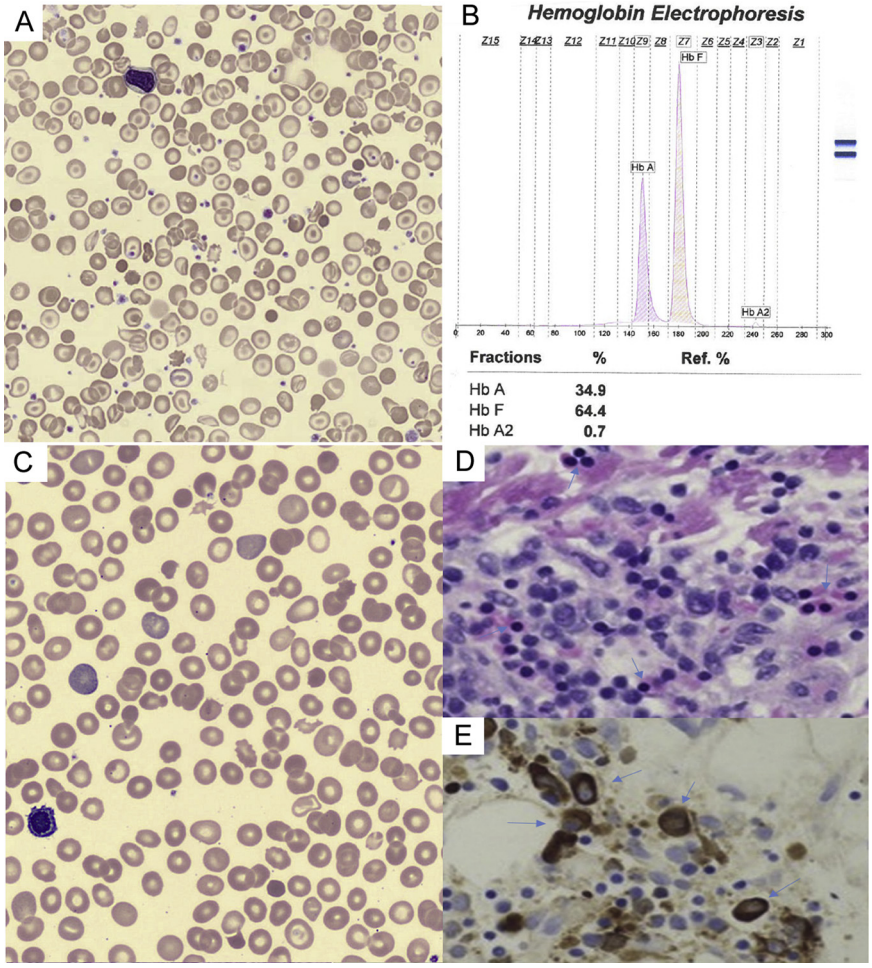


**Fig. 1.** Findings of peripheral blood, skin biopsy and hemoglobin electrophoresis. A. A Wright-Giemsa stained peripheral blood smear of case 1 revealed thrombocytosis, microcytic hypochromic red blood cells with anisopoikilocytosis, frequent target cells, some schistocytes and microspherocytes. B. Sebia capillary electrophoresis of case 1 showed normal hemoglobin electrophoresis pattern, no abnormal hemoglobin variant or evidence of beta thalassemia. C. A Wright-Giemsa stained peripheral blood smear of case 2 revealed prominent erythrocytic anisopoikilocytosis, polychromasia, occasional erythroblasts, and thrombocytopenia. D. An H&E stained skin biopsy section demonstrated frequent small- to medium-sized round cells with dark round nuclei and pink cytoplasm consistent with erythroblasts (arrows) admixed with medium- to large-sized mononuclear cells. E. The medium- to large-sized mononuclear cells were positive for myeloperoxidase (arrows) by immunohistochemical stain, and consistent with myeloid precursors.

