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LETTER TO THE EDITOR

Hb Fairfax [HBB:c.285_286insGAGCTGCACTGTGAC] in a Brazilian patient with severe hemolytic anemia—identification and functional study

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Dear Editor,

Hemoglobin (Hb) Fairfax [HBB:c.285_286insGAGCTGCACTGTGAC] is an unstable Hb variant first described in an African-American child [1] and then in an Iranian family [2]. It results from duplication of a 15-bp sequence (GAG-CTG-CAC-TGT-GAC) between codons 95 and 96 of the β-globin gene, with consequent insertion of five additional residues (-Glu-Leu-His-Cys-Asp-) in the interhelical FG region of the β-globin chain, and is associated with a dominant β-thalassemia phenotype [1–3]. We report here the third case of HbFairfax, detected as a de novo mutation in a Brazilian woman with severe hemolytic anemia. Functional and modeling studies were also performed.

The 25-year-old white female patient presented with severe hemolytic anemia (RBC = 2.15×10^{12}/L, Hb = 7.4 g/dL, Ht = 30.6%, MCV = 142.3 fL, MCH = 34.4 pg, RDW = 26.6%) and a history of marked hepatosplenomegaly, RBC-transfusion dependence (transfusions every 15 days, from 3 months to 8 years of age), and iron-chelation therapy; she was splenectomized when she was 8 years old after consecutive splenic sequestration crises and currently receives occasional transfusions. Neither her parents nor her siblings showed hematological alterations. In alkaline electrophoresis, HbFairfax migrated as HbS; in CE-HPLC (VARIANT II™, BioRad Laboratories, Hercules, CA, USA), it eluted slower than HbA and accounted for only 11.9% of total Hb (Fig. 1a) (HbA_2 = 3.6%; HbF = 3.1%); in RP-HPLC (Waters Corporation, Milford, CA, USA), the $\beta^{Fairfax}$ chain eluted as δ-chains (Fig. 1b). Tests for unstable Hb were positive [4]. The mutation was detected by direct sequencing of the β-globin genes (ABI PRISM® 3500, Applied Biosystems, Foster City, CA, USA) in a heterozygous state (Fig. 1c).

The O_2 affinity of the lysate containing HbFairfax and HbA was evaluated by determining the p50 values (Hemox Analyzer, TCS Scientific Corporation, New Hope, PA, USA), which were decreased (Table 1, Fig. 1d), indicating increased O_2 affinity. *Heme-heme* cooperativity, calculated by Hill coefficient (*n*), was also decreased as demonstrated in Table 1.

Predictions of the tertiary structure of $\beta^{Fairfax}$ by homology modeling (CPH models 3.2 server, University of Denmark) using the 1DXT.pdb coordinates as the native β coordinates in the *deoxy*-HbA form suggest that the insertion of the residues changed the structural plane of the interhelical region and the end of the helix, where the proximal histidine is located (Fig. 1e).

Four variants with single substitutions of βAsp95 have been described to date: HbGeldrop St Anna, HbBunbury, HbBarcelona, and HbChandigarh [5–9]. They all result in erythrocytosis because of increased O_2 affinity suggesting that the beginning of the FG region, specifically the position corresponding to the β95 residue, may play an active role in exposing the active site by means of conformational changes in the F

