resistant MDR cells.[10] The contribution of these proteins to As_2O_3 resistance in APL patients will need to be further examined.

Wing Y. Au, Chor Sang Chim, Albert Kwok Wai Lie, Annie Pang, Yok Lam Kwong

Department of Medicine, University of Hong Kong, Queen Mary Hospital, Hong Kong

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Correspondence: Dr. Yok Lam Kwong, MD, University Department of Medicine, Professorial Block, Queen Mary Hospital, Pokfulam Road, Hong Kong. Phone: international +852.2. 8554597. Fax: international +852.2.974 1165. E-mail: ylkwong@hkucc.hku.hk

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Identification and functional characterization of a new hemoglobin variant in Sardinia: Hb Muravera [β47 GAT→ GTT, (CD6) Asp→Val]

Hemoglobin *Muravera* [β47 Asp→Val] is a new, slightly unstable hemoglobin variant found in Sardinia during a screening for β-thalassemia. Purified Hb *Muravera* displays an oxygen affinity higher than that of HbA in the absence of 2,3 DPG, and a faster than normal rate of auto-oxidation. The functional alterations of Hb Muravera could be due to the structural modification induced by the type and position of the substituted amino acid.

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The β47 (CD6) residue of human hemoglobin (Hb)A is normally an aspartic acid that forms intrachain contacts with β53 Ala, β54 Val and β57 Asn.[1] The β47 (CD6) residue is in an external non-helical segment that, although not directly involved in the heme contacts, might contribute significantly to maintaining the structure of the heme pocket, therefore establishing the oxygen binding properties of the molecule.[2] We report here a new hemoglobin variant, resulting from a GAT→GTT mutation at codon 47 of the β globin gene, which predicts an Asp→Val amino acid substitution. This variant was detected during screening to identify β-thalassemia carriers in Southern Sardinia. Hemoglobin analysis of the proband performed by high performance liquid chromatography (Variant I, Bio-Rad, Milan, Italy) showed an abnormal peak eluting with HbA_2, in the amount of 33.3%. On cellulose acetate electrophoresis at alkaline pH, the abnormal band was in the HbS-like position, but the sickling test was negative. The proband had mild microcytosis (MCV = 78.1 fL) and hypochromia (MCH = 26.2 pg), but a normal hemoglobin level (Hb = 15.6 g/dL). HbA_2 determined by DE-52 microchromatography was 3.3% (Figure 1). The isopropanol test was weakly positive. Globin chain synthesis analysis showed an α/non-α globin chain synthesis ratio of 0.75, compatible with an α-thalassemia carrier state confirmed by α-globin gene analysis (genotype –3.7 α/αα). The proband's sister had mild reticulocytosis, a weakly positive isopropanol test, and some red blood cells containing inclusion bodies, after 1 h incubation at

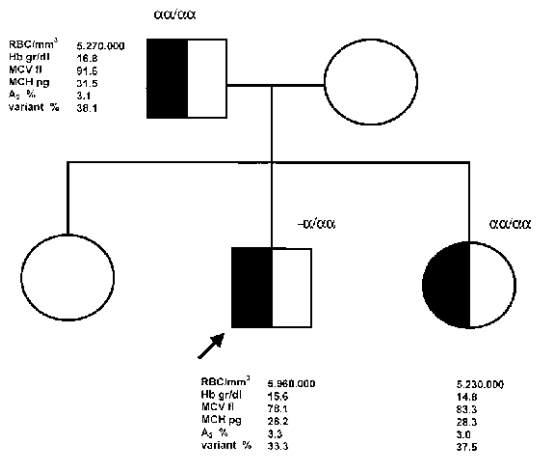

Figure 1. Pedigree of the family. The proband is indicated by the arrow.

