Clinical Hematology, Necker Hospital, Paris; [5]Université Paris Descartes, Paris, France

Keywords: pure red cell aplasia, renal transplant recipients, immune.

Acknowledgments: we would like to thank Dr S. Candon for assistance with FACS analysis.

Correspondence: Julien Zuber, M.D., Ph.D., Renal Transplant Unit, Hôpital Necker, 161 rue de Sèvres 75743 Paris Cedex 15, Paris, France. E-mail: julien.zuber@nck.aphp.fr

Citation: Zuber J, Beldjord K, Casadevall N, Thervet E, Legendre C, and Varet B. Immune-mediated pure red cell aplasia in renal transplant recipients. Haematologica 2008; 93:1750-1752. doi: 10.3324/haematol.13477

References

1. Croisille L, Tchernia G, Casadevall N. Autoimmune disorders of erythropoiesis. Curr Opin Hematol 2001;8:68-73.
2. Ramage JK, Hale A, Gane E, Cohen B, Boyle M, Mufti G, et al. Parvovirus B19-induced red cell aplasia treated with plasmapheresis and immunoglobulin. Lancet 1994;343: 667-8.
3. Pruijt JF, Haanen JB, Hollander AA, den Ottolander GJ. Azathioprine-induced pure red-cell aplasia. Nephrol Dial Transplant 1996;11:1371-3.
4. Sabnani I, Zucker MJ, Tsang P, Palekar S. Clonal T-large granular lymphocyte proliferation in solid organ transplant recipients. Transplant Proc 2006;38:3437-40.
5. Elimelakh M, Dayton V, Park KS, Gruessner AC, Sutherland D, Howe RB, et al. Red cell aplasia and autoimmune hemolytic anemia following immunosuppression with alemtuzumab, mycophenolate, and daclizumab in pancreas transplant recipients. Haematologica 2007;92: 1029-36.
6. Lacombe C, Casadevall N, Muller O, Varet B. Erythroid progenitors in adult chronic pure red cell aplasia: relationship of in vitro erythroid colonies to therapeutic response. Blood 1984;64:71-7.
7. Charles RJ, Sabo KM, Kidd PG, Abkowitz JL. The pathophysiology of pure red cell aplasia: implications for therapy. Blood 1996;87:4831-8.
8. Handgretinger R, Geiselhart A, Moris A, Grau R, Teuffel O, Bethge W, et al. Pure red-cell aplasia associated with clonal expansion of granular lymphocytes expressing killer-cell inhibitory receptors. N Engl J Med 1999;340:278-84.
9. Senechal B, Elain G, Jeziorski E, Grondin V, Patey-Mariaud de Serre N, Jaubert F, et al. Expansion of regulatory T cells in patients with Langerhans cell histiocytosis. PLoS Med 2007;4:e253.
10. Wlodarski MW, O'Keefe C, Howe EC, Risitano AM, Rodriguez A, Warshawsky I, et al. Pathologic clonal cytotoxic T-cell responses: nonrandom nature of the T-cell-receptor restriction in large granular lymphocyte leukemia. Blood 2005;106:2769-80.
11. Gabor EP, Mishalani S, Lee S. Rapid response to cyclosporine therapy and sustained remission in large granular lymphocyte leukemia. Blood 1996;87:1199-200.
12. Kirk AD, Hale DA, Swanson SJ, Mannon RB. Autoimmune thyroid disease after renal transplantation using depletional induction with alemtuzumab. Am J Transplant 2006; 6:1084-5.

Nonsense mutations of the α-spectrin gene in hereditary pyropoikilocytosis

Hereditary pyropoikilocytosis (HPP) is an inherited hemolytic anemia characterized by peripheral blood smear findings reminiscent of those seen in patients suffering severe thermal burns.[1,2] Erythrocytes from most HPP patients exhibit qualitative and quantitative abnormalities of the erythrocyte membrane protein spectrin, the principal structural component of the erythrocyte membrane skeleton. Qualitative spectrin defects are typically associated with missense mutations that lead to abnormal spectrin self-association, a process critical for membrane structure and function.[3]

The pathogenesis of qualitative spectrin defects, *i.e.* spectrin deficiency, in HPP erythrocytes is poorly understood. Whereas some HPP patients are compound heterozygotes or homozygotes for missense mutations in spectrin, others are heterozygotes for a missense mutation and possess a second, *thalassemia-like* α-spectrin allele *in trans* to the missense mutation.[4-6] This production-defective allele is associated with decreased or absent accumulation of α-spectrin on the erythrocyte membrane. With the exception of the original case described by Zarkowsky *et al.*, the molecular basis of the production-defective α-spectrin allele in HPP is unknown.

