α I 28 Arg→Ser (CGT→AGT) spectrin mutation associated with severe neonatal elliptopoikilocytosis in Spain

When faced with severe neonatal hemolytic anemia and markedly abnormal red cell morphology, the patient's red blood cells should be tested for their sensitivity to heat. We report a new case of hereditary elliptopoikilocytosis (HEP) and neonatal jaundice in a patient with a previously described gene mutation of α spectrin.

Hereditary elliptopoikylocytosis (HEP) is a rare cause of hemolytic anemia characterized by neonatal jaundice, markedly abnormal red blood cell (RBC) morphology and increased sensitivity of RBCs to heat.[1] HEP is inherited in an autosomal recessive manner and caused by mutations in either the spectrin (Sp) αI (SPTA1) or β1 (SPTB1) domains, leading to a failure of Sp heterodimers to self-associate into heterotetramers.

We describe here the first case of SPTA1 28 Arg→Ser mutation found in Spain in a patient who presented with fever, pallor, dark urine and splenomegaly 24h after delivery. This was associated with severe anemia, hyperbilirubinemia and jaundice requiring repeated transfusions and phototherapy. Hematologic data revealed a decreased hemoglobin concentration (Hb: 63 g/L) with reticulocytosis (220×10^9/L) and a markedly abnormal RBC morphology consisting in poikilocytosis, polychromasia, circulating nucleated RBCs, microspherocytes and a few elliptocytes (Figure 1). Plasma haptoglobin was nearly absent and serum lactic dehydrogenase (LDH) concentration greatly increased. Red cell fragility tests were normal but not-valuable due to the effect of transfusions. The RBC thermal stability test of Zarkowsky *et al*.,[2] which was performed on the patient's RBC just before the first transfusion, exhibited an enhanced sensitivity to heat. All other pertinent laboratory studies (direct antiglobulin test, red cell enzyme activities and isopropanol tests or hemoglobin electrophoresis) were non-contributory.

Family study revealed that both parents were clinically normal and only about 30% of elliptocytes were found in the father's peripheral blood. Interestingly, despite the severity of the HEP at birth, the patient's follow-up evaluation showed a well tolerated anemia with an excellent prognosis (Table 1).

Following polymerase chain reaction (PCR) amplification cloning and sequencing of SPTA1 exon 2, a CGT→AGT mutation at codon 28 was found in the propositus and her father. This mutation, first reported in a patient of English ancestry,[3] produces an arginine (Arg) for serine (Ser) substitution (Arg→Ser) and, as a consequence, a pronounced dimer self-association impairment. The same mutation was later reported in several other patients from diverse racial backgrounds also suffering from severe neonatal hemolytic anemia and poikilocytosis.[4-6] Interestingly, in all these cases, the α 28 mutation accounted for the occurrence in *trans* of a low-expression allele on αV/41 known as LELY (*low expression LYon*) leading to a much more severe expression of the membrane defect.[7,8] The same genotype was found in our family, in which an α^{LELY} allele was present in heterozygous form in both the patient and her mother (Figure 2).

The frequency of hereditary elliptocytosis is estimated to be about 1 every 50,000 births and HEP seems to be less common probably because this condition is frequently misdiagnosed as hereditary spherocytosis at birth. α^{LELY} allele is found in ethnic groups remote from one another with a uniform frequency (20-30% of all α-alleles). Its incidence in Caucasians is fairly high, being about 31%.[9] In 1993, we reported the first cases of Spanish HEP in two children whose mother had a symptomless elliptocytosis and whose father was normal. Spectrin maps demonstrated an α 469 CAT→CCT (His→Pro) mutation (Sp Barcelona) occurring in *trans* with α^{LELY} allele in both probands and their mother and the α^{LELY} allele in the father. It is worth mentioning

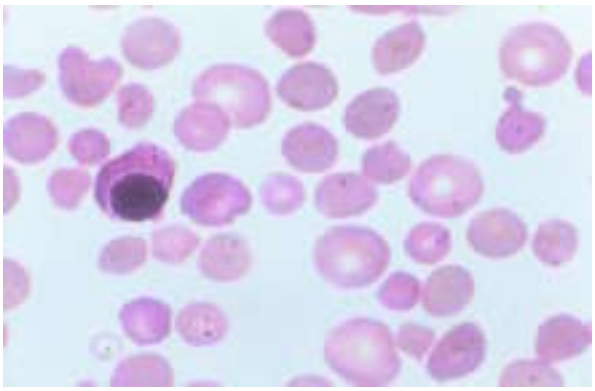
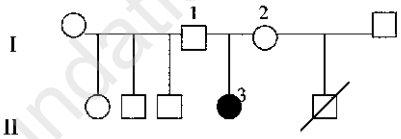


Figure 1. RBC morphology of the patient with HEP characterized by a marked anisopoikilocytosis and circulating erythroblasts (MGG stained blood smear).



1: Father ($Sp\alpha$ / $Sp\ \alpha^{28\ CGT\rightarrow AGT}$)
2: Mother ($Sp\alpha$ / $Sp\alpha^{LELY}$)
3: Propositus ($Sp\alpha^{LELY}$ / $Sp\alpha^{28\ CGT\rightarrow AGT}$)

Figure 2. Patient's family pedigree. The mother had had a previous abortion in a former marriage; the father had three healthy children from a first marriage.

Table 1. General hematologic data of the propositus (four years after diagnosis) and of her parents.

	Propositus	*Father*	*Mother*	*Reference values*
RBCs ($\times10^{12}$/L)	3.57	4.95	4,15	5.4 ± 1.5
Hb (g/L)	138	143	125	140 ± 25
MCV (fL)	83	83.7	91	89 ± 9
MCH (pg)	29	28.9	30.1	29.5 ± 2.5
MCHC (g/L)	337	345	331	42 ± 30
Reticulocytes (%)	3	2.5	1	0.2 – 1.5
RBC heat stability	Low	Normal	Normal	Normal
RBC morphology	Anisocytosis Few elliptocytes	Elliptocytes	Normal	Normal

that the present case of HEP was found in the same geographical region as the Sp Barcelona ones,[10] and also, that the patient exhibited a severe hemolytic anemia which appeared shortly after birth. Furthermore the comparison of the phenotype in our case with those of the other cases of α28 mutation (Arg→Ser) so far described, highlight the functional importance of α28 and

gives further support to the assumption that codon α28 is a *hot spot* for SPTA1 mutations. Finally, from a practical point of view, it should be mentioned that the diagnostic clues of HEP are the increased heat instability of patient's RBCs at birth and the finding that at least one of the proband's relatives has a classical hereditary elliptocytosis.

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