Hb I-Toulouse in association with homozygosity for the $\alpha^{3.7}$ deletion in a Pacific Island woman

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Abstract

Only four cases of Hb I-Toulouse have been reported to date. Current literature associates Hb I-Toulouse in the heterozygote with a mild chronic hemolytic anemia. The variant is mildly unstable with a tendency to form methHb. The quantity of the variant in heterozygotes has been reported as varying between 33 to 40%. This report confirms the finding from a single case, that a reduced percentage of Hb I-Toulouse along with microcytosis can be attributed to the co-inheritance of an abnormal α globin genotype. This current case was found in a woman of Pacific People ethnicity residing in New Zealand. There is a high prevalence of α thalassemia in this ethnic group and New Zealand has the highest Pacific population in the world. Therefore, if a reduced percentage of Hb I-Toulouse is found with microcytosis and normal iron studies, co-inheritance with α thalassemia should be considered.

Introduction

There have been very few reported cases of Hb I-Toulouse. The first was described in a previously healthy French Caucasian after he had an attack of gout and became mildly anemic.¹ A second case in association with Hb S trait was found in a seven-year-old white Nicaraguan girl.² Two more cases were reported in a 25 year-old pregnant Solomon Islander and her 10-year old paternal cousin.³ In Hb I-Toulouse the amino acid substitution occurs in the E10 region producing an alteration in the ionic bond between lysine in the position 66 of the β -chain and the propionic chain of the heme giving rise to a slightly unstable ferrihemoglobin associated with a mild hemolytic anemia in the heterozygous state.²

Case Report

Here, a case is reported of heterozygous Hb I-Toulouse with homozygosity for the $\alpha^{3.7}$ deletion in a 50 year old Solomon Island woman.

She presented with essential thrombocytosis and her full blood count was: hemoglobin 124 g/L, RBC $5.28 \times 10^{12}/L$, MCV 78 fL, MCH 23.5 g/dl and platelets $822 \times 10^9/L$. Her iron of 23 $\mu\text{mol/L}$ (10-30) and ferritin 273 $\mu\text{g/L}$ (20-200) were both within the normal range.

Screening tests for variant hemoglobin included: Alkaline electrophoresis, HPLC, isopropanol flocculation test for unstable hemoglobin and HbH staining for α thalassemia. Hemoglobin electrophoresis was performed on cellulose acetate at pH 8.6 using the Sebia Hydragel (E) Hemoglobin kit. Staining with amidoblack revealed the presence of two major bands; one with the mobility of Hb A and the other a fast band running in the Hb I position.

High performance liquid chromatography (HPLC) was undertaken using the Bio-Rad D10 system with the HbA2/F extended programme. HbA2 of 2.4% (1.7-3.8) and HbF <1% (<1%) as determined by HPLC were normal. The variant hemoglobin showed a peak of 31.1% running between the P3 and A₀ peaks with a retention time of 1.61 (Figure 1).

The variant hemoglobin was shown to be slightly unstable in the isopropanol flocculation test, with a light precipitate forming at 5 minutes and a flocculent precipitate occurring at 20 minutes. This was compared to no flocculation at 30 minutes for the normal control tested at the same time. A few HbH bodies were detected after incubating equal volumes of brilliant cresyl blue and whole blood at 37°C for 2 hours. As a variant hemoglobin was present, further investigations were undertaken to ascertain its identity. Direct analysis of whole lysate by electrospray ionization (ESI) mass spectrometry on a VG platform quadrupole mass spectrometer⁴ (Micromass, Manchester, U.K) showed a normal α chain mass of 15,126 Da and a β chain of 15,868 Da, consistent with the 1 Da increase associated with the suspected Glu→Lys mutation.

Further examination of lysate by reverse phase HPLC⁵ showed a more hydrophobic b chain representing 35% of the total β chain material (Figure 2). β chains were further characterized by tryptic peptide mapping which showed 0.4 and 0.3 increases in m/z of the +2 and +3 ions of peptide b8-9, suggesting a b66Lys→Glu mutation (Figure 3). To confirm this finding DNA sequencing of the b-gene was undertaken. DNA was extracted from the peripheral blood and Coding and non-coding regions of the beta globin gene (HBB) were analyzed by PCR based automated fluorescent DNA sequencing. The resulting sequence was compared to the GenBank reference sequence NM_000518.4. Result showed heterozygous HBB:c.199A>G, (AAA(Lys)→GAA(Glu)) substitution at codon 66 of the mature b-globin chain. This result supports the mass spectrometry findings with the variant previously reported as Hb I-Toulouse. As HbH bodies were

