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

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Supplemental Information

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Methods

Subjects and clinical data collection

The patient (II1) was a two year old boy from Zhaoqing city, in Guangdong Province, southern China (Figure 1A). He was diagnosed as a thalassemia patient at 16 days old and received blood transfusions once a month. The conventional biochemical investigations did not reveal anything unusual, such as G6PD, CK, AST, LDH, CRP, PT, Electrolyte, TBIL, TBA, T3, etc. Detection of common α-globin and β-globin gene mutations in Chinese populations identified him as homozygous for the SEA deletion ($--^{SEA}/--^{SEA}$), which is the most common genotype of homozygous α^0-thalassemia patients in China. His parents were not aware that they carried the SEA deletion ($--^{SEA}/\alpha\alpha$) until the diagnosis of II1. This meant that the mother received routine antenatal care and prenatal diagnosis of thalassemia was not performed. The younger twin, II2, died soon after birth and homozygous α^0-thalassemia was proposed as a possible cause, due to the hydropic features at birth and the genotype of his parents. Twin to twin transfusion syndrome was excluded due to normal amniotic fluid volume and bladder of both twins before 31 weeks of gestational age, using ultrasound (Table S2). However, further diagnosis of II2 was refused by his parents. With approval from the Ethics Committee of Nanfang Hospital of Southern Medical University and with informed consent, in accordance with the Helsinki declaration, the peripheral blood leucocytes from II1 and

his parents, II1's hair follicle and buccal mucosa were obtained for further study in our laboratory. Hematological parameters were analyzed as in a previous study[1]. Data on patient and parent characteristics, availability of antenatal care, antenatal ultrasonographic findings, perinatal course, presence of congenital malformations, subsequent neonatal and long-term neurodevelopmental outcome were collected. These data were compared with the data from 69 cases of homozygous α^0-thalassemia survivors[2, 3] and 308 cases of Hb H disease[4, 5].

Molecular analysis

Genomic DNA was extracted with the TIANamp Micro DNA Kit (DP316, TIANGEN, China). Total RNA was isolated with TRIzol Reagents (Thermo Fisher Scientific, USA), in accordance with the manufacturer's instructions.

The β-globin gene mutations were analyzed by direct DNA sequencing. Three common α-thalassemia deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$ and $--^{SEA}$) were detected by Gap-PCR. To detect copy number variations, a multiplex ligation dependent probe amplification (MLPA) assay (SALSA P140-C1 and P102-C1 kit, MRC-Holland, Amsterdam, Netherlands) was performed. Threshold ratios for deletion and duplication were set at <0.7 and >1.3, respectively.

The DNA and RNA levels of the α-globin gene and β-globin gene were

determined by quantitative real-time PCR (qRT-PCR), using the protocol provided by the manufacturer (Takara, Japan).

To analyze the source of the α-globin gene in the patient, the single nucleotide polymorphism (SNP) of rs57397665 by direct DNA sequencing was selected as it is inconsistent between the parents and located in the SEA deletion region. To analyze the source and ratio of chimerism, we used the technology of PCR-short tandem repeat (PCR-STR) as described in previous study[6]. The 19 STR loci and a segment of the X-Y homologous amelogenin gene were co-amplified using the Microreader™ 20A ID System (Microread Genetics Incorporation, China), following the manufacturer's instructions. Six STR loci (D12S391, FGA, D18S51, vWA, TPOX and Penta E) were selected, which chimeric genotype must be identified and STR peak must not be disturbed by stutter peaks. All primers for the PCR assay are listed in Table S4.

Table S1 General clinical characteristics of II1 and II2

Index	II1	II2	Normal	Deletional Hb H disease[4, 5]	No-deletional Hb H disease[4, 5]	Survivors[$] with homozygous α^0-thalassemia [2, 3]
Perinatal period						
Polyhydramnios	No	Yes	No	No	No	15%
Intrauterine intervention	No	No	No	No	No	No
Gestational weeks at birth	34	34	38-42	38-42	38-42	23-39
Means of delivery	Caesarean	Caesarean	NA	NA	NA	Caesarean (57%)
Birth weight of ≥ 10th centile	Yes	NA	Yes	Yes	Yes	81%
Hydropic feature at birth	No	Yes	No	No	No	55%
Neonatal period						
Apgar scores 1 min	9	1	7-10	7-10	7-10	0-6
Apgar scores 5 min	10	1	7-10	7-10	7-10	1-8
Required mechanical and/or assisted ventilation	Yes	NA	No	No	No	66%
Duration of mechanical and/or assisted ventilation required (days)	14	NA	No	No	No	4-60
Transfusion with the first 24 hours	No	NA	No	No	No	Yes
Congenital malformations						
Urogenital abnormalities	Hydrocele	NA	NA	NA	NA	48%

