

Hematologically Important Mutations: Band 3 and Protein 4.2 Variants in Hereditary Spherocytosis

Submitted 11/24/97

(communicated by Ernest Beutler, M.D., 11/24/97)

Patrick G. Gallagher¹, Bernard G. Forget²

Hereditary spherocytosis is a group of inherited disorders characterized by the presence of spherical erythrocytes on the peripheral blood smear (1-4). The principal cellular defect in HS is loss of erythrocyte membrane surface area relative to intracellular volume, accounting for the spherical shape as well as decreased deformability of the erythrocyte. Splenic destruction of these abnormal erythrocytes is the primary cause of the hemolysis experienced by HS patients. The clinical and laboratory features of HS have been the subject of recent reviews (3-6).

The primary biochemical defects in HS reside in the proteins of the erythrocyte membrane, particularly those proteins involved in the interactions between the membrane skeleton and the lipid bilayer: spectrin, ankyrin, band 3, and protein 4.2. This review focuses on the genetic defects in band 3 and protein 4.2 associated with HS. A future tabulation will include HS mutations in spectrin and ankyrin.

Band 3, the anion exchanger, is the major transmembrane protein of the erythrocyte. The NH₂-terminal cytoplasmic domain of band 3 binds several glycolytic enzymes and mediates interactions between band 3 and the membrane

skeleton via ankyrin, protein 4.1 and protein 4.2 (reviewed in 5,7-9). The COOH-terminal domain of band 3 is the site of anion exchange. A subset of HS patients with dominantly-inherited HS whose erythrocytes are deficient in band 3 has been described. These patients generally have mild to moderate HS and have been described as having pincerred spherocytes on peripheral blood smear.

Protein 4.2 (also known as band 4.2 or pallidin) is a peripheral membrane protein thought to aid in the linkage of the membrane skeleton to the lipid bilayer via interactions with band 3 and ankyrin (reviewed in 5,10,11). The precise function of protein 4.2, however, has yet to be determined. Protein 4.2-deficient patients, primarily from Japan, with recessively inherited HS have been described. These patients are homozygotes or compound heterozygotes for protein 4.2 defects. Protein 4.2 deficiency has also been observed in patients with defects in the cytoplasmic domain of band 3 (12,13), the region of band 3 involved in band 3-protein 4.2 interactions.

A variety of mutations in band 3 and protein 4.2 have been described. These are listed in Tables 1 and 2. The nucleotide numbers shown

¹ Reprint requests to: Patrick G. Gallagher, M.D., Department of Pediatrics, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208064, New Haven, CT 06520-8064, phone (203) 737-2896, fax (203) 785-5426, email: patrick_gallagher@qm.yale.edu.

² Hematology Section, Department of Internal Medicine, Yale University School of Medicine, 333 Cedar Street, P.O. Box 208021, New Haven, CT 06520-8021, phone (203) 785-4144, fax (203) 785-7232, email: bernard.forget@yale.edu.

in the table are based on the cDNA sequences listed in the GenBank/EMBL databanks, the accession numbers of which are: HUMAE1 (band 3 cDNA) (14) and M30646 (protein 4.2 cDNA)(15). In accordance with recommendations for the use of systematic names in nomenclature for mutations, the nucleotide numbers of the cDNA coding sequences presented here begin with +1, which refers to A in the ATG initiation

codon (16). This numbering is different from that used in the GenBank/EMBL databank files.

Other band 3 variants that affect erythrocyte morphology, as well as the blood group antigens that have been assigned to band 3, have recently been reviewed (9).