thalassemia (Fig. 1B). There was no deficiency in the RBC enzymes tested (glucose-6-phosphate dehydrogenase, pyruvate kinase, glucose phosphate isomerase, and hexokinase). The patient ended up requiring two blood transfusions in the first two months of life. Before transfusion, CBC of her blood sample with lowest hemoglobin (Hgb) level showed a Hgb level of 6.6 g/dL, mean corpuscular volume (MCV) 69.4 fL, mean cell Hgb (MCH) 23.2 pg, hematocrit (Hct) 19.7%, red blood cell (RBC) count $2.84 \times 10^{12}$/L, white blood cell (WBC) count $7.57 \times 10^{9}$/L, and platelet count $721 \times 10^{9}$/L.

The patient's genetic workup included SNP microarray analysis of a peripheral blood sample, which identified a 2.2 Mb loss of a genomic region at 11p15.4 containing nearly 77 genes. This pathogenic deletion removed the entire β-globin gene cluster including *HBE1, HBG1, HBG2, HBD,* and *HBB*, as well as the β-globin LCR, a finding consistent with $(\varepsilon\gamma\delta\beta)^{0}$-thalassemia (Fig. 2, Case 1). Additionally, the deleted region included exons 4–12 of the *STIM1* gene, indicating a carrier status for autosomal recessive immunodeficiency-10 (MIM number 612783), many olfactory receptor genes and a few other genes not currently known to be associated with a clinical phenotype.

The patient's family history was negative for similarly affected individuals. The patient's mother (Italian, British, and Irish descent) indicated that she had mild anemia all her life but had no reports of hemolysis, abnormal levels of bilirubin or liver enzymes. The patient's father (Lithuanian and German descent) is healthy and has no reported health issues. Targeted parental testing via quantitative PCR (qPCR) did not find the deletion in either parent, indicating that the deletion was likely *de novo* in origin. At 6 months of age, the patient was growing well, developing normally, and had a Hgb level in the normal range of 9.6 g/dL,while RBCs still showed features of thalassemia trait (increased RBC: $5.13 \times 10^{12}$/L, decreased MCV: 58.3 fL, and decreased MCH: 18.7 pg).

## 3. Case 2

A male, European Caucasian patient was born at 41 weeks gestation via spontaneous vaginal delivery to a 21-year-old G1P1 female with a history of HSV type I infection and O + type blood. The newborn, whose blood also typed as O+, presented with a "blueberry muffin" rash involving his face, arms, legs and torso on day 1 of life. He had significant and worsening respiratory distress, which required mechanical ventilation. A G-tube was placed due to an inability to achieve oral feeds. He was found to have hepatosplenomegaly, anemia and thrombocytopenia, and received multiple RBC and platelet transfusions. Before transfusion, the lowest Hgb level was 8.0 g/dL, and the lowest platelet count was $7 \times 10^{9}$/L. The first available MCV was 88 fL, which was tested on day 5 of life and after multiple RBC transfusions.

The patient developed seizures on day 6 of life and was placed on Keppra. EEG noted multifocal epileptiform discharges, and head ultrasound found a large temporal hemorrhage with midline shift. Investigation for intrauterine infections was negative. A newborn screen was negative for hemoglobinopathy and lysosomal storage disorders. Peripheral blood smear revealed prominent erythrocytic anisopoikilocytosis, polychromasia, occasional nucleated red blood cells and schistocytes (Fig. 1C). A skin punch biopsy of one of the "blueberry muffin" lesions on the patient's left thigh done on day 7 of life identified dermal cellular infiltrates with frequent small- to medium-sized round cells with dark round nuclei and pink cytoplasm consistent with erythroblasts (Fig. 1D). There were scattered or clustered mononuclear

