Pitfalls in prenatal diagnosis of β thalassaemia

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SUMMARY In this paper, we report a pregnancy at risk for β thalassaemia in which the fetal red blood cell volume was reduced while that of the mother was relatively great, so that the presence of a fetal red blood cell population in a mixed maternal-fetal sample was difficult to recognise. The molecular basis for these haematological phenotypes was clarified by follow up examination and α globin gene mapping. These indicated that the fetus was heterozygous for β thalassaemia and had deletion of three α globin structural genes, while the mother, heterozygous for β thalassaemia, also had deletion of two α globin structural genes. When the coinheritance of α thalassaemia is suspected, it is necessary to examine carefully the red blood cell distribution of a placental sample, so that the presence of a population of fetal red blood cells is not missed.

Prenatal diagnosis of β thalassaemia may be accomplished by amniocyte or trophoblast DNA analysis¹⁻⁵ or globin chain synthesis analysis on fetal blood.⁶⁻¹⁰ The presence of fetal red blood cells in the blood samples, obtained by placental aspiration or fetoscopy, is immediately monitored by means of analysis of the red cell volume distribution. The larger fetal red blood cells are easily differentiated from the microcytic red blood cells of the mother who is heterozygous for β thalassaemia.

In this paper, we report a case of prenatal diagnosis of β thalassaemia in which the differentiation between maternal and fetal red blood cell volume distribution was not clear cut, as the fetal erythrocytes had a pronounced reduction of red blood cell volume. Follow up examination and α globin gene mapping showed that this fetus was heterozygous for β thalassaemia and had deletion of three out of the four α globin structural genes, while the mother had deletion of two α globin structural genes in association with β thalassaemia trait.

Methods

Fetal blood sampling was carried out by placental aspiration with a 20 gauge needle at 19 weeks' gestation, according to previously described methods.¹¹ The presence of fetal red blood cells in the placental sample was immediately monitored by means of analysis of the red blood cell volume distribution with the Coulter Channelyzer (Coulter Electronics, Hialeah, Florida). Fetal red cell enrich-

ment was performed with $\text{NH}_4\text{Cl-NH}_4\text{HCO}_3$ differential lysis of maternal cells, and fetal blood analysis was carried out by globin chain synthesis on carboxyl-methyl-cellulose columns, as described previously.^{6,7,12,13} DNA was isolated from leucocytes according to Goossens and Kan.¹⁴ The α globin genotype was determined by digesting the DNA with *Bam*HI or *Bgl*II and hybridising with an α and ζ globin specific probe respectively.¹⁵⁻¹⁸ We used as the α globin gene probe a nick translated α globin cDNA cloned in the plasmid JW101.¹⁵ The ζ globin gene probe was a nick translated *Hinf*I fragment of pBr ζ plasmid, which is complementary to the whole ζ globin gene excluding the 5' part of the first exon.¹⁹

Case report

A couple, both of whom were carriers of β thalassaemia, requested prenatal diagnosis. As shown in table 1, both parents had haematological findings indicative of high Hb A₂ β thalassaemia trait. However, the mother showed a MCV of 78 fl, which is a borderline value, much higher than that usually found in β thalassaemia heterozygotes.

TABLE 1 Haematological findings.

	RBC ($\times 10^{12}/l$)	Hb (g/dl)	MCH (pg)	MCV (fl)	Hb A ₂ (%)	Hb Bart's (%)
Mother	4.44	12.0	27.1	78	5.54	----
Father	5.34	13.9	26.0	75	5.50	----
Infant						
At birth	7.58	18.7	24.8	82	0.46	19.45
At 5 months	6.62	11.1	16.7	51	4.01	2.1

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The placental sample, obtained by needling, was analysed for the presence of fetal red blood cells with the Coulter Channelyzer. At first sight, an apparently single distribution curve, partially overlapping that of maternal peripheral blood, was noted (figure). On closer inspection, however, the curve showed two peaks, one of which corresponded to that of the maternal red blood cell distribution; the other was slightly shifted to the left indicating the presence of a population of fetal red blood cells. The elimination of maternal cells from the placental sample was carried out with Ørskov lysis,²⁰ by which a pure sample of fetal red blood cells was obtained. The absence of maternal red blood cell contamination was confirmed with the Kleihauer method.²¹

The mean MCV of the fetal red blood cells was 87 fl, which is lower than the normal value (135 ± 0.2) at this gestational age.²² The β/γ ratio was 0.046, which indicates the heterozygous state for β thalassaemia. The $\alpha/\beta+\gamma$ ratio was 0.66 (normal value = 0.87 ± 0.09). Haematological examination of the

TABLE 2 α globin gene mapping.

	ζ probe		α probe		α globin genotype
	BglII	BamHI	BglII	BamHI	
Father	11.5-12.0 14.0	10.5-5.4	12.0-7.5	14.0	-/- $\alpha\alpha$
Mother	11.0-16.0	10.0-5.4	16.0	10.5	- α /- α
Proband	11.0-14.0 16.0	10.0-5.4	16.0	10.5	-/- α

infant at birth (table 1) showed microcytosis, hypochromia, and Hb Bart's of around 20%. At 5 months, this infant had pronounced hypochromia, microcytosis, Hb A₂ levels within the range of β thalassaemia heterozygotes, and Hb Bart's of around 2.1%; Hb H was not detected. These findings indicate that the fetus had the association of Hb H disease phenotype and heterozygous β thalassaemia. α globin gene mapping substantiated this diagnosis, showing that the proband had deletion of three α globin structural genes (-/- α). The mother was homozygous for deletion α^+ thalassaemia (- α /- α), while the father was heterozygous for α^0 thalassaemia (-/- $\alpha\alpha$) (table 2).

Discussion

This paper describes a pregnancy at risk for β thalassaemia, in which the fetal red blood cells showed a pronounced reduction of cell volume. The presence of fetal microcytosis in association with relatively large maternal red blood cells compared to those of classical β thalassaemia heterozygotes resulted in difficulty in recognising the presence of a fetal red blood cell population in a mixed maternal-fetal blood sample. Globin chain synthesis analysis showed a β/γ globin synthesis ratio within the range of β thalassaemia heterozygotes. The pronounced microcytosis made us suspect the presence of associated α thalassaemia. α globin gene mapping and follow up examination confirmed, in fact, that this fetus was heterozygous for β thalassaemia and showed, in addition, deletion of three out of the four α globin structural genes. At haematological maturity, this infant developed the haematological phenotype of β thalassaemia trait, with pronounced microcytosis and low Hb Bart's. The interaction of β^0 thalassaemia trait with the deletion of three α globin structural genes has already been described.²³ As in our case, the clinical phenotype was that of severe β thalassaemia trait. The mother, a carrier of a β thalassaemia gene, also showed deletion of two α globin structural genes, which explains the presence of relatively large maternal red blood cells, as compared to β thalassaemia heterozygotes with a full complement of four α globin structural genes.²³⁻²⁵

FIGURE Red blood cell volume distribution of the placental sample before (a) and after (b) Ørskov lysis.

The practical implication of our observation is to suspect the presence of associated α thalassaemia whenever the mean red blood cell volume of the mother is relatively large, and in these circumstances to make an accurate evaluation of the red blood cell distribution of the placental sample, in order that the presence of a population of fetal red blood cells is not missed.

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