We studied HPP probands from 2 HE/HPP kindreds. Both probands had typical hereditary pyropoikilocytosis. Laboratory findings included compensated hemolytic anemia, marked microcytosis (MCV<75 fL), and typical blood smears with erythrocyte morphology including elliptocytes, poikilocytes, microspherocytes, and fragmented cells. Informed consent was obtained in accordance with the Declaration of Helsinki.

One-dimensional SDS-PAGE analyses of erythrocyte membranes from both probands were qualitatively normal (*data not shown*). Quantitative analyses of spectrin content, measured by spectrin/band 3 ratios, demonstrated spectrin deficiency in both probands (Table 1). These are values typically seen in patients with HPP.

Limited tryptic digestion of normal spectrin followed by two-dimensional gel electrophoresis yields a pattern of five major proteolytically resistant domains of α-spectrin and 4 proteolytically resistant domains of β-spectrin.[7] The 80kDa αI domain encodes the NH_2-terminus of α-spectrin which interacts with sequences from the 17th repeat of β-spectrin to form the αβ binding site for spectrin self-association. Most HPP-associated spectrin mutations affect the 80kDa αI domain and yield peptide maps containing one or more fragments of the domain.

Both HPP probands exhibited abnormal tryptic spectrin maps, with the αI/50a kDa variant peptide. No normal αI 80kDa peptide was seen on maps from either of the HPP probands, implying homozygosity for the underlying

Table 1. Biochemical and genetic studies in hereditary pyropoikilocytosis patients.

	Patient 1	*Patient 2*
Spectrin/band 3 ratio	0.76+0.04	0.71+0.05
Spectrin tryptic phenotype	αI/50a kDa	αI/50a kDa
Missense mutation		
Exon number	Five	Six
DNA	CTG-CCG	CTG-CCG
Codon	Leu207Pro	Leu260Pro
Nonsense mutation		
Exon number	Forty-three	Thirty-five
DNA	GAA-TGA	CGA-TGA
Codon	Glu2018Stop	Arg1659Stop

spectrin mutation (Figure 1 and *data not shown*). Increased amounts of the αI/50akDa peptide have been associated with structural defects of spectrin in individuals with HE and HPP.

Mutations associated with the variant αI/50α peptide have been identified in exon 5 or 6 of the α-spectrin gene, part of the region encoding the spectrin self-association site. Exon 5 and 6 were amplified from genomic DNA from the HPP probands and subjected to nucleotide sequence analysis. This revealed missense mutations in exon 5 (patient 1) or exon 6 (patient 2) of the α-spectrin gene (Table 1). These mutations have previously been associated with hereditary elliptocytosis or HPP.

To determine the basis of the observed disparity between spectrin tryptic maps and DNA sequence analyses, nucleotide sequence analysis of the remainder of the coding exons of the α-spectrin gene was performed on genomic DNA from the probands. Nonsense mutations were identified *in trans* in both patients, revealing the basis of the apparent homozygosity seen on spectrin tryptic maps (Table 1). The nonsense mutations found in these patients likely led to nonsense-mediated α-spectrin mRNA decay[8] or protein proteolysis, as there was no evidence of a truncated α-spectrin peptide on one or two dimensional gel electrophoresis.

The presence or absence of the low expression allele, α^{LELY}, was determined in the probands and family members. Patient 1 was heterozygous for the α^{LELY} allele, in *cis* to the L207P mutation. Patient 2 did not carry the α^{LELY} allele on either allele.