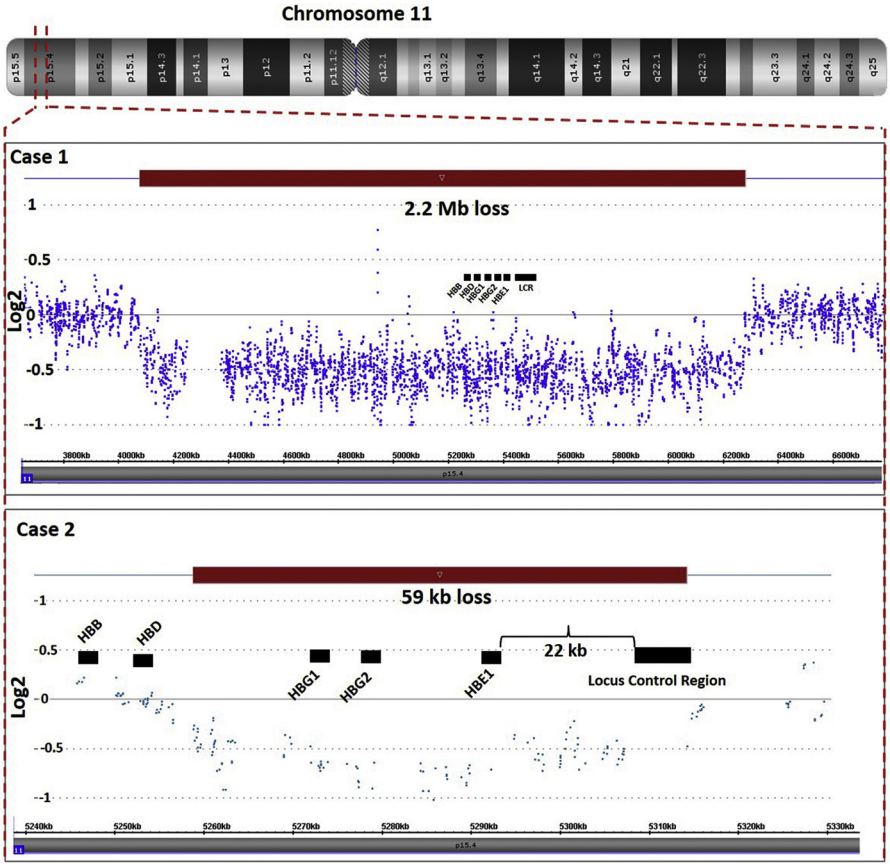


**Fig. 2.** Thermofisher Cytoscan HD SNP microarray showing probe distribution consistent with a single copy number loss in the region of 11p15.4. Case 1: the deleted region (chr11:4073950_6274517, hg19) contained 77 genes including the β-globin locus control region (LCR), which controls the expression of the genes within the β-globin gene cluster, and all five genes in the β-globin gene cluster: epsilon (*HBE1*), gamma (*HBG1* and *HBG2),* delta (*HBD*) and beta *(HBB*). Case 2: the deleted region (chr11:5257699_5316491, hg19) included three genes in the β-globin gene cluster: epsilon (*HBE1*) and gamma (*HBG1* and *HBG2)*, as well as the β-globin LCR.

cells containing large nuclei and open chromatin, which were highlighted by myeloperoxidase immunohistochemistry stain (Fig. 1E), and were consistent with myeloid precursors. Overall, the findings of the skin biopsy were consistent with cutaneous extramedullary hematopoiesis (CEH).

The patient's initial genetic diagnostic workup included carbohydrate-deficient transferrin testing for congenital disorders of glycosylation, sequencing and deletion/duplication analysis of 32 genes associated with thrombocytopenia, and symptom-driven exome sequencing, all of which were non-diagnostic. SNP microarray analysis of a peripheral blood sample revealed a 59 kb single copy loss of a genomic region at 11p15.4. Genetic material lost included 3 genes in the β-globin gene cluster:*HBE1, HBG1,* and *HBG2,*as well as the β-globin LCR (Fig. 2, Case 2), consistent with $(\varepsilon\gamma\delta\beta)^0$-thalassemia.

