Citation: Naresh KN. Morphological evaluation of monocytes and monocyte precursors in bone marrow trephine biopsies - need for establishing diagnostic criteria. Haematologica 2009;1623-1624 doi: 10.3324/haematol.2009.013235

References

1. Goasguen JE, Bennett JM, Bain BJ, Vallespi T, Brunning R, Mufti GJ; for the International Working Group on Morphology of Myelodysplastic Syndrome (IWGM-MDS). Morphological evaluation of monocytes and their precursors. Haematologica 2009;94:994-7.
2. Ngo NT, Lampert IA, Naresh KN. Bone marrow trephine morphology and immunohistochemical findings in chronic myelomonocytic leukaemia. Br J Haematol 2008; 141:771-81.
3. Orazi A, Chiu R, O'Malley DP, Czader M, Allen SL, An C, Vance GH. Chronic myelomonocytic leukemia: The role of bone marrow biopsy immunohistology. Mod Pathol 2006;19:1536-45.
4. Orazi A, Bennett JM, Germing U, Brunning RD, Bain BJ, Thiele J. Chronic myelomonocytic leukaemia, in Swerdlow SH, Campo E, Harris NL et al. editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France, IARC, 2008, p. 76-9.
5. Arber DA, Brunning RD, Orazi A, Porwit A, Paterson L, Thiele J, Le Beau MM. Acute myeloid leukaemia, not otherwise specified, in Swerdlow SH, Campo E, Harris NL et al. editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France, IARC, 2008. p. 130-9.
6. Torlakovic EE, Naresh K, Kremer M, van der Walt J, Hyjek E, Porwit A. Call for a European programme in external quality assurance for bone marrow immunohistochemistry; report of a European Bone Marrow Working Group pilot study. J Clin Pathol 2009;62:547-51.

Chronic hemolytic anemia due to novel α-globin chain variants: critical location of the mutation within the gene sequence for a dominant effect

Approximately 30 hemoglobin (Hb) α-chain variants may entail chronic hemolytic anemia (CHA).[1,2] In some, interaction between heme and globin is hampered, leading to an unstable Hb. In others, the change affects the domain which binds AHSP and associates with the β-chain partner.[3] Variants underlying CHA may also result from in-frame deletion or insertion, leading to a shortened or elongated chain, or from frameshift (FS) deletions and insertions leading to a premature stop codon and a profoundly altered C-terminus.[4]

We herewith report 3 variants associating with CHA in the heterozygous state, a picture in contrast with that of many unstable α chain variants in which only borderline α thalassemia is displayed.[4]

Hb Sens was found in a French Caucasian patient (Table 1). The T>A heterozygous substitution at CD43(α2) changes Phe for Ile at CE1, one of the two invariable residues among all globin chains. This Phe maintains the heme in the proper position for interaction with the globin chain. Missense mutants at this position lead to severe unstable Hbs, with the anemia state aggravated by the consecutive shift of the heme towards the lower oxygen affinity conformation. Comparable variants are Hb Torino (Phe>Ser) and Hb Hirosaki (Phe>Leu) for the α chain, or Hb Hammersmith (Phe>Ser), Hb Sendagi (Phe>Val), and Hb Louisville (Phe>Leu) for the β chain (*see HbVar for details*).[1] It is likely that these mutations do not impair the formation of tetramers but lead to unstable molecules which precipitate into Heinz bodies when submitted to oxidative stress.

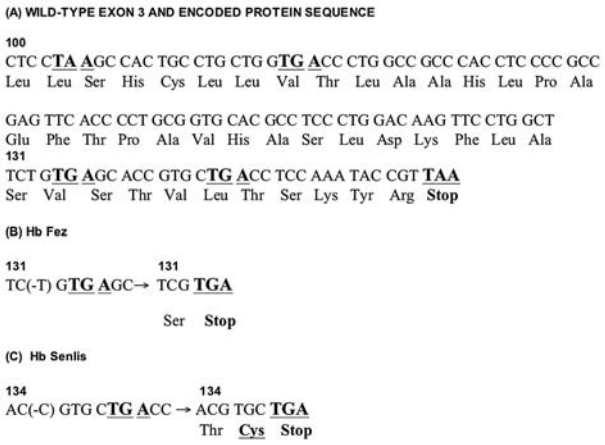

Figure 1. Nucleotide sequence of exon 3 in wild-type and mutated α globin genes, with resulting protein sequences. (A) Wild-type sequences of exon 3 in the α gene showing the potential stops (bold and underlined) that may result from FS. (B) In *Hb Fez*, the 3rd nucleotide of codon 131 is deleted, leading to FS and occurrence of a stop at CD132. (C) In Hb Senlis, deletion of a C within CD134 leads to a stop at CD136 and to a Cys as the outermost residue of the C-terminus.