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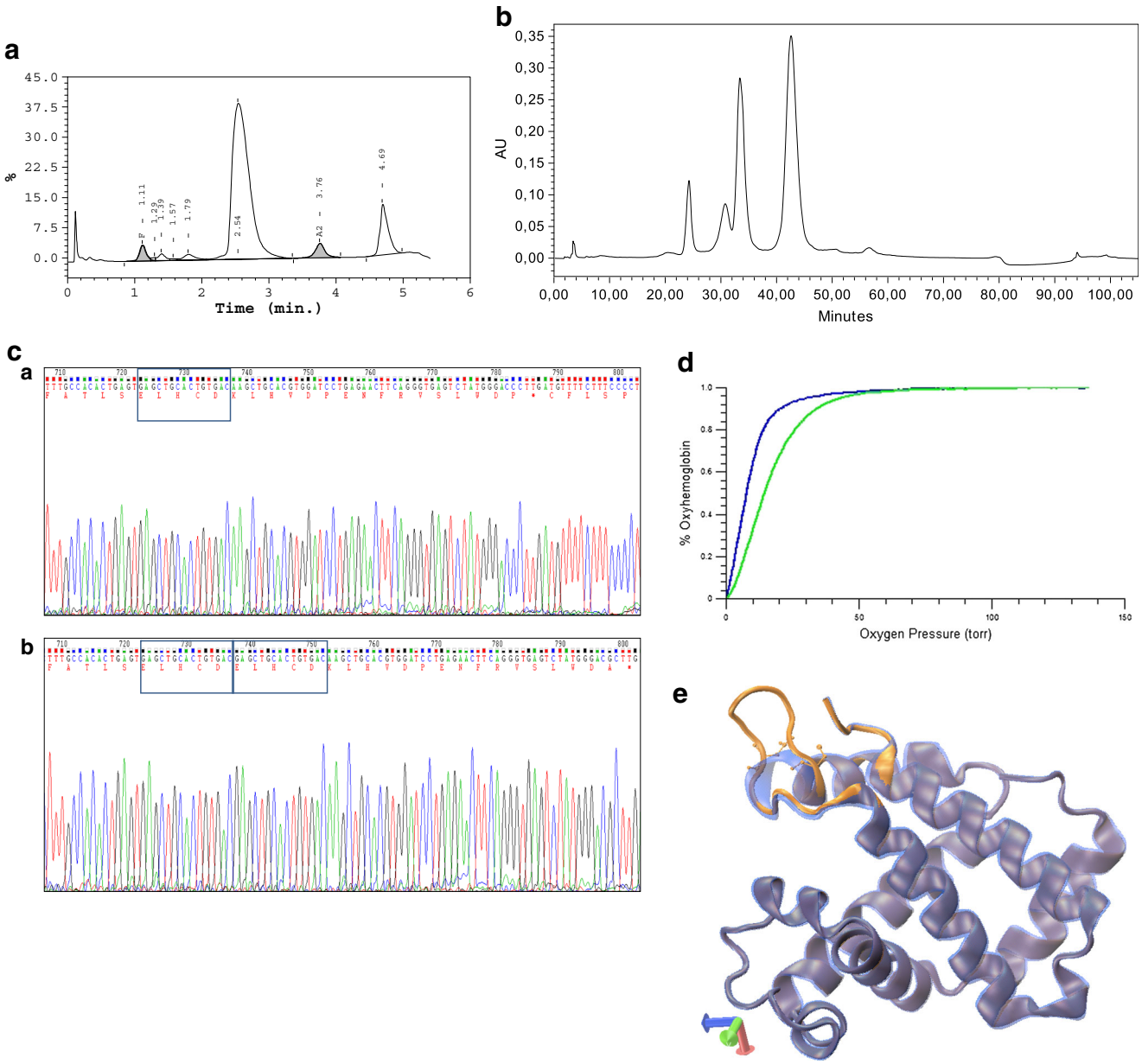

Fig. 1 **a** CE-HPLC chromatogram showing the anomalous retention peak (arrow). **b** RP-HPLC chromatogram showing co-elution of the $\beta^{Fairfax}$ chains with the δ-chains (arrow). **c** β-globin gene sequencing showing the wild-type (A) and mutant (B) allele sequences. **d** Oxygen-Hb dissociation curve of native HbA (in green) and HbFairfax RBC lysates (in blue) demonstrating the increased O_2 affinity conferred by the presence of the Hb variant. **e** Representation of the tertiary structure of β-Fairfax (in orange) and the native β structure (in blue). Detail showing the FG interhelical region. β-Fairfax (in orange) and native β (in blue)

helix. Hence, the insertion of five residues in the region could, besides causing instability in the functional tetramer, modify the exposure plane and binding of O_2 to the heme group, reducing the mobility of the region and further exposing the active site, which may result in increased affinity for the ligand. This exposure could increase instability due to autoxidation without, however, complete denaturation of the tetramer, as a small amount of the variant was detected in the patient's peripheral blood.

Table 1 p50 values and *heme-heme* cooperativity (by the Hill coefficient, *n*) determined from the dissociation (*deoxy*) and O_2 saturation (*oxy*) curves

	p50 (*deoxy* curves)	Hill, *n* (*deoxy* curves)	p50 (*oxy* curves)	Hill, *n* (*oxy* curves)
HbA	12.26 (SD 0.19)	2.86 (SD 0.15)	11.78 (SD 0.13)	2.21 (SD 0.12)
HbA + HbFairfax	8.13 (SD 0.52)	1.97 (SD 0.16)	7.89 (SD 0.4)	1.81 (SD 0.13)

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Compliance with ethical standards

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008 (5). The patient gave her written consent for the investigation.

Conflict of interest The authors declare that they have no conflict of interest.

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