Keywords: hereditary spherocytosis, mutation, band 3, protein 4.2, erythrocyte

Table 1. Band 3 Mutations Associated with Hereditary Spherocytosis

Variant	Systematic Name	Type	Amino Acid Variation	Domain ¹	Reference
Montefiore	c118A	missense	40 Glu→Lys	Cyto	13
Foggia	c162del	deletion	54-55→frameshift	Cyto	17
Kagoshima		deletion	56→frameshift	Cyto	18
Hodonin	c243A	nonsense	81 Trp→Stop	Cyto	19
Napoli I	c298T299ins	insertion	99-100→frameshift	Cyto	17
Fukayama I		deletion	112-113→frameshift	Cyto	18
Nachod	IVS5 -3A ²	splicing	117-121 GTVLL deleted	Cyto	19
Fukuoka	c388A	missense	130 Gly→Arg	Cyto	18
Osnabruck I	c448T	nonsense	150 Arg→Stop	Cyto	20
Lyon	c448T	nonsense	150 Arg→Stop	Cyto	21
Worcester	c515G516ins	insertion	170-172→frameshift	Cyto	19
Fukuyama II		insertion	183→frameshift	Cyto	18
Campinas	IVS8 +1T ³	splicing	203→frameshift	Cyto	22
Bohain	c241 del	deletion	241→frameshift	Cyto	23
Princeton	c822C823ins	insertion	273-275→frameshift	Cyto	19
Boston	c854A	missense	285 Ala→Asp	Cyto	19
Tuscaloosa	c980G	missense	327 Pro→Arg	Cyto	12
Noirterre	c988T	nonsense	330 Gln→Stop	Cyto	24
Bruggen	c1257del	deletion	419→frameshift	TM	20
Benesov	c1365A	missense	455 Gly→Glu	TM	19
Bicêtre II	c1366 del	deletion	456→frameshift	TM	23
Pribram	IVS12 -1A ⁴	splicing	477→frameshift	TM	19
Coimbra	c1462A	missense	488 Val→Met	TM	25
Bicêtre I	c1468T	missense	490 Arg→Cys	TM	23
Evry	c1474 del	deletion	492→frameshift	TM	23
Milano	c1498-69nt ins	duplication	498 23AA insertion	TM	26
Dresden	c1552T	missense	518 Arg→Cys	TM	20
Smichov	c1848del	deletion	616→frameshift	TM	19
Trutnov	c1884A	nonsense	628 Tyr→Stop	TM	19
Hobart	c1939c1940del	deletion	646-647→frameshift	TM	19
Osnabruck II	c1987-1989del	deletion	663-664 →frameshift	TM	20
Most	c2120C	missense	707 Leu→Pro	TM	19
Okinawa	c2140A	missense	714 Gly→Arg	TM	18

Table 1. Band 3 Mutations Associated with Hereditary Spherocytosis (Cont'd)

Variant	Systematic Name	Type	Amino Acid Variation	Domain ¹	Reference
Prague II	c2279A	missense	760 Arg→Gln	TM	27
Kumamoto		missense	760 Arg→Gln	TM	18
Hradec Kralove	c2278T	missense	760 Arg→Trp	TM	27
Chur	c2312A	missense	771 Gly→Asp	TM	28
Napoli II	c2438A	missense	783 Ile→Asn	TM	17
Jablonec	c2422T	missense	808 Arg→Cys	TM	27
Nara		missense	808 Arg→His	TM	18
Prague	c2463-10nt ins-2464	insertion	822→frameshift	TM	29
Birmingham	c2501C	missense	834 His→Pro	TM	19
Philadelphia	c2510T	missense	837 Thr→Met	TM	19
Prague III	c2608T	missense	870 Arg→Trp	TM	27
Vesuvio	c2689 del	deletion	984→frameshift	TM	30

¹ Abbreviations: Cyto = cytoplasmic, TM = transmembrane

² In frame deletion of 15 nucleotides

³ Skipping of exon 8, frameshift

⁴ Intron 12 inserted into coding sequence, frameshift

Table 2. Protein 4.2 Mutations Associated with Hereditary Spherocytosis

Variant	Systematic Name	Type	Amino Acid Variation	Reference
Lisboa	c265del	deletion	89→frameshift	31
Fukuoka	c357A	missense	119 Trp→Stop	32
Nippon	c424A	missense	142 Ala→Thr	33
Komatsu	c523T	missense	175 Asp→Tyr	34
Notame	IVS6 +1A	splicing ¹	308→frameshift	35
Tozeur	c929A	missense	310 Arg→Gln	36
Shiga	c949T	missense	317 Arg→Cys	37