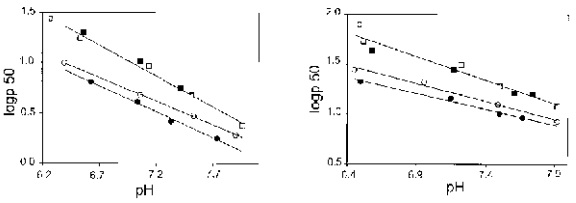

Figure 2. Böhr effect at 20°C (a) and 37°C (b) of total hemolysate (open symbols) and purified Hb Muravera (closed symbols) in 0.1 M Bis/Tris-HCl buffer + 0.1 M NaCl in the absence (○) and in the presence (□) of 5 mM 2,3 DPG; O_2 pressure is expressed in Torr units.

37°C with brilliant cresyl blue. Clinical examination of the proband, his sister and father did not demonstrate any pathologic finding; their hematologic characteristics are reported in Figure 1. DNA analysis of polymerase chain reaction (PCR) amplified β globin gene by direct sequencing,[3] revealed a GAT→GTT mutation at codon 47, predicted to encode an Asp→Val substitution. This new Hb variant was named Hb Muravera from the Sardinian village in which the proband was born. The functional studies were performed on the proband total hemolysate and on hemoglobin components isolated by ion-exchange chromatography on a column (2.5×20 cm) of DEAE-cellulose. The column was first equilibrated with 20 mM Tris-HCl buffer, at pH 8.0, which was then decreased to 7.0 with a linear gradient. The components were checked for purity by isoelectric-focusing. Oxygen dissociation curves were determined spectrophotometrically by the tonometric method[4] in 2-[bis (2-hydroxyethyl) amino]-2-(hydroxymethyl)-propane-1,3-diol (Bis-Tris) and Tris [hydroxymethyl] aminomethane (Tris) buffer, both in the absence and in the presence of the 2,3-diphosphoglycerate (2,3-DPG). The oxygen affinity at 20°C and 37°C of the proband's total hemolysate was not different from that of normal hemoglobin A. Cooperativity of oxygen binding, as indicated by the value of the Hill coefficient n_{50} (the slope of the binding curve at 50% saturation) was not affected by mutation, being very similar to that of the native human hemoglobin (n_{50} = 2.8). The purified hemoglobin showed an intrinsic oxygen affinity higher than that of the total hemolysate. In fact in the absence of 2,3-DPG the log p50 value of the purified Hb was lower than that of total hemolysate at all pH values examined. However, this difference was abolished after addition of 2,3 DPG (Figure 2).

Hb Muravera offered us the opportunity of determining the effect of a hydrophobic residue replacing a hydrophilic residue at position β47. Position β47 is an external residue that, normally, as for the stereochemical model proposed by Perutz,[2] is not directly involved in determining functional alterations in the tetrameric molecule. In all the hemoglobin variants at the same position so far described,[5-10] the reported hematologic findings and the functional studies are normal. In Hb Muravera there is a normal hemoglobin level despite the mild instability and the presence of α-thalassemia, likely as a consequence of the increased oxygen affinity. The substitution of a hydrophilic residue (Asp) by a hydrophobic one (Val) could produce some conformational change, resulting in modified oxygen affinity. This effect is abolished by 2,3-DPG, probably because the β globin chain interactions, affected by the organic phosphate, produce structural modifications that hide those caused by the substitution at the β47 residue. The effect of this substitution is, on the contrary, evident on the molecule stability. In fact in the presence of valine, replacing the aspartic acid, the heme group is probably more exposed to oxidation.

These results are a further demonstration of the remarkable heterogeneity in functional characteristics of the hemoglobin molecule, as a consequence of the position of the mutation and, much more, of the type of amino acid substitution.

Marcella Corda, Antonella Fais,* Luciana Perseu,° Loretta Cipollina,@ Susanna Barella,# Renzo Galanello@*

**Dipartimento di Scienze Applicate ai Biosistemi, Università di Cagliari, Monserrato (CA), Italy; °CNR, #Ospedale Regionale Microcitemie, @Dipartimento di Scienze Biomediche e Biotecnologie, Cagliari, Italy*

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Correspondence: Prof. Renzo Galanello, Dipartimento di Scienze Biomediche e Biotecnologie, via Jenner s.n., 09121 Cagliari, Italy. Phone: international +39.070.6095508. Fax: international +039.070.6095509. E-mail: renzo.galanello@mcweb.unica.it

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