

Unusual survival of a twin with homozygous α^0 -thalassemia due to chimerism

Hydrops fetalis is a common birth defect in obstetrics. Non-immune hydrops fetalis (NIHF) accounts for almost 90% of cases of hydrops and its prevalence is estimated to be 1 in 1,700-3,000 pregnancies.¹ Worldwide, cardiovascular abnormalities are the most common cause of

NIHF and account for approximately 20% of cases. However, in the Southeast Asia (SEA) region, which includes southern China, homozygous α^0 -thalassemia is the most common cause of NIHF (approximately 55%), since α -thalassemia has a high prevalence in this region.² It has been reported that there are at least 26,000 at risk pregnancies and 6,600 affected fetuses per year.³ Consequently, homozygous α^0 -thalassemia is a key disease for prenatal diagnosis in this region.

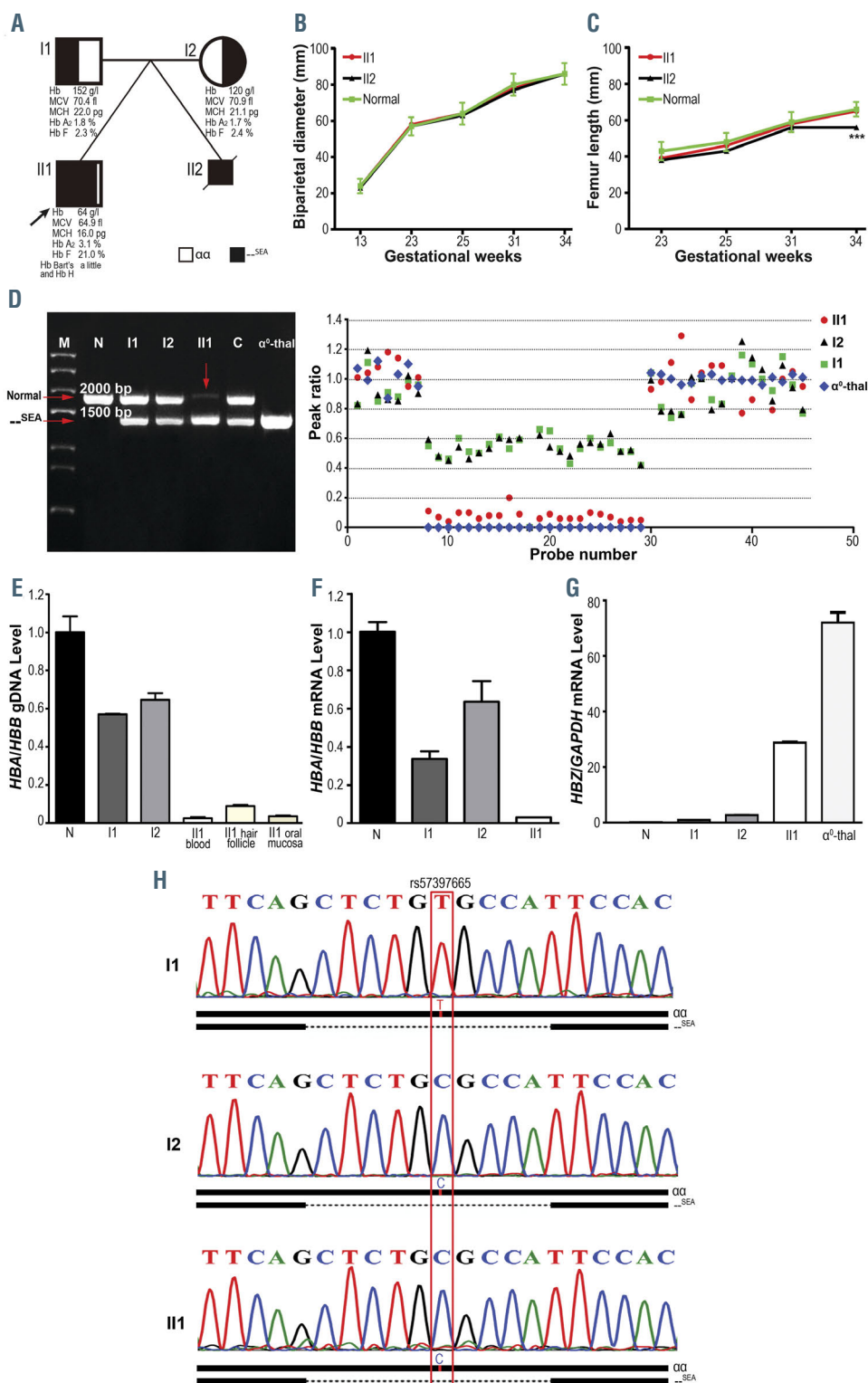


Figure 1. The family pedigree, growth status of the twins *in utero* and the molecular analysis of α -globin gene in patient II1. (A) Pedigree of the Zhaoqing family. The patient II1 is labeled with an arrow. The hematological phenotype data of I1, I2 and II1 are listed. The II1 patient had a blood transfusion. (B) The biparietal diameter of the twins *in utero*, compared to the standard population.^{7,8} (C) Femur length (FL) of twins *in utero*, compared to the standard population.^{7,8} The II2 patient's FL did not increase after gestational week 31. (D) The gap-polymerase chain reaction and multiplex ligation-dependent probe amplification (MLPA) results for the family. The normal band (1,800 bp) and α^0 -SEA band (1,300 bp) were observed in I1 and I2. A faint normal band was also observed in II1, along with a bright α^0 -SEA band (left). Scattering of II1 (red circles) shows the low level of the α -globin gene in the SEA deletion region, from probe 8 to probe 29 (chr16:217274-231141, hg19), while α^0 -thal samples (blue rhombus) usually drop to "0" in this region (right). (E) The quantitative analysis of the α -globin gene shows the existence of the gene in genomic DNA (gDNA) from blood, hair follicle and oral mucosa samples from II1. The expression of α -globin mRNA (F) and ζ -globin mRNA (G) were detected in blood cells from II1. (H) Haplotype analysis of one SNP in the SEA deletion region shows that the residual α -globin gene in II1 was inherited from his mother (I2). M: marker; N: normal control; α^0 -SEA: carrier of α^0 -SEA/ $\alpha\alpha$ control; α^0 -thal: Homozygous α^0 -thalassemia control; SEA: Southeast Asian; SNP: single nucleotide polymorphism.

