

Transfusion Dependent Homozygous α -Thalassemia in Patients Associated with Hypospadias in Three Survivors

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Received date: Nov 24, 2014, Accepted date: Jan 23, 2015, Publication date: Jan 30, 2015

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Abstract

Alpha-thalassemia results from a dysfunction of the α -globin gene. Types of mutations include large deletions and point mutations. The most severe form of α -thalassemia is hydrops fetalis, which is caused by homozygosity of certain types of either deletion or point mutations, and some cases were the results of a combination of both. Here, we describe three cases of homozygous α -thalassemia who continue to survive, all with hypospadias. The first two cases were 5-year-old twins that were diagnosed with homozygous SEA deletion and the first description of a 20-month-old child with the genotype of the homozygous Cd 59 (GGC>GAC) mutation of the HBA2 gene. Prognoses for any α -thalassemia mutation types that are known to lead to hydrops fetalis in male fetuses should be informed about the potential survival associated with hypospadias.

Keywords: Homozygous $\alpha 2$ Cd 59 (GGC>GAC); Homozygous SEA deletion; α -thalassaemia major; Hypospadias

Introduction

Alpha-thalassemia disorders are a group of hereditary anemias leading to variable disease severity. The least extreme are asymptomatic due to one or two of four dysfunctional α -globin genes. The most severe form is Hemoglobin (Hb) Bart's hydrops fetalis, where the deletion of all four α -globin genes usually leads to severe anemia with resulting hypoxia, heart failure, and fatal α -thalassemia disease either in-utero or at stillbirth. Initial laboratory testing includes mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) determination, as well as a quantitative assessment of the HbA2 and HbF. Carrier screening is difficult due to unchanged HbA2 and HbF in their hypochromic microcytic red blood cells (RBCs), whereas Hb Bart and Hb H are detectable by Hb electrophoresis. Genotyping known deletions and point mutations of α -thalassemia can be achieved by amplification refractory mutation system (ARMS) and Gap-Polymerase Chain Reaction (Gap-PCR).

Due to severe ineffective erythropoiesis, hydropic fetuses have massive organomegaly, severe albuminemia, heart failure, body edema, growth failure, and intrauterine demise [1]. High incidences of congenital abnormalities, such as limb defects, hydrocephalus, microcephaly, atrial septal defects, pulmonary hypoplasia, hypospadias, and skeletal dysplasia, have also been reported [2]. Survival with Hb Bart's hydrops fetalis is possible following premature delivery and treatment with post-delivery transfusions [3] or intrauterine umbilical vein transfusions (IUT) [4]. A blood transfusion with iron chelator provides symptom relief, whereas allogeneic stem cell transplantation [5] is the only curative therapy for thalassemia.

Recently, an in-utero hematopoietic cell transplantation (IUHCT) was attempted [6].

The intermediate form is Hb H, where three α -globin genes are affected by deletions or by compound heterozygous point mutations. Hb H resulting from a combination of a deletion and a point mutation may have a more severe phenotype than most three-deletional α -thalassemia [7]. Clinically, they are characterized by moderate to severe microcytic, hypochromic, hemolytic anemia, mild jaundice, and moderate hepatosplenomegaly [8]. Hb H may also lead to hydrops fetalis, as presented in a case report by Lorey et al. [9] in a fetus with Cd 35 (TCC>CCC) and α -Filipino deletion [9], as well as in a patient that inherited Mediterranean deletion compounded with α TSaudi (polyA AATAAA>AATAAG) [10]. A more severe outcome was further evident in point mutations of α -thalassemia in a case report by Naiggolan et al. (2010) where hydrops fetalis resulted from the homozygous point mutation Cd 59 (GGC>GAC) [11]. Here, we present cases of surviving children born with hypospadias, one with the same genotype of homozygous Cd 59 (GGC>GAC) and twin siblings homozygous for SEA deletions. They are transfusion-dependent and are managed as thalassemia major patients.

Hypospadias is a congenital malformation in which the urethral opening is displaced along the ventral side of the penis. It is classified into anterior, middle, and posterior hypospadias based on the location of the urethral meatus [12]. The etiology of hypospadias is multifactorial, involving both genes and environmental factors [13]. Different molecular factors have been identified as being associated with syndrome-associated and isolated hypospadias cases. For instance, the homeobox gene A13 (HOXA13) in knockout mice caused hypospadias [14]. The mutation HOXA13 in hand foot-genital syndrome frequently presented with hypospadias [15]; however, no sequence variant was found in hypospadias patients without the

syndrome [16]. Genetic studies have found many candidate genes associated with hypospadias; however, only variants in the X-chromosomal gene DGKK encoding diacylglycerol kinase κ were consistently associated with anterior and middle forms of hypospadias [17-19]. Hypospadias was also reported in male infants with homozygous SEA deletions. It was speculated that the deletion might either generate a gene product arising from the deletion's breakpoint on the chromosome 16p13.3 or create an imbalance transcription on the -14 gene harboring the HS40 promoter of the α -globin [20]. However, no cryptic mRNA and potential exons were found around the deletion breakpoint, and a comparable amount of transcription was found in the -14 gene of SEA deletion/hypospadias survivor cDNAs [21].