Hb Fez and *Hb Senlis* (Table 1) are specified by a single nucleotide deletion leading to FS and a premature stop codon. *Hb Fez* was identified in a Moroccan patient with Heinz bodies observed on the blood film, without any visible abnormal Hb. The (-T) deletion, within CDα1-131, leads to a synonymous change at CD132 (TCT>TCG, both encoding Ser), followed by TGA (Stop), and a resulting 131-residue long protein. Hb Senlis was identified in a French Caucasian patient without any apparent abnormal Hb. The (-C) deletion within CDα1-134 leads to FS with two novel residues (Thr and Cys) encoded by codons 134 and 135 (ACG and TGC, respectively), followed by a Stop codon (TGA), and a resulting 135-residue long protein.

Deletion within the 3rd exon has a different outcome whether affecting the α- or β globin-encoding genes. In the α genes, the meaningfulness of the FS is consequent upon its occurrence within the gene sequence (Figure 1). When the deletion involves a nucleotide located between CD100 and 106, a stop is met at position 101 or 107, leading to a protein where helix H is missing, and thus unable to interact with AHSP or the β chain to form α1β1 dimers. Such mutants are likely to be α-thalassemic. A nucleotide deletion occurring between CD107 and 132 (the next potential stop), will more or less significantly alter the structure of helix H, depending how early it occurs within the sequence. As for *Hb Fez* and *Hb Senlis*, with stop codons at positions 132 or 136, respectively, the deleted residues are located by the end of helix H, in a region that does not interact with either AHSP or the β-chain partner.[6] Therefore, an abnormal, unstable, Hb tetramer may likely form and rapidly precipitate within the erythrocyte, accounting for the observed CHA. Furthermore, in *Hb Senlis*, a Cys residue occupies the C-terminus, allowing for possible interchain S-S bonds. When FS occurs more distal within an α2 gene sequence, the next stop is at position 147, leading to Hb Wayne,[7] a relatively stable molecule. In the α1 gene, the nearest potential stop following codon 136 will occur only at position 173, a possibility unreported to this day.

In the β globin gene, deletion of 1 or 2 nucleotides

Table 1. Hematological and DNA sequence data from the 3 patients.*

Variant	Mutation	Gender Age (years)	RBC 10^{12}/L	Hb g/dL	Ht %	MCV fl	MCH pg	Reticulocytes %	HbA_2 %
Hb Sens** (a243(CE1)Phe>Ile)	α2 CD 43 (TTC>ATC)	M 54	3.2	10.5	33.5	104	30	n.d	1.8
Hb Fez	α1 CD131 (TCT>TC-)	M 40	2.9	8.5	26.0	91	29.3	4.4	1.7
Hb Senlis	α1 CD134 (ACC>AC-)	F 62	2.5	8.8	27.0	105	34	3.9	1.9

** Molecular studies were performed as described in Moradkhani et al.[5] **splenectomized 18 years earlier.*

within the 3rd exon leads to FS with occurrence of a stop codon at position 156. In such cases where FS occurs proximal within the sequence, helix H is drastically altered by incorporation of several hydrophobic residues (six Leu, two Ile, four Trp), leading to dominant β thalassemia syndrome.[8] When the deletion is located at the very end of the 3rd exon, as in Hb Tak,[9] the mere outcome is a 10-residue-long C-terminal tail leading to moderate instability and high oxygen affinity.

Unlike most other α gene defects reported, the mutations described here have a dominant effect. Thus, the biological consequences of those mutations, whether missense or FS, are highly dependent upon their occurrence within the gene sequence.

Claude Préhu,[1,2] Kamran Moradkhani,[1,2] Jean Riou,[1] Michel Bahuau,[1] Pierre Launay,[2] Natacha Martin,[1] Henri Wajcman,[1,2] Michel Goossens,[1,2] and Frédéric Galactéros[3]

[1]AP-HP, Groupe Hospitalier Henri Mondor-Albert Chenevier, Service de Biochimie et de Génétique, 94000 Créteil; [2]INSERM U 955, IMRB, Département de Génétique Moléculaire, Equipe 11, 94000 Créteil; [3]AP-HP, Groupe Hospitalier Henri Mondor-Albert Chenevier, Unité des Maladies Génétiques du Globule Rouge, 94000 Créteil, France

Key words: hemolytic anemia, α hemoglobin variants, unstable hemoglobin, frameshift mutant, truncated chains.