Only a few production-defective alleles of the α-spectrin gene, α^{LELY}, α^{LEPRA}, $\alpha^{LELY\text{-}Bicentre}$, and spectrin$^{St.\ Louis}$ have been described,[9-12] and only the α^{LELY} and spectrin$^{St.\ Louis}$ alleles have been associated with the HPP phenotype. The $\alpha^{LELY\ (Low\ Expression\ Lyon)}$ allele is characterized by a C-T mutation at -12 of intron 45 associated with partial in-frame skipping of exon 46. Deletion of the amino acids in exon 46 disrupts the folding of α-spectrin repeat 21, which participates in αβ spectrin nucleation. This inhibits assembly of the shortened peptide into spectrin dimers, leading to proteolytic degradation, with a resulting ~50% decrease in spectrin available for membrane assembly.[12] In general, HE patients heterozygous for missense mutations of α-spectrin on one allele and α^{LELY} *in trans* are more severely affected, presumably because the decreased amount of α^{LELY} spectrin incorporated into the membrane increases the relative incorporation of spectrin containing the mutation *in trans.*

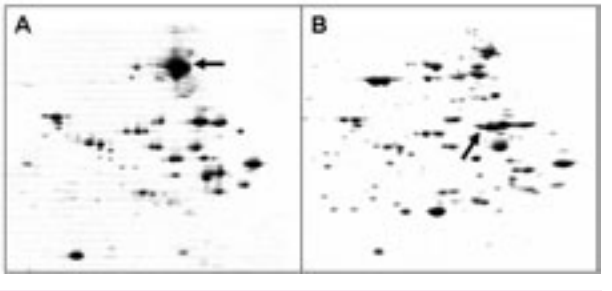

Figure 1. Limited tryptic digestion of spectrin. Erythrocyte spectrin extracted from erythrocytes of a normal control (A) and hereditary pyropoikilocytosis proband 2 (B) was partially digested with trypsin and fractionated by two-dimensional gel electrophoresis with IEF (SDS/PAGE with IEF). The proband's spectrin digests demonstrate increased amounts of a 50kDa spectrin peptide (arrow) and no normal 80kDa αI domain peptide. In tryptic digests from the control (A), the 80kDa αI domain peptide (arrow) is normal.

There is abundant evidence that there are non-α^{LELY} production-defective α-spectrin alleles associated with HPP. In some patients, α^{LELY} in trans does not worsen clinical severity. In others, similar to patient 1, α^{LELY} is found *in cis* to an α-spectrin mutation, suggesting the inheritance of a non-α^{LELY} production-defective α-spectrin allele *in trans*. Non-α^{LELY} production defective α-spectrin alleles are characterized by reduced α-spectrin mRNA levels and diminished α-spectrin synthesis.[4] In one HPP kindred, the molecular basis of the production defective allele is known.[9] A mutation in the donor splice site of intron 22 of the α-spectrin gene, spectrin$^{St.\ Louis}$, leads to aberrant splicing producing α-spectrin cDNAs containing in-frame premature termination codons. These studies demonstrate that nonsense mutations cause spectrin deficiency in some HPP patients, and, together with the spectrin$^{St.\ Louis}$ allele, reveal considerable genetic heterogeneity in the molecular basis of HPP.

Whitney Tolpinrud,[1] Yelena D. Maksimova,[1] Bernard G. Forget,[2] and Patrick G. Gallagher[1]

[1]Departments of Pediatrics, [2]Genetics and Internal Medicine, Yale University School of Medicine, New Haven, CT, USA

Funding: this work was supported in part by grants HL65448 (PGG) and DK019482 (BGF) from the National Institutes of Health.

Key words: anemia, spectrin, erythrocyte membrane, mutation.

Correspondence: Patrick G. Gallagher, Department of Pediatrics, Yale University School of Medicine, 333 Cedar Street, P. O. Box 208064, New Haven, CT 06520-8064, USA. Phone: international +203.6882896. Fax: international +203.7856974. E-mail: patrick.gallagher@yale.edu

Citation: Tolpinrud W, Maksimova YD, Forget BG, and Gallagher PG. Nonsense mutations of the a-spectrin gene in hereditary pyropoikilocytosis. Haematologica 2008; 93:1752-1754. doi: 10.3324/haematol.13639