A three-generation family pedigree was obtained and was negative for individuals with birth defects, genetic disease, multiple miscarriages, early infant death, or known consanguinity. No relatives with microcytic erythrocytes were documented. The mother of the patient is of mixed Caucasian/German background and his father is of Irish descent. Parental testing to determine the inheritance of the deletion was not performed due to the unavailability of samples. A general checkup visit at 2.5 years of age found that the patient was healthy, developmentally on track, normal for height/weight, weaned off of Keppra, and had a Hgb level in the low-normal range of 11.4 g/dL, while RBCs still showed features of thalassemia trait (increased RBC: $6.18 \times 10^{12}$/L, decreased MCV: 59.1 fL, and decreased MCH: 18.4 pg).

## 4. Discussion

Anemia and thrombocytopenia are the most common hematological problems in neonates. These problems can occur due to a variety of causes, which can be broadly classified into three categories: blood loss, increased blood cell destruction, and low production [4,5]. The etiology may be difficult to determine in some patients with complex medical conditions.

$(\varepsilon\gamma\delta\beta)^0$-thalassemia, as illustrated in the cases above, usually presents as a severe neonatal microcytic anemia that is Coombs-negative. It results from variably-sized hemizygous deletions in the β-globin gene cluster that abolish all regional globin chain gene expression from that allele [6,7]. On the molecular level, the deletions can affect either all or most of the β-globin cluster including *HBB* (group I), or the β-globin LCR leaving *HBB* intact (group II) [8]. Our case 1 represents one of the largest reported group I deletions to date, while case 2 represents a novel group II deletion. Both patients described here had anemia requiring multiple transfusions. As others have previously documented, neither the severity of the anemia, nor the clinical presentation, is dependent on the type of deletion (group I or II) or the size of the region deleted. The second patient had a more severe clinical presentation compared to the first, but genetically had a much smaller deletion. The second patient also presented with CEH, a feature rarely reported in association with this disorder. In general, CEH is a rare finding that is usually associated with conditions such as congenital infections, erythroblastosis fetalis, twin-twin transfusion syndrome, and hereditary spherocytosis [9–11]. In our second case, $(\varepsilon\gamma\delta\beta)^0$-thalassemia was the most likely cause for the anemia and CEH, while the thrombocytopenia might have been related to blood loss, sequestration, and/or decreased megakaryopoiesis in the bone marrow.

Loss of both copies of the β-globin gene cluster region has never been reported and is presumed to be incompatible with life. In contrast to other thalassemias causing neonatal anemia, such as β-thalassemia major or Hgb H disease, the anemia seen in $(\varepsilon\gamma\delta\beta)^0$-thalassemia usually self-resolves within the first year of life. However, RBC microcytosis persists throughout life [6,7]. Thus, the hematological lab profile (increased RBC count, microcytosis, and hypochromasia), as seen in the samples from our patients from their most recent clinic visit, may mimic β-thalassemia minor except that Hgb A2 is not increased. Hgb

electrophoresis and High Performance Liquid Chromatography (HPLC) are two of the most useful methods for diagnosing thalassemia. β-thalassemia major is usually diagnosed or suspected when no Hgb A is found, while Hgb H disease is diagnosed or suspected by the presence of Hgb Barts and/or Hgb H. However, these methods are not useful for detecting $(\varepsilon\gamma\delta\beta)^0$-thalassemia due to its normal Hgb electrophoresis pattern and the absence of abnormal peaks or bands. To establish the diagnosis, molecular testing capable of detecting large gene deletions, such as SNP-based DNA microarrays, multiplex ligation-dependent probe amplification (MLPA), Southern Blot analysis, or whole genome sequencing, is required.

In summary, we describe the detailed clinical information, histopathologic and molecular findings of two $(\varepsilon\gamma\delta\beta)^0$-thalassemia cases with novel deletions. As an exceedingly rare cause of neonatal anemia, $(\varepsilon\gamma\delta\beta)^0$-thalassemia is commonly undiagnosed or misdiagnosed. Our cases highlight the importance of recognizing the clinical features of $(\varepsilon\gamma\delta\beta)^0$-thalassemia and implementing appropriate testing to diagnose and manage this blood disorder.

## Declaration of Competing Interest

The authors declare no conflicts of interest.

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