A family study of congenital X linked sideroblastic anaemia

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Abstract

We report on the cytogenetic findings in a family study of pyridoxine responsive, X linked sideroblastic anaemia. An increase in the number of X chromosomes was observed in a small proportion of metaphases prepared from five female members, but these findings did not strictly correlate with the carrier status of the condition. No consistent cytogenetic abnormality could be identified or associated with this rare familial condition. The diagnosis and counselling of carriers of this condition is discussed.

Congenital X linked sideroblastic anaemia (SA) is a rare, well recognised condition consisting of a hypochromic microcytic anaemia with deformed erythrocytes affecting the male members of families. The anaemia is often responsive to pyridoxine, which significantly improves the well being of these patients, although it does not return the haemoglobin or the red cell values to normal. The condition is diagnosed by the presence of a hypochromic, microcytic anaemia associated with ringed sideroblasts in the bone marrow. The female carriers are generally not anaemic and typically show two populations of red blood cells (normocytic, normochromic and hypochromic, microcytic) yielding a dimorphic appearance on the blood film.¹ The carrier prevalence and incidence of affected births are unknown.

The condition has a variable clinical course and can cause death in infancy. Dangers in adult life arise from the accumulation of iron owing to increased iron absorption to support an increased rate of ineffective erythropoiesis, inappropriately

prescribed iron tablets, and blood transfusions. Since the female carriers also have an increased rate of erythropoiesis in order to compensate for the ineffective nature of half of their red cell production, they are also thought to be at risk of iron overload in later life,² especially if iron tablets are taken unnecessarily over long periods of time.

The primary defect in congenital X linked SA is not known. Decreased delta aminolaevulinic acid synthase (ALAs) and haem synthase activities are frequent findings, but there is as yet no convincing or substantiated evidence for the primary abnormality being an abnormal or absent enzyme of haem synthesis.³ The two populations of red cells in the female carriers of this condition argue against the inheritance being via maternal mitochondria; therefore, some abnormality must reside on the X chromosome. It was hoped that cytogenetic studies on stimulated lymphocytes from the members of the family described here would show a visible X chromosome abnormality that would be informative regarding the position of the affected gene on this chromosome, would help in neonatal and prenatal diagnosis of the condition, and would help focus more detailed DNA studies. The locus for idiopathic acquired sideroblastic anaemia (IASA) is believed to be at Xq13.⁴

Case report

We report a study of a family with X linked sideroblastic anaemia. The proband II.5 (fig 1) presented at the age of 8 years with hypochromic, microcytic anaemia (Hb=7.5 g/dl), normal white cells, normal platelets, and slight splenomegaly. Thalassaemia was excluded by the presence of normal levels of HbA₂ and HbF and a normal globin chain synthesis ratio.⁵ Unstable haemoglobin tests were negative. Serum ferritin levels were increased. Bone marrow examination showed 75% ringed sideroblasts, thus confirming the diagnosis of sideroblastic anaemia.

Further investigations indicated a low red cell protoporphyrin level. Addition of ALA increased erythroblast iron incorporation into haem but did not restore it to normal, nor did it prevent the production of ringed sideroblasts in vitro.⁶ In vitro

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Figure 1 Family pedigree. II.5 is the proband.

bone marrow protoporphyrin production in the presence of desferrioxamine to block haem synthase was increased much more than normal by the addition of ALA. The enzymes between ALAs and haem synthase are therefore present and active, but there seems to be a greater than normal block at ALAs. These studies suggest a dual defect or inhibition of erythroblast ALAs and haem synthase, typical of this condition.³

Treatment with pyridoxine (200 mg/day) increased his haemoglobin level by 2 to 4 g/dl to a steady state value of about 9 g/dl. His red cell protoporphyrin increased to normal and his MCV increased slightly. Although his mother showed only one population of red cells and normal haematology, his sister and her daughter showed two populations of red cells by Coulter Channelyzer studies and otherwise normal haematology, consistent with their being carriers of X linked sideroblastic anaemia.

An extended family study was then undertaken (fig 1). No other affected males were found. Altogether, five female carriers were identified by the presence of a dimorphic population of cells seen on blood films, characterised by a bimodal distribution of the red cell size distribution curve (fig 2) obtained with an automated blood counter (Technicon H1). Serum B12, folate, and ferritin concentrations were normal. The proportion of red cells in the peripheral blood of the carriers that were abnormal varied greatly. Only in the woman with the highest number of abnormal cells (III.3) did the MCV and MCH become low. None was anaemic. Those women who were detected as female carriers and had not already received counselling were referred to their regional medical genetics service.