References

1. Gallagher PG. Hereditary elliptocytosis: spectrin and protein 4.1R. Semin Hematol 2004;41:142-64.
2. Iolascon A, Perrotta S, Stewart GW. Red blood cell membrane defects. Rev Clin Exp Hematol 2003;7:22-56.
3. Tse WT, Lux SE. Red blood cell membrane disorders. Br J Haematol 1999;104:2-13.
4. Gallagher PG, Tse WT, Marchesi SL, Zarkowsky HS, Forget BG. A defect in α-spectrin mRNA accumulation in hereditary pyropoikilocytosis. Trans Assoc Am Physicians 1991;104:32-9.
5. Hanspal M, Hanspal JS, Sahr KE, Fibach E, Nachman J, Palek J. Molecular basis of spectrin deficiency in hereditary pyropoikilocytosis. Blood 1993;82:1652-60.
6. Coetzer T, Palek J, Lawler J, Liu SC, Jarolim P, Lahav M, et al. Structural and functional heterogeneity of α spectrin mutations involving the spectrin heterodimer self-association site: relationships to hematologic expression of homozygous hereditary elliptocytosis and hereditary pyropoikilocytosis. Blood 1990;75:2235-44.
7. Speicher DW, Morrow JS, Knowles WJ, Marchesi VT. Identification of proteolytically resistant domains of human erythrocyte spectrin. Proc Natl Acad Sci USA 1980; 77:5673-7.
8. Isken O, Maquat LE. Quality control of eukaryotic mRNA: safeguarding cells from abnormal mRNA function. Genes Dev 2007;21:1833-56.
9. Costa DB, Lozovatsky L, Gallagher PG, Forget BG. A novel splicing mutation of the α-spectrin gene in the original hereditary pyropoikilocytosis kindred. Blood 2005;106: 4367-9.
10. Delaunay J, Nouyrigat V, Proust A, Schischmanoff PO, Cynober T, Yvart J, et al. Different impacts of alleles αLEPRA and αLELY as assessed versus a novel, virtually

null allele of the SPTA1 gene in trans. Br J Haematol 2004;127:118-22.
11. Wichterle H, Hanspal M, Palek J, Jarolim P. Combination of two mutant α spectrin alleles underlies a severe spherocytic hemolytic anemia. J Clin Invest 1996;98:2300-7.
12. Wilmotte R, Maréchal J, Morlé L, Baklouti F, Philippe N, Kastally R, et al. Low expression allele αLELY of red cell spectrin is associated with mutations in exon 40 (α V/41 polymorphism) and intron 45 and with partial skipping of exon 46. J Clin Invest 1993; 91:2091-6.

First case of γ-thalassemia in association with a βS allele: a pitfall in the neonatal screening for sickle cell disease

Numerous deletional thalassemias have been reported so far, involving one or several globin genes in combination.[1] A deletion specifically targeted on the fetal β-like genes was first described in 1983 and was characterized as a deletion-fusion removing the 3' end of Gγ-gene and the 5' end of Aγ-gene.[2] This deletion was found after the identification of several newborns with an abnormally low Gγ/Aγ ratio, in the course of an Hb F expression study in newborns from various origins (Asian, European and African-American).[3] This γ-gene deletion is clinically silent either in heterozygous or in homozygous conditions and was found in 1.42% of the tested newborns (mainly from China, India, Japan and the former Yugoslavia). In the present investigation, we have identified the first case of such a fetal γ-gene deletion found in association with a Sickle β-globin gene (βS).

The proband is a newborn screened at birth, in the course of the French neonatal screening program for Sickle Cell Disease (SCD). He was suspected of having SCD because of the presence of HbS and a very low level of HbA detected by IEF and HPLC, on a dry blood sample collected after three days of life (Figure 1A). At six weeks of age, control hemoglobin analysis on a venous blood sample revealed the following Hb rates: HbF 55%, HbS 27% and HbA 15% suggesting a compound heterozygosity for β⁺-thalassemia and βS alleles. However, molecular analysis of the β-globin gene showed, as a single defect, heterozygosity for the prevalent sickle cell mutation transmitted by the father. The proband's mother showed no Hb abnormality and normal red blood cell indices.

Nonetheless, the baby was included in the prevention program and a regular clinical follow-up was defined. At eight months of age, a control Hb analysis revealed a typical profile of βS heterozygote with Hb S: 35%, Hb A: 55% in contradiction with the neonatal diagnosis.

