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UK NEQAS International Quality Expertise

Learning from EQA: Hb Knossos

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Introduction

UK NEQAS Haematology supplies a comprehensive range of external quality assessment (EQA) programmes designed to support the quality assurance needs of participating laboratories.

The DNA Diagnostics for Haemoglobinopathies (DN) EQA programme is for specialist laboratories that offer molecular haemoglobinopathy testing as part of their diagnostic repertoire. It is designed to performance assess how laboratories identify mutations of the alpha and beta globin genes and the interpretation of the results obtained in context of the patient's clinical background and other haematology.

Assay material is supplied as DNA in Tris-EDTA (TE) buffer and is suitable for all molecular haemoglobinopathy techniques. Each specimen is supplied with clinical case details, gender and ethnic background and haematology results.

Background

HB Knossos is a Hb variant caused by a mutation in Codon 27 that changes the amino acid Alanine to Serine. This mutation has been identified in multiple individuals with origins in the Mediterranean basin, including North African countries. In addition to creating a Hb variant protein, this mutation activates a cryptic splice site, resulting in many of the transcripts to be degraded; therefore, the variant has a relatively lower expression than a normal beta globin chain and acts as a beta plus thalassaemia mutation (the variant is still functional to some degree). Hb Knossos migrates with Hb A using most methods of haemoglobin separation, so the variant peak is not obvious in heterozygotes.

This mutation is typically identified in cis with a delta zero thalassaemia mutation (HBD c.179delA), causing the detectable Hb A2 to be reduced by around 50%. This means that in the heterozygous state it is associated with a Hb A2 of 1.8-2.4% and in the homozygous state there is no Hb A2 present at all.

A DNA specimen from a child with this mutation was used in a UKNEQAS exercise in November 2022 (distribution 2203DN). This particular child was heterozygous for Hb Knossos and the delta zero thalassaemia mutation (HBD c.179delA). They were also compound heterozygous for alpha+ thalassemia due to the 3.7kb alpha+ deletion and a non-deletional IVS1(-5nt) mutation.

The clinical details stated that the child is of Moroccan origin with microcytic anaemia, normal HPLC, and that his father has beta thalassaemia intermedia.

Methods

48 laboratories participated in this survey. Participants were asked to test the specimen as per their usual diagnostic workflow for an ordinary patient specimen for both alpha and beta globin genotyping. Gamma and delta genotyping is not currently assessed; however, 2/48 labs did notify us that delta gene testing was performed which revealed the presence of the c.179delA mutation.

Methods used 2203DN2 (some laboratories use more than one method)

Alpha genotype	Beta genotype
Sanger Sequencing (21)	Sanger Sequencing (35)
MLPA (19)	MLPA (12)
Multiplex Gap PCR (18)	Vienna labs strip assay (3)
Vienna labs strip assay (8)	NGS (5)
Gap PCR (8)	Reverse Dot Blot (2)
NGS (5)	PCR + Reverse Hybridisation (1)
Reverse Dot Blot (1)	Multiplex GAP PCR (2)
ARMS PCR (1)	PCR-other (1)
PCR + Reverse Hybridisation (1)	Real-time PCR assay (discrimination of alleles S and C) (1)
Multiplex Triple PCR (1)	

Table 1. Methods used by UKNEQAS participants for alpha and beta globin genotyping

Results

Alpha globin genotyping:

35/47 participants (74%) returned the expected result: $-\alpha^{3.7}/\alpha^{IVS1(-5nt)}\alpha$

12 participants reported the 3.7kb alpha globin gene deletion but did not report the IVS1(-5nt) mutation. One failed to detect it by Sanger sequencing and received an adverse score. The remaining 12 did not detect it because they used assays that detect deletional mutations only e.g. MLPA or GAP PCR.

Beta globin genotyping:

44/48 participants (92%) returned the expected result: $\beta^{A}/\beta^{Codon27(GCC>TCC)}$

4 participants did not detect Hb Knossos due to the limitations of the assay used. None of these labs performed Sanger or Next Generation sequencing; 2 of them used Reverse dot blot, 1 used the Vienna labs strip assay and 1 used PCR + reverse hybridisation.

