resistant MDR cells. The contribution of these proteins to As2O3 resistance in APL patients will need to be further examined.

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

Hemoglobin Muravera ([β47 (CD6) 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 (HbA) is normally an aspartic acid that forms intrachain contacts with β53 Ala, β54 Val and β57 Asn. The β47 (CD6) residue is in an external non-helical segment, which is not directly involved in the heme contacts, with a small contribution to maintaining the structure of the heme pocket. The oxygen binding properties of the molecule. We report here a new hemoglobin variant, resulting from a GAT–GTI mutation at codon 47 of the β globin gene, which predicts an Asp→Val amino acid substitution. This variant was diagnosed during screening to identify β-thalassemia carriers in Southern Sardinia. Hemoglobin analysis of the proband performed by high performance liquid chromatography (Variant II, Bio-Rad, Milan, Italy) showed an abnormal peak eluting with HbAΔ, in the amount of 33.3%. On cellulose acetate electrophoresis at alkaline pH, the abnormal band was in the HbF-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). HbAΔ was determined by DE-52 microchromatography to be 3.3% (Figure 1). The isosmolar test was weakly positive. Globin chain synthesis analysis showed an α/β ratio of 0.75, compatible with an α-thalassemia carrier state confirmed by 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 isosmolar 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.

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37°C with brilliant cresyl blue. Clinical examination of the
proband, his sister and father did not demonstrate any patho-
logic finding; their hematologic characteristics are reported
in Figure 1. DNA analysis of polymerase chain reaction (PCR)
amplified β-globin gene by direct sequencing, revealed a GAT→GT
mutation at codon 47, predicted to encode an Asp→Val substi-
tution. This new Hb variant was named Hb Muravera from the
Sardinian village in which the proband was born. The function-
al studies were performed on the proband total hemolysate and
on hemoglobin components isolated by ion-exchange chro-
matography 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 spectrophotomet-
ically by the tonometric method in 0.1 M Bis/Tris-HCl buffer + 0.1 M NaCl in the
absence (C) and in the presence (D) of 5 mM 2,3 DPG; O2
pressure is expressed in Torr units.

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 (C) and in the presence (D) of 5 mM 2,3 DPG; O2
pressure is expressed in Torr units.

37°C with brilliant cresyl blue. Clinical examination of the

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.

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