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SHORT COMMUNICATION

Hb L'AQUILA [β106(G8)Leu→Val, *C*TG→*G*TG]: A NOVEL THALASSEMIC HEMOGLOBIN VARIANT

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□ *A new β-globin variant at codon 106 (CTG→GTG), and which we named Hb L'Aquila [β106(G8)Leu→Val], was detected by DNA analysis. The proband and her father presented with the features of a mild β^+-thalassemia (thal), confirmed by their α/β-globin chain biosynthesis ratios.* 10

Keywords Hemoglobin (Hb) variants, β-Thalassemia (thal), splicing region

More than 900 hemoglobin (Hb) variants have been described so far (1), but only a few of those lead to hematological disorders. Their characterization, in molecular genetic screening, is of pivotal relevance since their association with a defect in Hb synthesis has been shown to modify clinical 15 expression and could lead to thalassemia intermedia. In this study, we report a novel β-globin gene mutation that was detected in a 26-year-old Italian female, who presented with a hypochromic, microcytic anemia without any hemolytic trait and any peculiar medical record (Table 1). This novel variant was named Hb L'Aquila after the place of birth of the 20 proband.

After direct DNA sequencing of the entire β-globin gene, a *C*TG→*G*TG transversion located at codon 106 and predicting a Leu→Val amino acid change, was detected (Figure 1). A novel amplification refractory mutation system (ARMS) analysis was designed to confirm the DNA sequence data, 25

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TABLE 1 Hematological Findings in the Proband and Her Family

Parameters	Proband	Mother	Father	Brother
Sex-Age	F-27	F-55	M-61	M-35
Hb (g/dL)	10.8	12.6	13.8	15.0
RBC (10^{12}/L)	4.35	4.25	5.71	4.98
MCV (fL)	76.0	88.0	74.0	86.0
MCH (pg)	24.8	29.7	24.1	30.1
Reticulocytes (%)	21.0	18.0	15.0	19.0
Ferritin (ng/mL)	42.0	38.0	133.0	124.0
Hb A_2 (%)	4.3	2.6	4.8	2.9
Hb F (%)	0.9	0.3	0.9	1.1
Hb L'Aquila heterozygosity	[+]	[−]	[+]	[−]
Isopropanol test	[−]	n.d.	[−]	n.d.
Heat test	[−]	n.d.	[−]	n.d.
Globin chain synthesis	1.15	n.d.	1.37	n.d.

n.d.: not determined.

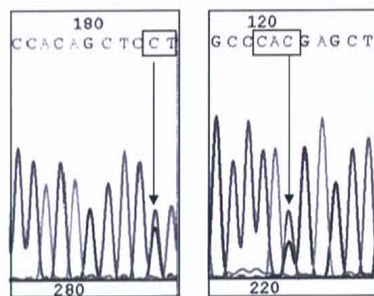

FIGURE 1 The β-globin DNA sequence of the Hb L'Aquila carrier, showing the *CTG*→*GTG* point mutation at codon 106. The left panel shows the forward sequence; the right panel shows the reversed sequence.

thus showing the fragment with the predicted size for the mutation described above (Figure 2). 35

Cation exchange high performance liquid chromatography (HPLC) (VARIANT II™; Bio-Rad Laboratories, Hercules, CA, USA) showed a Hb A_2 level of 4.3% (normal range 2.0–3.2), a Hb F level of 0.9% and no Hb variant. The variant could not be detected either by electrophoresis on citrate agar 40 (pH 6.0) or on cellulose acetate (pH 8.6), or by capillary electrophoresis

FIGURE 2 The ARMS-PCR (polymerase chain reaction) analysis for detection of the mutation at codon 106. Allele-specific mutant reverse primer: 5'-GCA CAC AGA CCA GCA CGT TGC CAA C-3'. This primer was coupled with the forward common primer: 5'- CAA TGT ATC ATG CCT CTT TGC ACC-3'. The 285 bp product seen in lane 1 indicates the presence of the mutation, while the absence of this band in lane 2 indicates its absence. The 330 bp internal control product in both lanes indicates the PCR efficacy.

(Capillarys™ 2; Diagnostic Systems, Bagno a Ripoli (FI) Sebia Italia Srl, Florence, Italy). Heat stability and isopropanol tests failed to detect any abnormal Hb precipitate in the hemolysate. For biosynthetic studies, the globin chains were separated by reversed phase HPLC, using a Vydac large pore C_4 column (The Separations Group, Hesperia, CA, USA). The α/β-globin biosynthesis ratio was 1.15 in the proband and 1.37 in her father, which are in the range of values found in β^+-thalassemia (thal) heterozygotes (the value in normal individuals being ~1). The presence of the most common α-globin deletional thalassemic mutations and of the $\alpha\alpha\alpha^{anti\ 3.7}$ allele have been excluded.

The proband's family was also examined: her mother and brother were hematologically normal, whereas her father, who carried the mutation, showed very similar hematological and Hb features (Table 1). All family members had normal iron metabolism indices.

Three other variants have previously been described involving position β106(G8): Hb Tubingen, in which the leucine residue is replaced by glutamine. The leucine residue at G8 has a hydrophobic contact with the heme group and its substitution by a hydrophilic residue allows water to enter into the heme pocket, thus causing a moderate instability of this variant with high oxygen affinity and increased auto-oxidation rate (2). Hb Southampton

(also known as Hb Casper), in which the leucine residue is replaced by proline. This substitution causes a distortion of the G helix destroying a heme contact and leading to a very unstable Hb with a severe hemolytic anemia (3). Hb Terre Haute, in which the leucine residue is replaced by arginine. This substitution introduces a positive charge into the hydrophobic heme contact, giving rise to the extreme instability of this Hb variant. For this reason, the variant could not be detected by standard techniques of Hb identification (4).

Our variant, Hb L'Aquila, caused by substitution of valine for leucine, has shown normal stability, likely because the genetic alteration gives rise to a conservative replacement between neutral and hydrophobic residues, thus explaining the absence of hemolytic anemia, polycytemia or cyanosis in the proband and in her father.

Using the ESE finder program (http://rulai.cshl.edu/tools/ESE), we have found that the described mutation modifies the splicing region, since the SRp40 motif has disappeared, similar to Hb Terre Haute and Hb Southampton but different from Hb Tubingen.

This novel β-globin variant, Hb L'Aquila, was completely silent in alkaline, acid or capillary electrophoreses and by cation exchange HPLC. The only difference with respect to normal individuals was observed using globin chain chromatographic separation, which detected the higher pre-β peak in the proband. This larger size could be due to the presence of a variant globin chain that comigrates with the pre-β peak. An electrospray ionization-mass spectrometry (ESI-MS) measurement might be useful to detect and establish at which level this silent Hb variant is eventually present, but both the proband and her father are not disposed to collaborate for further analyses.

We hypothesize that the unbalanced globin chain synthesis ratio is caused by the mutation affecting the splicing region (5): the amount of the correctly spliced mRNA is then lower than normal, resulting in a decreased concentration of the β-L'Aquila chain compared to the β^A chain. As such, the reduction in normal splicing might be the molecular basis for the minimal pathological effect of β^+-thal associated with this structural variant.

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