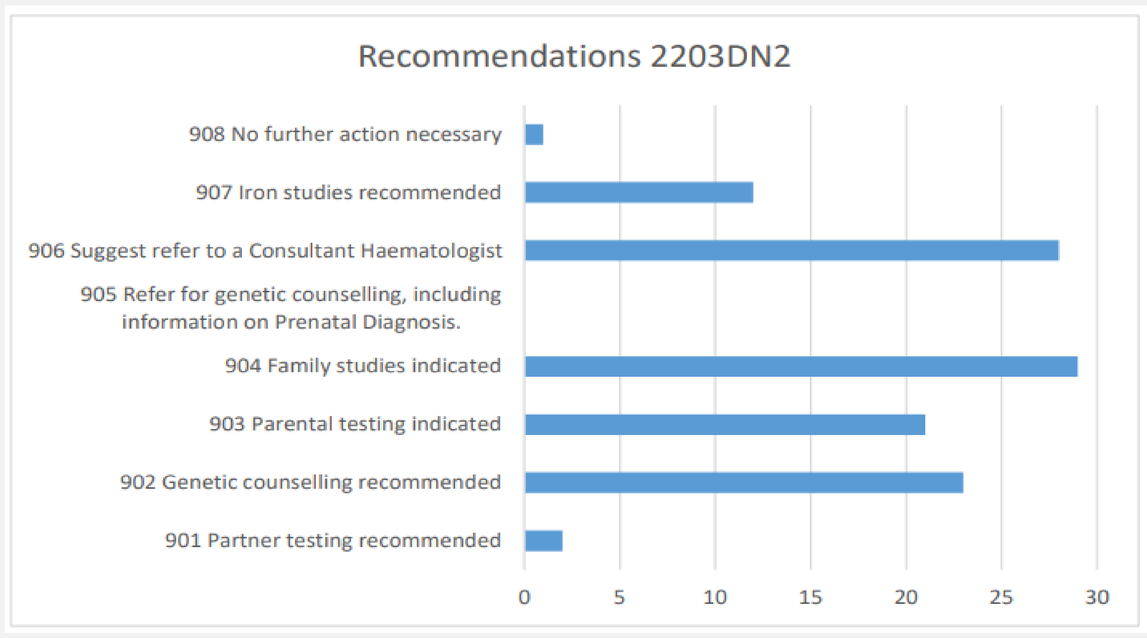

Table 2. Recommendations made by UKNEQAS participants for a child with Hb Knossos

Conclusions

This case highlights the importance of interpreting Hb genotyping with the clinical details in mind. Initial review of the details would lead to suspicion of alpha thalassaemia only. However, a key detail was that the child's father has a diagnosis of beta thalassaemia intermedia, implying that the child is likely to have inherited a beta thalassaemia mutation from his father. It also showed that delta globin gene sequencing was the key to explaining the normal range Hb A2 seen in this child.

Carriers of Hb Knossos are almost undetectable on HPLC/CE because the abnormal variant is hidden within the Hb A peak and the Hb A2 peak is normal, although they do have thalassaemic indices. This mutation typically results in beta thalassaemia intermedia when homozygous or inherited in combination with other beta thalassaemia mutations, so it is important to be able to detect it in cases of anaemia or to establish reproductive risks. When discussing reproductive options or family testing it is worthwhile mentioning that this beta thalassaemia can be missed or misdiagnosed as alpha thalassaemia using conventional screening tests, so it might be worth considering using genetic testing to obtain an unambiguous carrier diagnosis in these families.

This is the first time that this atypical beta thal-assaemia mutation has been included within this molecular EQA scheme and it is encouraging that the majority of labs detected and interpreted it correctly.

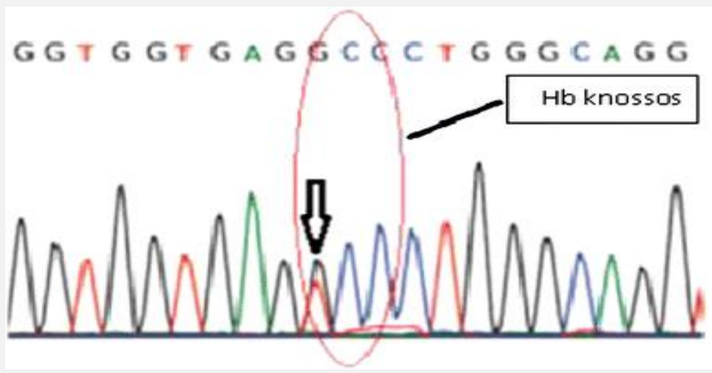

Figure 1. Codon 27 GCC>TCC: Hb Knossos as detected by DNA Sanger Sequencing. Nasouhipur, H., *et al. Indian J Hem Blood Transfus* **30**; 243–245 (2014).