Cytogenetic findings

One hundred metaphases from each member of the family except III.6, III.7, and III.9 were studied. In no case was there any abnormality found that could be linked with the sideroblastic abnormality found

Figure 2 Red cell size distribution curves of the proband (II.5) (Hb 9.3 g/dl, MCV 52 fl, MCH 16.1 pg), and two female carriers: III.1 (Hb 12.5 g/dl, MCV 81 fl, MCH 27.1 pg) and III.3 (Hb 12.2 g/dl, MCV 78 fl, MCH 25.9 pg). That of II.5 was obtained on a different machine from those of III.1 and III.3 and the scales along the bottom are similar but not identical.

in the proband and the carriers. In particular, high resolution analysis showed no deletion or rearrangement at Xq13. However, there were some changes. In family member I.1 two metaphases were seen with trisomy 8, one of which also contained a deletion of 5q in the region q13-q22, features characteristically observed in the bone marrow of patients with myelodysplastic syndromes.⁷ No other significant chromosome rearrangements were seen in any family member. The incidence and distribution of gaps and breaks appeared consistent in all cases with that expected after induction of fragile site expression by methotrexate and thymidine synchronisation. Greater than expected X chromosome gain⁸ was observed in five females (I.1, II.1, II.2, II.4, and III.1) but this did not strictly correlate with carrier status of this condition (table). No X chromosome gain was seen in any male family member studied including the proband.

X chromosome gain.

Family member	Age (y)	Carrier status	X chromosome gain (% of metaphyses)	Age related mean normal value for hyperdiploidy* ⁸ (% of metaphyses)
I.1	65	No	4	1.8
II.1	47	Yes	3	0.77
II.2	45	No	2	
II.4	35	Yes	1	0.67
III.1	25	Yes	1	0.50
III.3	22	Yes	0	0.33
III.4	21	No	0	
III.8	17	Yes	0	0.0-33

*These figures represent an upper limit for X chromosome gain.

Discussion

In this family no consistent constitutional abnormality of the X chromosome was detected by cytogenetic studies. It remains to be seen whether this is the case in all families affected by this disorder. The presence of metaphases showing trisomy 8 and one showing a 5q deletion has not been explained.

There are several possible explanations for the apparent lack of carrier status in the mother (I.1) of the proband. It may be that there is no previous family history of this condition. She may represent a de novo mutation such that her haemopoiesis remains unaffected but her ova contain the defective X chromosome. Alternatively, there may be extreme Lyonisation making her clone producing microcytic red cells undetectable. Finally, her abnormal population of cells may be subject to such ineffective erythropoiesis that release of these cells into the peripheral circulation does not occur.

It is then possible that other female family members not showing a dimorphic red cell population may be carriers. Taking this and the age related normal values into account, it becomes possible that the X chromosome gain observed could be correlated with carrier status.

The diagnosis of a female carrier of this condition consists of the often incidental finding in a healthy, non-anaemic woman of two populations of red cells, one small and one normal, using a Technicon H1 or similar automated blood counter. Examination of a peripheral blood film will confirm the different degrees of haemoglobinisation. Iron deficiency can be excluded by carrying out a serum ferritin estimation and family studies should show its inherited nature.

Early treatment with pyridoxine may benefit those children who are severely affected and avoid unnecessary transfusion. However, it is not known to what extent this disorder is expressed during fetal life or if it can be detected at birth. Supplementation of the carrier mother with pyridoxine and folic acid

might be of benefit to an affected fetus. At birth the cord blood should be examined for anaemia, hypochromia, an altered red cell size distribution, and the presence of ringed sideroblasts among the erythroblasts of the buffy coat. As the age at which this disorder manifests itself is unknown, regular monitoring of the child's haemoglobin and red cell size distribution over the first few years of life would be advisable.

Diagnosis of a carrier should also be accompanied by a caution about taking unnecessary iron tablets with the consequent risk of iron overload. Any suspected iron deficiency should be confirmed by a decreased serum ferritin concentration. At the time of diagnosing the proband, his sister was found to have an increased transferrin iron saturation and increased serum ferritin. The serum ferritin had returned to normal by the time this study was completed. It would be heartening to think that this was a result of counselling.

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