Most homozygous α^0 -thalassemia incidences in SEA are caused by a homozygote SEA deletion ($-^{SEA}/-^{SEA}$).⁴ In homozygous α^0 -thalassemia patients, absence of α -globin results in aggregations of Hb Bart's (γ_4), which cannot release oxygen in hypoxic tissue due to its extremely high oxygen affinity. This can damage maturing erythroid precursors and causes ineffective erythropoiesis.^{4,5} Most homozygous α^0 -thalassemia fetuses die in week 24 to 38 of gestation or shortly after delivery. Recently, increasing numbers of homozygous α^0 -thalassemia survivors have been reported worldwide.^{3,6} However, most underwent at least one intrauterine intervention and the mechanism of homozygous α^0 -thalassemia survival, without intrauterine intervention, has been little explored.

Here, we report twins with homozygote α^0 -thalassemia: the elder twin (II1 in Figure 1A) survived, whilst his twin brother (II2) was a hydrops fetus who died. Further analysis found that the elder twin's index was significantly better than that of the younger and other reported survivors with homozygous α^0 -thalassemia (*Online Supplementary Table S1*) due to a chimera with a small amount of functional α -globin. It is the first discovery of a homozygous α^0 -thalassemia survivor related to chimerism and it is also a rare report which explores the mechanism of natural survival of homozygous α^0 -thalassemia patients.

The II1 was a 2-year-old boy from Zhaoqing city, in the Guangdong Province, southern China, and was diagnosed with homozygote of α^0 -thalassemia at 16 days old, but he survived naturally, until birth, without intrauterine treatment. His parents were carriers of the SEA deletion (hematological data showed in Figure 1A). The mother, who was 20 years old at conception, received routine antenatal care in a local hospital. This was her first pregnancy and she did not receive the specific fetal DNA analysis for thalassemia as she did not know that she was a carrier at that time. The twin had a normal ultrasonic index before the gestational age (GA) of 31 weeks, compared with the standard population.^{7,8} Hydrops, placentomegaly and cardiomegaly were not detected (*Online Supplementary Table S2*; Figure 1B and C). However, the femur length of II2 did not increase after 31 weeks (Figure 1C). The mother had some complications, such as polyhydramnios and pre-eclampsia (*Online Supplementary Table S3*), but the symptoms were not serious before 31 weeks.

