Two familial cases of Hb Tyne confirm instability as cause of low expression

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Abstract

We report a second occurrence of hemoglobin (Hb) Tyne, [$\beta 5$ (A2) Pro>Ser] *HBB*:c.16C>T(p.Pro6Ser), which like the first case was associated with normal hematology. We verified the variant was mildly unstable by showing it was greatly enriched in isopropanol precipitates. This minor instability accounts for the slightly decreased expression of the new β chain. The variant was picked up as an interfering component on HbA1c testing using cation exchange high performance liquid chromatography (HPLC). However, this may be an advantage in detecting electrophoretically silent variants. Furthermore, this report also highlights the importance of uneven or sloping baselines on HPLC, which could reflect the presence of a variant hemoglobin even in the presence of normal electrophoresis and full blood count.

Introduction

There has been only one previous report of Hb Tyne where it was identified in two unrelated diabetic males.¹ There was no obvious hematological abnormality associated with either case and full blood counts were normal. The variant Hb was detected by cation exchange HPLC where it eluted close behind HbA. However, Hb Tyne did not separate by any electrophoresis technique, *viz* cellulose acetate at pH 8.4, agar gel at pH 6.0 or isoelectrofocusing on a pH 6-9 gradient. The HbA₂ and HbF levels were normal. Isopropanol stability tests however, were equivocal; one case giving a negative result and the other weak positive. Both cases were heterozygous and had approximately 37% Hb Tyne.

Case Report

Here, two further cases are described in a Caucasian mother and daughter, aged 75 and 52 respectively. Both of these women presented separately a few months apart with atypical chest pain and had cardiac investigations. The daughter developed an anterolateral STEMI. Part of their cardiac workup involved HbA1c testing, and both cases showed an interfering component present on ion-exchange HPLC. HbA1c (IFCC) results by affinity method were similar for both mother and daughter at 41 and 44 mmol/mol ($nr < 40$) respectively. Both had normal full blood counts, electrophoresis, ferritin, HbF and HbA₂ (Table 1).

High performance liquid chromatography (HPLC) was initially undertaken using a Bio-Rad D10 (Hercules, California, USA) instrument, column and reagents and the HbA₂/F extended programme. This showed an unusual sloping baseline (Figure 1A) with no other remarkable features. Subsequent analyzing on a Bio-Rad Variant II with a β -Thalassaemia column, showed an aberrant peak of approximately 41% eluting as a trailing shoulder on the HbA₀ peak (Figure 1B).

Examination of lysate by electrospray mass spectrometry on an Agilent 6230 time-of-flight instrument (Agilent Technologies, Santa Clara, CA, USA) showed a new β -chain with a mass decrease of 10 Da indicating a Pro→Ser substitution; the only point mutation capable of generating this

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mass shift (Figure 2 top). The new β chain represented 42% of the total β material and this low expression level appears to be due to intrinsic instability, because when a standard isopropanol test was extended to 40 min and the light precipitate that formed

Figure 1. A) Bio-Rad D10 HPLC profile showing sloping baseline; B) Variant II profile showing aberrant peak eluting as a trailing shoulder on the HbA₀ peak.

Table 1. Hematology and chemistry results for these reported cases of heterozygous Hb Tyne.

	Haemoglobin g/L	RBC X10 ¹² /L	MCV fL	MCH pg	Ferritin/L µg	HbA1c mmol/mol	Hb F % (D10 results)	Hb A ₂ % (D10 results)	Hb H bodies
Mother (Age: 75)	135	4.23	96	33	98	41	<1	2.3	None seen
Daughter (Age: 52)	134	4.22	96	32	60	44	<1	3.1	None seen

Figure 2. Transformed mass spectra of whole lysate (top) and redissolved isopropanol precipitate (bottom) from patient heterozygous for Hb Tyne showing normal (15,867 Da) and variant (15,827 Da) β chains. The precipitate is enriched with 15,857 Da chains confirming instability.

Figure 3. Tryptic peptide map showing a decrease in intensity at 952.5 m/z and a new signal at 942.5 m/z confirming a $\beta 5\text{Pro} \rightarrow \text{Ser}$ substitution.

recovered, it was found to be greatly enriched in the variant β chain (Figure 2 bottom).

Tryptic peptide mapping was used to locate the mutation and digests of total globin showed a decrease in intensity of the $[M+1H]$ ion of peptide $\beta\text{T-1}$ (952.5 m/z) which had a new companion ion at 942.5 m/z indicating a $\beta 5(\text{A2})\text{Pro} \rightarrow \text{Ser}$ substitution (Figure 3). This mutation has been reported on one previous occasion as Hb Tyne.

Discussion

Similar to the first two reported cases, these cases were picked up incidentally as an interfering component on HbA1c testing. Ion-exchange HPLC separates hemoglobin species based on charge differences between HbA1c and other hemoglobins, so the utility of the method can be adversely affected by the presence of hemoglobin variants.^{2,3} When this occurs, another method, *e.g.* boronate affinity, should be used for confirmation of HbA1c levels. With boronate affinity methods, *m*-aminophenylboronic acid reacts specifically with the *cis*-diol groups of glucose bound to Hb. This measures total glycated Hb (GHb),

including HbA1c and Hb glycated at other sites and so it tends to demonstrate the least interference from the presence of hemoglobin variants and derivatives.²

However, an advantage of ion exchange HPLC over boronate or immunoassay based methods for HbA1c measurement, is that aberrant chromatograms can indicate presence of clinically silent variants.³ Though, knowledge and awareness of how the variants affect the HbA1c measurements is essential.

Similar amounts of the variant were detected in the heterozygotes, approximately 41% and 37% for present and original cases respectively. Similarly, this case was weakly positive for isopropanol stability testing as was one of the first cases. Both had normal haematological parameters with a normal Hb electrophoresis. As yet, there is no separation electrophoretically by any known technique, including IEF.⁴ The number of variant hemoglobins will be underestimated if conventional electrophoretic methods are used alone as a screening test for hemoglobinopathies.⁵ Ideally, both HPLC and capillary electrophoresis should be used in combination since the techniques are complimentary and can provide a correct assessment for the detection and identification of Hb variants.^{6,7} Even using that approach, vigilance is required when read-

ing HPLC chromatograms. As shown in this case, an uneven baseline on HPLC was the only indication of a variant Hb present.

Conclusions

In electrophoretically silent variants, this report validates the usefulness of cation exchange HPLC for HbA1c determination and that conventional Hb electrophoresis must not be used alone as a first line screening method for variant hemoglobins. This report highlights the importance of undertaking further investigations when an uneven baseline is detected on HPLC machines for hemoglobinopathy testing, even when the full blood count parameters and findings on conventional Hb electrophoresis are apparently normal.

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