Case Report

Case 1

These patients are twin sibling, mentally normal boys. They presented with severe anemia at birth, and they were prematurely delivered at 34 weeks gestation. The first child (twin 1) had a failure to thrive, while the second child (twin 2) had poor growth. Both had organomegaly, requiring monthly blood transfusions, and they were noted to have penoscrotal hypospadias. No pre-transfusion indices were available for either of them. A full blood film of twin 1 showed anisopoikilocytosis and hypochromic RBCs. Many nucleated RBCs, microspherocytes, spherocytes, schistocytes, and polychromatic cells were observed. Occasionally, myelocytes and atypical lymphocytes were seen. In twin 2, the presence of poikilocytosis, hypochromic cells, microcytes, target cells, and nucleated RBCs were seen. Occasional Howell-Jolly bodies and myelocytes were also observed. DNA analysis revealed both parents as carriers of the common SEA deletion, and the twins were both homozygous for SEA deletions.

Case 2

The infant boy was born to a woman of Iban descent, gravida 2, para 1. At term, she had high blood pressure and she underwent a caesarean section for impending eclampsia. During earlier antenatal examinations, her blood pressure was normotensive, and she did not have polyhydramnios or any history of antepartum hemorrhage. The proband was delivered with a weight of 1.68 kg at 35 weeks gestation. His Hb at birth was 7.1 g/dL, and thrombocytopenia was seen. Upon abdominal examination, hepatosplenomegaly was seen. Peripheral blood film revealed anisopoikilocytosis with marked hypochromic, macro, and microcytic cells. Moderate polychromasia was seen with some teardrop, bite, pencil shape, and target cells observed. He was immediately transfused after birth with a subsequent transfusion 9 days later. He was also found to have hypospadias without other abnormalities. Since birth, repeated blood transfusions were given and his recent full blood count showed a retic count of 8%, and H inclusion was not observed. At birth, his enlarged liver and spleen were 6 cm and 5 cm below the costal margins, respectively. His recent examination showed his liver and spleen had shrunk to 2 cm and 3 cm below costal margins, respectively. Hb electrophoresis was not performed at birth, his parents were investigated for any hemoglobinopathies with FBC, and a hemoglobin analysis was performed.

A molecular analysis of DNA sequencing confirmed both parents as carriers of the α 2-globin gene Cd 59 (GGC>GAC) and the index homozygous for Cd 59 (GGC>GAC) (Figure 1). An FBC and

hemoglobin typing analysis of the referred blood samples are shown in Table 1.

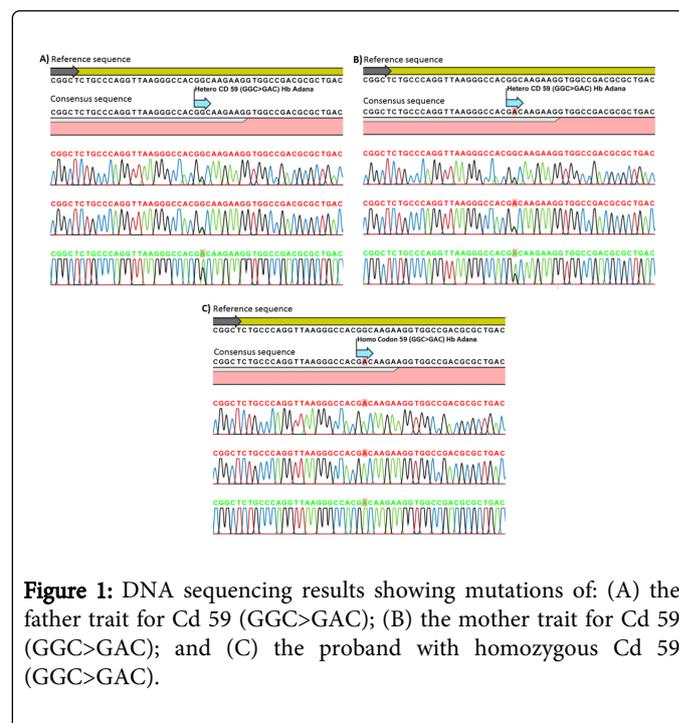


Figure 1: DNA sequencing results showing mutations of: (A) the father trait for Cd 59 (GGC>GAC); (B) the mother trait for Cd 59 (GGC>GAC); and (C) the proband with homozygous Cd 59 (GGC>GAC).