In an attempt to further explore this peculiar phenotype, the whole β-globin locus was analyzed by means of MLPA technical procedures (Xservices, the Netherlands) that allowed us to evidence a heterozygous deletion of the region localized between the fetal globin genes, Aγ and Gγ (Figure 1B). This deletion was confirmed by genomic qPCR (Applied Biosystem 7500) (Figure 1C) and

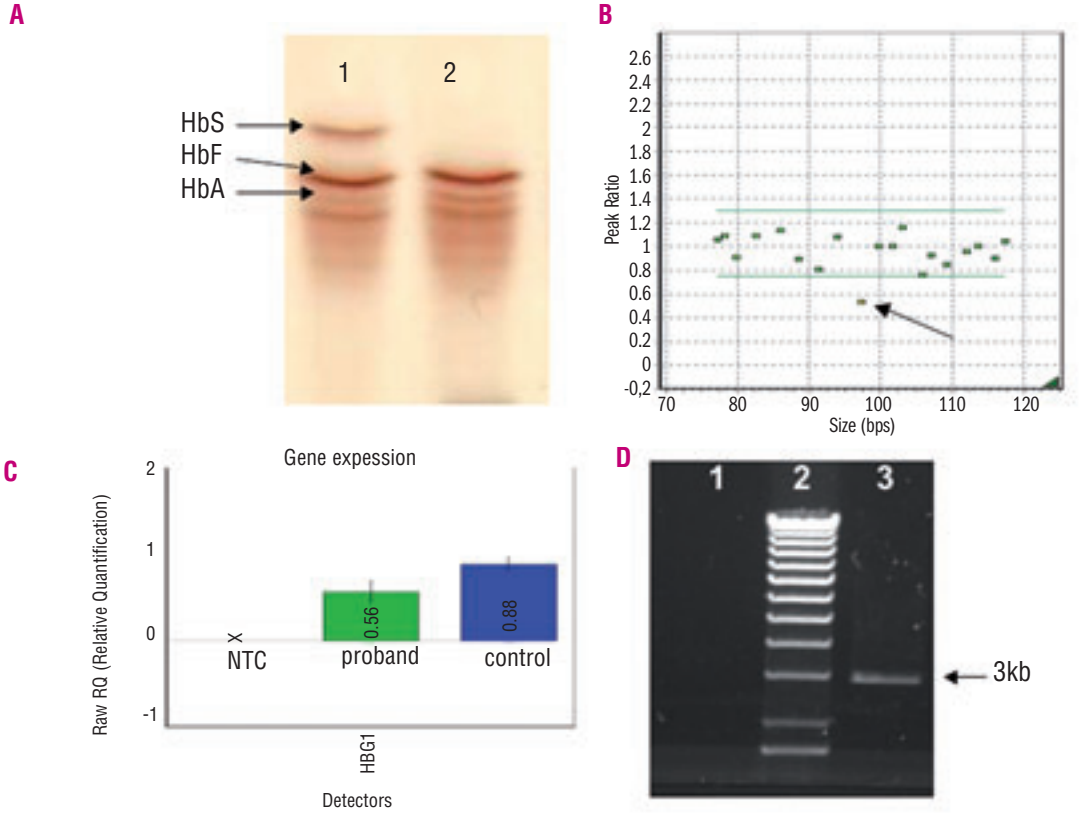

Figure 1. β-globin locus analysis. (A) Isoelectrofocalisation from 1:the proband's dried blood, 2: a normal newborn. (B) MLPA of the whole β-globin locus using the commercial kit from Xservices, the Netherlands (the arrow shows the probe located within the deletion). (C) Genomic Quantitative PCR with a set of primers located between G and Aγ genes (forward: aaatgtgtgtctttctggcctttt; reverse: ccttcatgatcctgtgtaaagcttata); NTC: No Template Control. (D) Standard PCR with primers flanking Gγ and Aγ genes: Forward 5'acaagtgtctttactgctttatttgct3' and Reverse 5'ccaaggtcatggatcgagtt3'; 1:Normal Control (no amplification); 2: Ladder 12kb (Invitrogen); 3: proband presenting a 3 kb amplicon.