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101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Defining Hereditary Pyropoikilocytosis (HPP) in the Molecular Diagnostic Era

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Misconceptions continue in distinguishing hereditary elliptocytosis (HE) with hemolysis in early perinatal period and hereditary pyropoikilocytosis (HPP). In 1975, Zarkowsky and colleagues described a group of children with transfusion dependent congenital hemolytic anemia with the unique morphology of "microspherocytes, measuring 2-3 microns in diameter, and cells with blunted projections or triangular in shape", a morphology resembling the heat induced damage to normal erythrocytes - hence the name hereditary pyropoikilocytosis (HPP). Subsequently, similar cases were described with or without demonstrable heat sensitivity (Ravindranath & Johnson, 1985). Regardless, the common denominators were 1) transfusion dependency early in infancy - resolved with splenectomy, 2) severe microcytosis, often <60 fL, and 3) a decrease in membrane bound spectrin (decreased spectrin/band3 ratio). Family studies showed normal RBC morphology in both parents (Ravindranath & Johnson, 1985) or in some cases, one parent with HE (Zarkowsky *et al.*, 1975). Protein chemistry studies showed decreased tetramer formation and tryptic digests showed mutations within the n-terminal alpha 1 domain of alpha spectrin - the dimer/dimer interaction site. Thus, homozygosity or double heterozygosity of mutations in the dimer/dimer interaction site of alpha spectrin (alpha 1 domain of alpha spectrin) defined HPP while classical HE cases showed heterozygous mutations in the alpha 1 domain. A fact unexplained was how this results in spectrin deficiency, specifically, alpha spectrin deficiency (Ravindranath & Johnson, 1985).

Using the molecular analytical tool AnemiaID (courtesy of Agios Pharmaceuticals, Inc.), we show that HPP is defined by the co-inheritance of two *SPTA1* mutations and *Alpha-LeLy* polymorphism (*αLeLy* rs28525570; SPTA1[c.5572C>G;c.6531-12C>T]) in one or both alleles, thus explaining the increased propensity for red cell fragmentation and the spectrin deficiency noted on acrylamide gels *[Table: Clinical and molecular characteristics of our cases]*. Of note, homozygosity for *αLeLy* polymorphism and one *SPTA1* variant did not result in clinical HPP phenotype *[HE-2 in Table]*. At a clinical laboratory level, the extreme microcytosis in HPP is evident in the markedly diminished band3 content on eosin-5'-maleimide (EMA) binding test- mean channel fluorescence (MCF) of <275.0 (control: 473.0-549.0) while HE (including neonatal HE with hemolysis) show a double peak pattern in EMA (the minor peak representing the fragmented cells while the major peak had MCF higher than control MCF) and the characteristic trapezoid pattern on osmotic gradient ektacytometry (not shown).

Disclosures No relevant conflicts of interest to declare.

Table: Clinical and molecular characteristics of our cases

Diagnosis-PatientID	Hb (g/dL)	MCV (fL)	RDW (%)	Trx	Spx	Tryptic fragments	*SPTA1 gene mutation*	*Alpha LeLy*	EMA MCF
									 Control (Range: 473.0-549.0)
HE-Neonatal	7.2	90.3	33.2	Yes	No		c.1124 T>C; p.Phe375Ser (heterozygous)	-/-	 Minor Peak: 261.2 Major Peak: 485.2
HE-1	10.4	88.3	16.2	No	No		c.460_462dup p. Leu154dup (heterozygous)	+/-	 Minor Peak: 302.6 Major Peak: 611.0
HE-2	13.5	83	15.2	No	No		c.620T>C; p. Leu207Pro (heterozygous)	+/+	 Minor Peak: 312.0 Major Peak: 608.6
HE-3	10.7	74.9	15.7	No	No		c.460_462dup p. Leu154dup (heterozygous)	-/-	 Minor Peak: 333.1 Major Peak: 574.8
HE-3a	11.5	74.7	12.9	No	No		c.460_462dup p. Leu154dup (heterozygous)	-/-	 Minor Peak: 325.8 Major Peak: 616.0
HE-3b	12.6	79.7	12.8	No	No		c.460_462dup p. Leu154dup (heterozygous)	-/-	Not Done
HPP-1a	10.9	54.5	>40.0	Yes	Yes	α1-74/50	1. c.83G>A; p. Arg28His 2. c.620T>C; p. Leu207Pro	+/+	Not Done
HPP-1b HbC trait	11.0	54.6	30.2	Yes	Yes	α1-74/50	1. c.83G>A; p. Arg28His 2. c.620T>C; p. Leu207Pro	+/+	 219.8
HPP-2	7.9	75.6	25.7	Yes	Yes		1.c.83G>A; p.Arg28His 2. c.1406-1408 del; p. His469del	+/-	 178.7
HPP-3	10.1	56.7	32.4	Yes	Yes	α1-46/ 46	Not Done	Not Done	 264.5

HE: hereditary elliptocytosis
HPP: hereditary pyropoikilocytosis
Hb: hemoglobin (in g/dL)
MCV: mean corpuscular volume (in fL)
RDW: red cell distribution width (in %)
Trx: transfusions
Spx: splenectomy
EMA: eosin-5'-maleimide binding test
MCF: mean channel fluorescence
HbC trait: hemoglobin C trait

Of Note: Patients HE-3, 3a and 3b are related; Patients HPP-1a and 1b are siblings

Figure 1

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