Correspondence: Henri Wajcman, AP-HP, Groupe Hospitalier Henri Mondor-Albert Chenevier, Service de Biochimie et de Génétique, 94000 Créteil, France.
E-mail: henri.wajcman@inserm.fr

Citation: Préhu C, Moradkhani K, Riou J, Bahuau M, Launay P, Martin N, Wajcman H, Goossens M, and Galactéros F. Chronic hemolytic anemia due to novel α-globin chain variants: critical location of the mutation within the gene sequence for a dominant effect. Haematologica 2009; 94:1624-1625.
doi: 10.3324/haematol.2009.012971

References

1. Hardison RC, Chui DHK, Giardine B, Riemer C, Patrinos GP, Anagnou N, et al. HbVar: a relational database of human hemoglobin variants and thalassemia mutations at the globin gene server. Hum Mutat 2002;19:225-33.
2. Giardine B, van Baal S, Kaimakis P, Riemer C, Miller W, Samara M, et al. HbVar database of human hemoglobin variants and thalassemia mutations: 2007 update. Hum Mutat 2007;28:206.
3. Vasseur C, Domingues-Hamdi E, Brillet T, Marden MC, Baudin-Creuza V. The α-hemoglobin stabilizing protein and expression of unstable a-Hb variants. Clin Biochem 2009 May 29. [Epub ahead of print].
4. Wajcman H, Traeger-Synodinos J, Papassotiriou I, Giordano PC, Harteveld CL, Baudin-Creuza V, et al. Unstable and thalassemic α chain hemoglobin variants: a cause of Hb H disease and thalassemia intermedia. Hemoglobin 2008;32:327-49.
5. Moradkhani K, Mazurier E, Giordano PC, Wajcman H, Préhu C. An α 0-thalassemia-like mutation: Hb Suan-Dok [α109(G16)Leu_Arg] carried by a recombinant -α(3.7) gene. Hemoglobin 2008;32:419-24.
6. Feng L, Gell DA, Zhou S, Gu L, Kong Y, Li J, et al. Molecular mechanism of AHSP-mediated stabilization of α-hemoglobin. Cell 2004;119:629-40.
7. Seid-Akhavan M, Winter WP, Abramson RK, Rucknagel DL. Hemoglobin Wayne: a frameshift mutation detected in human hemoglobin α chains. Proc Natl Acad Sci USA 1976;73:882-6.
8. Préhu C, Pissard S, Al-Sheikh M, Le Niger C, Bachir D, Galactéros F, et al. Two French Caucasian families with dominant thalassemia-like phenotypes due to hyper unstable hemoglobin variants: Hb Sainte Seve [codon 118 (-T)] and codon 127 [CAG_TAG (Gln_Stop]). Hemoglobin 2005;29:229-33.
9. Flatz G, Kinderlerer JL, Kilmartin JV, Lehmann H. Haemoglobin Tak: a variant with additional residues at the end of the β-chains. Lancet 1971;1:732-3.

Safety of cardiovascular magnetic resonance gadolinium chelates contrast agents in patients with hemoglobinopathies

Myocardial fibrosis/necrosis has been documented by histological and Cardiovascular Magnetic Resonance (CMR) studies in patients with hemoglobinopathies.[1] The delayed enhancement CMR technique with intravenous administration of gadolinium (Gd) chelates contrast agents is the only validated approach for detecting myocardial fibrosis non-invasively.[2]

Recently, serious concerns have been raised regarding the safety of Gd chelates. In particular, a link between gadolinium and nephrogenic systemic fibrosis (NSF) has emerged, due to the presence of gadolinium in skin samples of NSF patients.[3] However, NSF has also been diagnosed in patients who had not been exposed to gadolinium. NSF is a scleroderma-like disease mainly involving the skin and it is closely related to severe kidney failure (glomerular filtration rate (GFR) < 30 mL/min/1.73 m^2).[4] No cases have been reported in patients with GFR > 60 mL/min/1.73 m^2. Although cause and effect have not been proven for the NSF-gadolinium link, avoidance and care have been strongly recommended.[5] In addition, unconfirmed doubts have been raised about the use of Gd-chelate contrast agents in patients with hemoglobinopathies, characterized by heavy co-morbidity due to iron overload, which can also damage the kidneys and could be a co-factor, further enhancing the risk for NSF development.[6] To date, there have been no dedicated clinical studies on the safety of the Gd chelates in these