The twins were born by cesarean section at 34 weeks due to severe maternal pre-eclampsia and the development of symptoms of polyhydramnios and ascites in II2. After birth, II2 showed abdominal distension and tension and died quickly. The clinical features of abdominal distension, hepatosplenomegaly and anasarca led to II2

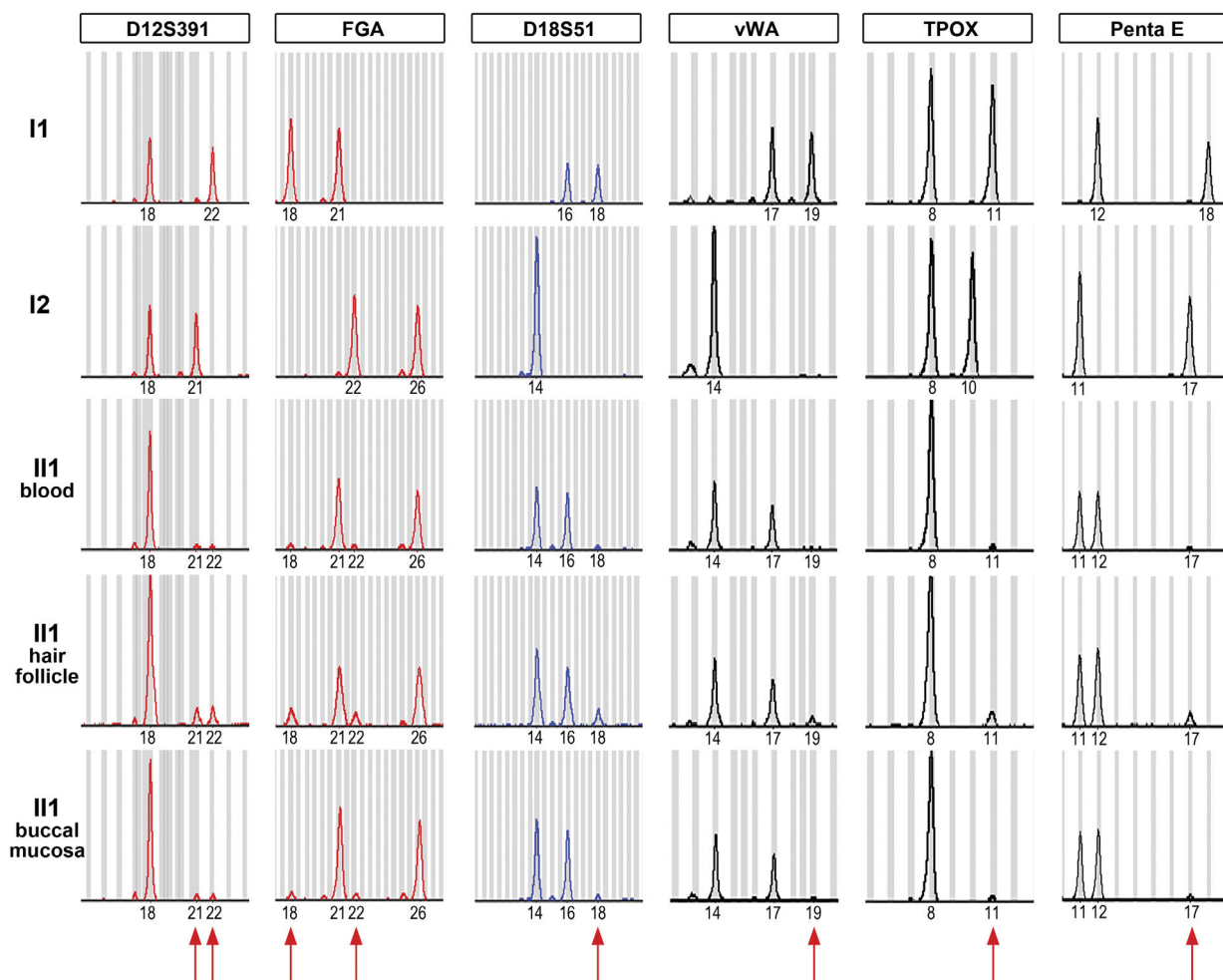


Figure 2. Chimeric ratio analysis by polymerase chain reaction of short tandem repeat loci. There are more than two short tandem repeat (STR) peaks at one STR locus in the blood, hair follicle and oral mucosa sample from II1 and all of them were inherited from his parents.

being diagnosed with lethal hydrops fetalis. The birth weight of II1 was 1.9 kg, which is approximately the same as a normal child born at 34 weeks. The Apgar scores were normal and a physical examination showed that he had no congenital abnormalities, except for hydrocele. He was cared for as a premature infant in the neonatal department for 16 days. He was not transfused for the first 24 hours and did not enter the intensive care unit. The full postpartum clinical characteristics of the twins are summarized in the *Online Supplementary Table S1*.