Limb abnormalities	No	NA	NA	NA	NA	16%
Atrial septal defect	No	NA	NA	NA	NA	10%
Long-term outcomes						
Growth retardation	No	NA	No	Rare	15%	approximately 50%
Neurodevelopmental retardation	No	NA	No	NA	NA	43.5%
Hemoglobin data						
Hemoglobin (g/dL)	9.5	NA	NA	5.7-10.2	4.6-9.9	3.7-8.0
Age at first transfusion	14 d	NA	NA	11 ± 5.5 Y	1.5 ± 2.1 Y	1 d
Transfusion frequency per year	12	NA	NA	0-10.7	0.12-8.0	10.4-26
Hepatosplenomegaly after transfusion	Yes	NA	NA	NA	NA	Yes

NA not available.

[$] These survivors did not receive intrauterine intervention.

Table S2. Pre-natal clinical characteristics of II1 and II2

Anatomic area	Gestational weeks							
	13		23		25		31	
	II1	II2	II1	II2	II1	II2	II1	II2
Head	Roundness and ventricle without dilation		Intact ring of skull, centered line of cerebrum, ventricle without dilation, posterior cranial fossa without dilation and visible muffle					
Neck	NA		With no impression and streamer of umbilical cord					
Spine	Vertebrae in order							
Chest	Normal shape		Normal cardiothoracic proportion, regular heart rhythm, normal atrioventricular ratio and cardiac structure					
Abdomen	Normal shape		Normal liver, stomach vesicle, double kidney and bladder					
Extremities	Visible		Visible long bone and without abnormal shape				Some limbs unclear[$]	
Umbilical cord[&]	NA		2.78[a]	2.88[a]	3.25[a]	3.33[a]	2.86[a]	2.88[a]
	NA		145[b]	152[b]	146[b]	150[b]	153[b]	145[b]

Placenta[!]	22		26		37		30	
Amniotic fluid[&@]	48	47	62	63	70	72	68	73

NA, not available.

[$] Due to fetal position and gestational age

[&] These data are unclear between II1 and II2 due to the lack of tags

[a] Ratio of fetal umbilical artery systolic and diastolic

[b] Heart rate (beats per minute)

[!] Location in anterior wall of the uterus and thickness (millimeter)

[@] The largest diameter line (millimeter)

Table S3. Maternal complications

Complication[$]	Maternal condition
Previous pregnancies	
Abortion	No
Still birth/neonatal death	No
Antepartum	
Polyhydramnios	Yes
Oligohydramnios	No
Intrauterine infection	No
Preeclampsia	Yes
Abruptio placenta	No
Delivery	
Preterm delivery	Yes
Malpresentation	Yes
Assisted vaginal delivery	No
Caesarean section	Yes
Postpartum	
Postpartum hemorrhage	No

[$] Reference from report of Songdej D, Blood, 2017

Table S4. Primer sequences for molecular studies

Primer name	Primer sequences (5’-3’)
βF	AACTCCTAAGCCAGTGCCAGAAGAGC
βR	ATGCACTGACCTCCCACATTCCCT
α2/3.7-F	CCCCTCGCCAAGTCCACCC
α2-R	AAAGCACTCTAGGGTCCAGCG
3.7-R	AGACCAGGAAGGGCCGGTG
4.2-F	GGTTTACCCATGTGGTGCCTC
4.2-R	CCCGTTGGATCTTCTCATTTCCC
SEA-F	CGATCTGGGCTCTGTGTTCTC
SEA-R	AGCCCACGTTGTGTTCATGGC
SNP-F	CACCCCCAGAAGAGACCAAA
SNP-R	GTGCATCCCTGCTCATGAAA
α-F1	CTGACTGTGAGTCGGCCAAA
α-R1	AGTTCTCCAAACTACCGGGC
β-F1	GAAATTGGACAGCAAGAAAGCGA
β-R1	AAGAATTCACCCCACCAGTGC
α-F2	TGCCGACAAGACCAACGTCA
α-R2	GTGGGGAAGGACAGGAACAT
β-F2	ATGGTGCATCTGACTCCTGA
β-R2	TGGACAGATCCCCAAAGGAC
ζ-F	CGGTCAACTTCAAGCTCCTGT

ζ-R	CGGTCAGGACAGAGGATACG
GAPDH F	ACCCACTCCTCCACCTTTGA
GAPDH R	CTGTTGCTGTAGCCAAATTCGT

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