Parameters	Patient*	Mother	Father
Age (year)/sex	1Y10M/M	31/F	36/M
Hb (g/dL)	7.1	14.3	15.1
RBC (106/ μ L)	3.84	5.18	5.6
MCV (fL)	78.9	82.8	80.2
MCH (pg)	21.5	27.6	27.0
MCHC (g/dL)	27.1	33.3	33.6
RDW-CV (%)	NA	NA	NA
HbA (%)	76.7	86.9	85.7
HbA ₂ (%)	2.0	2.6	2.8
HbF (%)	10.3	1.4	1.3

Table 1: Hematological parameters and molecular analysis. NA- not available; *Post-transfusion.

Discussion

Homozygous SEA deletion and the α 2-globin gene Cd 59 (GGC>GAC) are among several α -thalassemia mutation types that cause hydrops fetalis, though the frequencies of homozygous SEA deletions are much higher due to high SEA deletion carriers among Asians. Many cases of non-hydropic newborns have been previously reported, and the most striking associations were hypospadias among the surviving male newborns with known α -thalassemia mutations. The first case was reported in 1985 by Beaudry and his team in a boy of Chinese-descent; however, the exact mutation on the α -globin gene

could not be confirmed due to the lack of DNA analyses. To date, 10 cases of non-hydrops male newborns with homozygous SEA deletions associated with genitalia defects have been reported [22-25]; however, none have been reported involving homozygous Cd 59 (GGC>GAC).

Hb Adana (Cd 59, GGC>GAC) was first encountered in the α 1-globin gene in Turkish patients [26]. Subsequently, the same nucleotide substitution was found in the α 2-globin gene (HBA2) in an Albanian patient [27]. It was also reported that compound heterozygous α 1-globin gene Cd 59 (GGC>GAC) with α^0 -thalassemia-1 deletion leads to severe hemolytic anemia requiring regular blood transfusions [26].

Homozygous α 2-globin Cd 59 (GGC>GAC) was also found to manifest hydrops fetalis [11], thus prompting an extensive molecular diagnosis of mutations within α -, and the β -globin gene which may have ameliorated the clinical outcome of the patient case 2. Our extensive tests involved PCR amplification of multiplex amplification refractory mutation system (MARMS), multiplex Gap-PCR, multiplex ligation-dependent probe amplification (MLPA), sequencing of the α - and β -globin point mutations and deletions, and α -triplication tests. We did not detect any β -thalassemia mutations, deletions, or α -triplication. Then, we were informed by the pediatrician of his accompanying hypospadias. To our knowledge, this is the first report of Hb Adana associated with hypospadias.

Hypospadias was possibly induced in utero, and/or the edema secondary to hydrops fetalis could have led to a failure of the normal fusion of the urogenital folds. Hypospadias could also possibly be due to a defect of another gene located at the chromosome 16p13.3 region [24]. Genetically, syndrome-associated hypospadias could probably be due to the different mutations in a single gene that can cause different phenotypes due to unknown genetic factors involved in genital determination and/or due to a differentiation located in an altered chromosomal location due to inversion, deletion, or insertion mutations [28]. However, homozygous Cd 59 (GGC>GAC) is a point mutation (base substitution) not involved in the fusion of an altered gene at the chromosome 16p13.3.

The variable outcomes of the same mutation types show the complex factor influencing disease severity. The predictive outcomes of α -thalassemia based on mutation types are not always straightforward; thus, it complicates genetic counselling. Prenatal care for the fetus for homozygous α -thalassemia leading to hydrops fetalis must also include an examination of genital formation. Parent carriers of SEA deletion and α 2-globin Cd 59 (GGC>GAC) should be informed of the possible survival of their fetus if hypospadias is observed.

Conclusions

We report our cases of non-hydropic homozygous α -thalassemia SEA deletions, as well as the first case of homozygous α 2-globin Cd 59 (GGC>GAC), leading to transfusion-dependent α -thalassemia major that occurred in Chinese twin boys and an Iban ethnic boy, respectively. This case provides valuable information regarding the clinical manifestations of such genotypes when associated with hypospadias.

Acknowledgments

The authors would like to thank Director General of Health for the permission to publish this case report. We would also like to thank the

National Prevention and Control Program for Thalassemia Fund for supporting our efforts.

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