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present, α -globin gene analysis was undertaken to establish the genotype. DNA was isolated from peripheral blood leukocytes and was screened for six common deletions and one triplication using a multiplex PCR amplification. A normal (α) 2 gene was not detected. These results indicate homozygosity for the -3.7kb α gene deletion, which abolishes one α globin gene copy from each affected chromosome. This is consistent with a diagnosis of α thalassemia of genotype $-\alpha^{3.7}/-\alpha^{3.7}$.

Discussion

Hematological and clinical features of the original French patient and the Nicaraguan girl, as reported by Rosa *et al.*¹ and Tejuca *et al.*² respectively were very similar. Both were normocytic and macrocytic, Rosa *et al.*¹ reported an MCV of 120 microns³, and Tejuca *et al.*² reported value was 110 fL. This case and Hendy and Cauchi's³ 25 year-old pregnant Solomon Islander had MCV's of 72 and 78 fL respectively and both showed hypochromia and microcytosis. The 10-year old child of Hendy and Cauchi³ had an MCV of 75 fL, which was on the lower edge of normal (75-95) but showed no microcytosis. The percentage of Hb I-Toulouse (31.1%) detected in this case was similar to that observed in Hendy and Cauchi's³ 25 year-old pregnant Solomon Islander of 33%, but lower than the 40% in Hendy and Cauchi's³ 10-year old child or the first reported case by Rosa.¹

Figure 1. High-performance liquid chromatography using Bio-Rad D10 showing the hemoglobin variant peak of 31.1% running between the P3 and A0 peaks with a retention time of 1.61 (arrow).

Figure 2. Reverse phase high-performance liquid chromatography profile of whole lysate from subject (lower trace) and control (upper trace). Subject is heterozygous for more hydrophobic I chain making up 35% of the total.

Figure 3. Electrospray ionization mass spectra of tryptic digests of the normal (top) and abnormal β -chain (bottom).

Hendy and Cauchi reported that the differences in laboratory features observed between their two patients, viz microcytosis and lower percentage of Hb I-Toulouse, in the presence of similar low iron levels (5 and 9), could be attributed to the difference in the α -globin genotype.³ The 25 year-old pregnant Solomon Islander's genotype was $-\alpha^{3.7}/-\alpha^{3.7}$, whilst the 10-year old child's genotype was $\alpha\alpha/-\alpha^{3.7}$. Similarly, this case was found to have a genotype of $-\alpha^{3.7}/-\alpha^{3.7}$ in the presence of normal iron and ferritin levels of 23 $\mu\text{mol/L}$ and 273 $\mu\text{g/L}$ respectively. It is interesting to note that all three cases (both Hendy and Cauchi's³ and this case) that were diagnosed with Hb I-Toulouse and concomitant α thalassemia, were from the same ethnic background. The presence of concomitant α thalassemia in these cases may not be surprising as there is an increased prevalence of genes causing α thalassemia amongst Māori and Pacific Peoples as well as people of Chinese, South East Asian, Southern European, Mediterranean, Middle Eastern, Indian subcontinent and African ancestry.⁶⁻⁸ Additionally, in this case found in NZ, the largest Pacific population (represented primarily by Samoan, Cook Islands, Tongan, Niuean, Fijian and Tokelauan groups, Tuvalu, Kiribati, Papua New Guinea, Vanuatu, Solomon Islands and island states of Micronesia) in the world resides in Auckland, and comprises 14.6% of NZ's population.⁹ At present almost one in four persons in New Zealand's 4.3 million population is of ethnic origin.¹⁰ The Asian ethnic (which includes Chinese, Indian, Korean, Filipino, Japanese, Sri Lankan, Cambodian and Thai) population in NZ is 11.8%.¹¹ As New Zealand's cultural diversity is predicted to increase substantially, one needs to be aware of the possibility of Hb I-Toulouse appearing with concurrent α thalassemia.

Conclusions

In the case presented here a low level of the variant Hb I-Toulouse is partially explained by the reported instability of Hb Toulouse which has a tendency to form met Hb, but also due to the homozygosity of the $-\alpha^{3.7}$ thalassemia deletion.

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