The II1 received regular blood transfusions once a month from the age of 14 days due to anemia, and the anemia countenance and splenomegaly was comparable to a 1.5-year old. He grew normally, weighed 12 kg and was 85 cm in height at the age of 2 years. This matches the data of normal peers in southern China. He also had

normal intellect, in accordance with the evaluation standard of the Chinese population.⁹

In order to clarify the reasons for the difference between the twins, we performed molecular analysis. Preliminary thalassemia mutation analysis in a local hospital showed that the parents were both heterozygous and II1 was homozygous for the SEA deletion without β -globin gene mutations. The II2 twin was not tested due to lack of sample, but his symptoms indicated that he was also likely to be homozygous for the SEA deletion. However, in our laboratory, a residual β -globin gene was detected in the blood cells of II1, based on the results of gap-polymerase chain reaction (gap-PCR) and multiplex ligation-dependent probe amplification (MLPA) analyses (Figure 1D). This residual β -globin gene was also found in II1's blood, hair follicle and oral mucosa cells with a different level ($2.58 \pm 0.64\%$, $8.91 \pm 0.65\%$ and $3.58 \pm 0.49\%$,

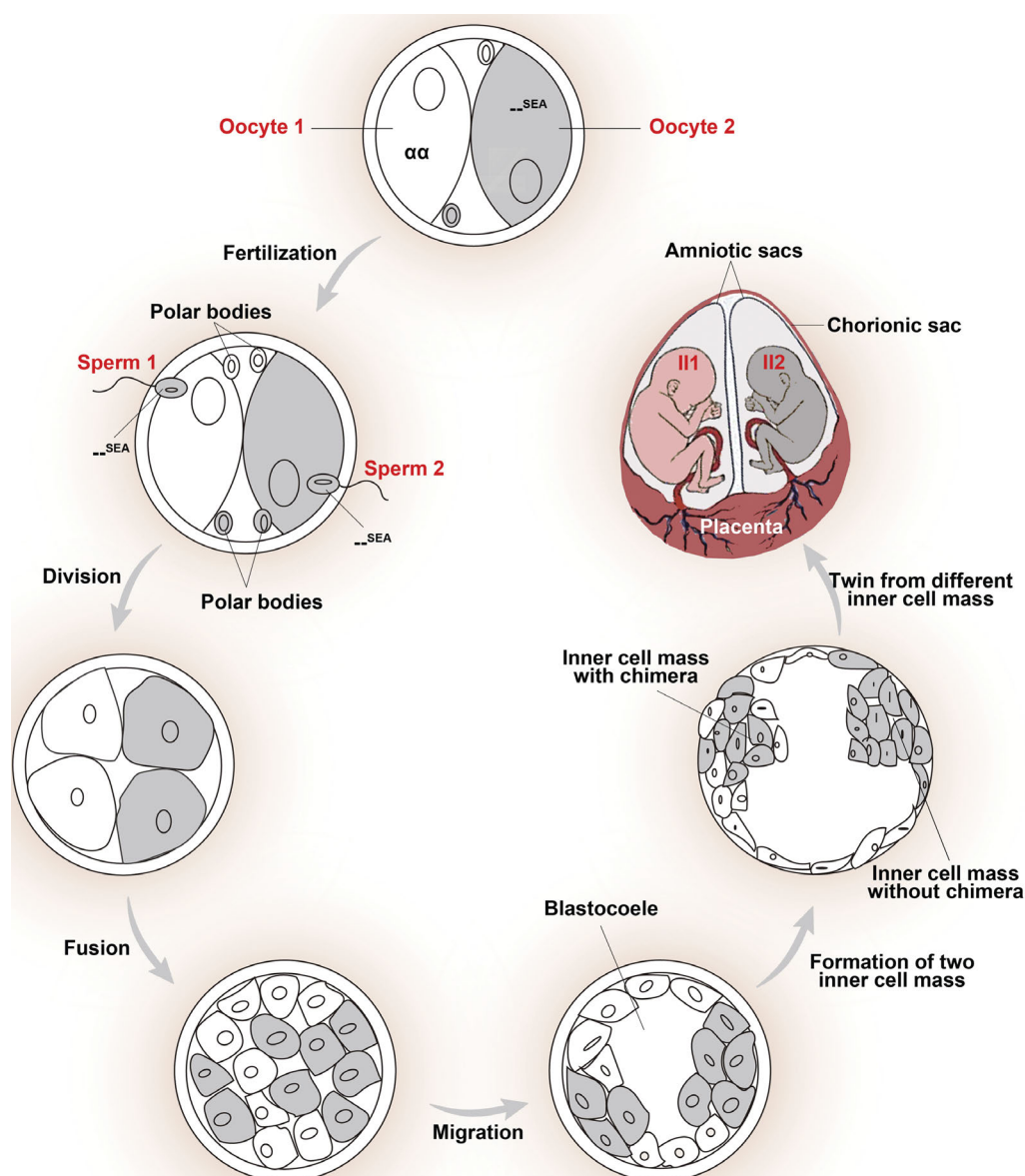


Figure 3. A hypothetical model to explain the etiology of the chimerism in II1. Two oocytes arise in a single *zona pellucida* and are fertilized by two individual sperm. The fertilized eggs divide and then the blastomeres fuse during the morula stage. Two inner cell masses are formed along with the division and migration of the blastomeres in the blastocyst (whether the trophoblastic layer is chimeric is unclear due to a lack of research). Two individuals then form from the inner cell mass, one of which (II1) is a chimera with $-\text{SEA}/-\text{SEA}$ and $-\text{SEA}/\alpha\alpha$ and the other (II2) carries the $-\text{SEA}/-\text{SEA}$ genotype.

respectively, analyzed by quantitative reverse transcriptase PCR [qRT-PCR] (Figure 1E). Meantime the qRT-PCR analysis of the mRNA level in II1's blood showed a $3.16 \pm 0.48\%$ expression level of this residual α -globin gene (Figure 1F), and a low expression level of ζ -globin (embryonic α -like globin) gene comparing with other homozygous α^0 -thalassemia patient (Figure 1G). In addition, the haplotype analysis also confirmed that this residual α -globin gene was inherited from his mother (Figure 1H). Therefore, II1 was assumed to be a chimera with two genotypes: $--^{SEA}/--^{SEA}$ (major) and $--^{SEA}/\alpha\alpha$ (minor), which is the same as analysis result of six short tandem repeat (STR) loci showing a low degree of chimerism in II1's blood, hair follicle and oral mucosa samples ($5.22 \pm 0.83\%$, $19.32 \pm 1.03\%$ and $6.70 \pm 0.28\%$ in cells with the $--^{SEA}/\alpha\alpha$ genotype, respectively) (Figure 2).