¹Skipping of exon 6

REFERENCES

1. Becker P, Lux S. Disorders of the red cell membrane skeleton: Hereditary spherocytosis and hereditary elliptocytosis. In Scriver C, Beaudet A, Sly W, et al. *The Metabolic Basis of Inherited Disease*. New York: McGraw-Hill. pp. 529-632, 1995.
2. Gallagher PG, Ferreira JD. Molecular basis of erythrocyte membrane disorders. *Curr Opin Hematol* 4:128-135, 1997.
3. Hassoun H, Palek J. Hereditary spherocytosis: a review of the clinical and molecular aspects of the disease. *Blood Rev* 10:129-147, 1996.
4. Delaunay J. Genetic disorders of the red cell membranes. *FEBS Lett* 369:34-37, 1995.
5. Lux SE, Palek J. Disorders of the red cell membrane. In: Handin RI, Lux SE, Stossel TP. *Blood: Principles and Practice of Hematology*. Philadelphia: JB Lippincott. pp. 1701-1816, 1995.
6. Palek J, Jarolim P. Clinical expression and laboratory detection of red blood cell membrane protein mutations. *Semin Hematol* 30:249-283, 1993.
7. Wang DN. B and 3 protein: structure, flexibility and function. *FEBS Lett* 346:26-31, 1994.
8. Tanner MJ. Molecular and cellular biology of the erythrocyte anion exchanger (AE1). *Semin Hematol* 30:34-57, 1993.
9. Bruce LJ, Tanner MJ. Structure-function relationships