An international registry of homozygous α^0 -thalassemia survivors has proved that intrauterine transfusion increases the chance of survival *in utero*.³ In addition, the persistent expression of the ζ -globin gene was assumed to be one explanation for the phenotypic diversity in homozygous α^0 -thalassemia survivors. However, the II1 survived naturally, until birth, without intrauterine treatment, and the expression level of the ζ -globin gene does not increase comparing with other homozygous α^0 -thalassemia patient (Figure 1G). Previous research had shown that even a very small amount of chimerism of the Y chromosome can make girls appear with ambiguous genitalia or hermaphroditism.^{10,11} Therefore, we considered a low level of expression (approximately 3% of that of normal individuals, Figure 1F) of α -globin, originating from approximately 5% chimerism of $--^{SEA}/\alpha\alpha$ in blood cells (Figure 2), can ensure a natural birth and significant improvement in growth and neurodevelopment.

We have further considered the generation of chimerism. The II1 and II2 patients were monozygotic twins, based on the ultrasound findings and STR loci analysis. Previous research¹² indicated that there are three hypotheses to explain the generation of chimeric monozygotic twins. Hypothesis 1: placental anastomoses result in inter twin transfer of blood cells, with subsequent blood cell chimerism. However, the STR analysis of II1 indicated that the chimeric ratio of hair follicle cells is higher than that of blood cells (Figure 2). Hypothesis 2: fusion of elements of two genetically distinct zygotes. This is more normal in an assisted reproductive technologies pregnancy, whilst our case was a natural pregnancy. Therefore, we propose a model (Figure 3) based on hypothesis 3: "fertilization of a binovular follicle", were two oocytes arising in a single *zona pellucida* are fertilized by two individual sperm and form two inner cell masses by division, fusion and migration. The cell mass then differentiates into two fetuses: II1 (chimera, survives) and II2 (non-chimera, deceased).

In summary, this is the first report of a homozygous α^0 -thalassemia patient who survived, without intrauterine intervention, due to chimerism. This data may be valuable in providing accurate diagnosis and genetic counseling in similar cases.

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Contributions: DP, DC, YC, XW and FH collected samples and performed the research; DP, FY and HL analysed the data; DP, XS and XX designed the study and wrote the paper; XX supervised the research and all authors reviewed, edited and approved the manuscript.

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