- of band 3 variants. *Cell Mol Biol* (Noisy-Le-Grand) 42:953-973, 1996.
10. Yawata Y. Red cell membrane protein band 4.2: phenotypic, genetic and electron microscopic aspects. *Biochim Biophys Acta* 1204:131-148, 1994.
 11. Cohen CM, Dotimas E, Korsgren C. Human erythrocyte membrane protein band 4.2 (pallidin). *Semin Hematol* 30:119-137, 1993.
 12. Jarolim P, Palek J, Rubin HL, Prchal JT, Korsgren C, Cohen CM. Band 3 Tuscaloosa: Pro327---Arg327 substitution in the cytoplasmic domain of erythrocyte band 3 protein associated with spherocytic hemolytic anemia and partial deficiency of protein 4.2. *Blood* 80:523-529, 1992.
 13. Rybicki AC, Qiu JJ, Musto S, Rosen NL, Nagel RL, Schwartz RS. Human erythrocyte protein 4.2 deficiency associated with hemolytic anemia and a homozygous 40glutamic acid-lysine substitution in the cytoplasmic domain of band 3 (band 3Montefiore). *Blood* 81:2155-2165, 1993.
 14. Lux SE, John KM, Kopito RR, Lodish HF. Cloning and characterization of band 3, the human erythrocyte anion-exchange protein (AE1). *Proc Natl Acad Sci USA* 86:9089-9093, 1989.
 15. Sung LA, Chien S, Chang LS, et al. Molecular cloning of human protein 4.2: a major component of the erythrocyte membrane. *Proc Natl Acad Sci USA* 87:955-959, 1990.
 16. Beutler E, McKusick VA, Motulsky AG, Scriver CR, Hutchinson F. Mutation nomenclature: nicknames, systematic names, and unique identifiers. *Hum Mutat* 8:203-206, 1996.
 17. Miraglia del Giudice E, Vallier A, Maillet P, et al. Novel band 3 variants (bands 3 Foggia, Napoli I and Napoli II) associated with hereditary spherocytosis and band 3 deficiency: status of the D38A polymorphism within the EPB3 locus. *Br J Haematol* 96:70-76, 1997.
 18. Kanzaki A, Takezono M, Kaku M, et al. Molecular and genetic characteristics in Japanese patients with hereditary spherocytosis: frequent band 3 mutations and rarer ankyrin mutations. *Blood* 90(suppl):6b, 1997.
 19. Jarolim P, Murray JL, Rubin HL, et al. Characterization of 13 novel band 3 gene defects in hereditary spherocytosis with band 3 deficiency. *Blood* 88:4366-4374, 1996.
 20. Eber SW, Gonzalez JM, Lux ML, et al. Ankyrin-1 mutations are a major cause of dominant and recessive hereditary spherocytosis. *Nat Genet* 13:214-218, 1996.
 21. Alloisio N, Maillet P, Carre G, et al. Hereditary spherocytosis with band 3 deficiency. Association with a nonsense mutation of the band 3 gene (allele Lyon), and aggravation by a low-expression allele occurring in trans (allele Genas). *Blood* 88:1062-1069, 1996.
 22. Lima PRM, Gontijo JAR, Lopes de Faria JB, Costa FF, Saad STO. Band 3 Campinas: a novel splicing mutation in the band 3 gene (AE1) associated with hereditary spherocytosis, hyperactivity of Na⁺/Li⁺ countertransport and an abnormal renal bicarbonate handling. *Blood* 90:2810-2818, 1997.
 23. Dhermy D, Galand C, Bournier O, et al. Heterogenous band 3 deficiency in hereditary spherocytosis related to different band 3 gene defects. *Br J Haematol* 98:32-40, 1997.
 24. Jenkins PB, Abou-Alfa GK, Dhermy D, et al. A nonsense mutation in the erythrocyte band 3 gene associated with decreased mRNA accumulation in a kindred with dominant hereditary spherocytosis. *J Clin Invest* 97:373-380, 1996.
 25. Alloisio N, Texier P, Vallier A, et al. Modulation of clinical expression and band 3 deficiency in hereditary spherocytosis. *Blood* 90:414-420, 1997.
 26. Bianchi P, Zanella A, Alloisio N, et al. A variant of the EPB3 gene of the anti-Lepore type in hereditary spherocytosis. *Br J Haematol* 98:283-288, 1997.
 27. Jarolim P, Rubin HL, Brabec V, et al. Mutations of conserved arginines in the membrane domain of erythroid band 3 lead to a decrease in membrane-associated band 3 and to the phenotype of hereditary spherocytosis. *Blood* 85:634-640, 1995.
 28. Maillet P, Vallier A, Reinhart WH, et al. Band 3 Chur: a variant associated with band 3-deficient hereditary spherocytosis and substitution in a highly conserved position of transmembrane segment 11. *Br J Haematol* 91:804-810, 1995.
 29. Jarolim P, Rubin HL, Liu SC, et al. Duplication of 10 nucleotides in the erythroid band 3 (AE1) gene in a kindred with hereditary spherocytosis and band 3 protein deficiency (band 3PRAGUE). *J Clin Invest* 93:121-130, 1994.
 30. Perrotta, S, Nigro V, Polito R, Nobili B, et al. Hereditary spherocytosis due to an elongated band 3: Band 3 Vesuvio. *Blood* 90(suppl):270a, 1997.
 31. Hayette S, Dhermy D, dos Santos ME, et al. A deletional frameshift mutation in protein 4.2 gene (allele 4.2 Lisboa) associated with hereditary hemolytic anemia. *Blood* 85:250-256, 1995.
 32. Takaoka Y, Ideguchi H, Matsuda M, Sakamoto N, Takeuchi T, Fukumaki Y. A novel mutation in the erythrocyte protein 4.2 gene of Japanese patients with hereditary spherocytosis (protein 4.2 Fukuoka). *Br J Haematol* 88:527-533, 1994.
 33. Bouhassira EE, Schwartz RS, Yawata Y, et al. An alanine-to-threonine substitution in protein 4.2 cDNA is associated with a Japanese form of hereditary hemolytic anemia (protein 4.2 NIPPON). *Blood* 79:1846-1854, 1992.
 34. Kanzaki A, Yawata Y, Yawata A, et al. Band 4.2 Komatsu: 523 GAT-TAT (175 Asp-Tyr) in exon 4 of

- the band 4.2 gene associated with total deficiency of band 4.2, hemolytic anemia with ovalostomatocytosis and marked disruption of the cytoskeletal network. *Int J Hematol* 61:165-178, 1995.
35. Matsuda M, Hatano N, Ideguchi H, Takahira H, Fukumaki Y. A novel mutation causing an aberrant splicing in the protein 4.2 gene associated with hereditary spherocytosis (protein 4.2^{Notame}). *Hum Mol Genet* 4:1187-1191, 1995.
36. Hayette S, Morle L, Bozon M, et al. A point mutation in the protein 4.2 gene (allele 4.2 Tozeur) associated with hereditary haemolytic anaemia. *Br J Haematol* 89:762-770, 1995.
37. Kanzaki A, Yasunaga M, Okamoto N, et al. Band 4.2 shiga: 317 CGC→TGC in compound heterozygotes with 142 GCT→ACT results in band 4.2 deficiency and microspherocytosis. *Br J Haematol* 91:333-340, 1995.