Dominant β-thalassaemia with unusually high Hb A_2 and Hb F caused by $\beta^{CD121(-G)}$ (HBB:c.364delG) in exon 3 of β-globin gene

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ABSTRACT

We describe a dominant β-thalassaemia caused by a deletion of G at nucleotide position 364 in exon 3 of the β-globin gene. The heterozygosity of this mutation was found in a 36-year-old Thai patient who had moderate hypochromic microcytic anaemia with haemolytic blood picture. Haemoglobin (Hb) analysis revealed relatively higher Hbs A_2 (6.8%) and F (4.7%) as compared with those of β^0-thalassaemia (n=278) and β^+-thalassaemia (n=55) carriers in our series. Secondary structure prediction of the elongated β-globin chain showed that the α-helix at the C-terminal is disrupted dramatically by the random coil and β-sheet, which should result in a highly unstable β-globin variant, undetectable in peripheral blood and a dominant clinical phenotypic feature.

INTRODUCTION

β-thalassaemia is an inherited disorder caused by absent or reduced β-globin chain synthesis. The β-thalassaemia mutations described so far are mostly inherited in an autosomal recessive manner. These mutations can lead to clinical symptoms in patients only when they are found in a homozygote or compound heterozygote state. However, some mutations could be considered dominant forms of thalassaemia, in which clinical symptoms present in a heterozygote state.[1] At present, around 402 mutations have been reported worldwide. While most of them are recessive β-thalassaemia, 61 mutations are recognised as having a dominantly inherited manner.[2] In Thailand, more than 20 mutations have been reported. Only one of them, the haemoglobin (Hb) Khon Kaen (HBB:c.370_378delACCCCACCA), has been documented as dominant β-thalassaemia.[3] Unfortunately, no pure heterozygotic form was described to ascertain the clinical phenotypic feature in heterozygote state. We report another dominantly inherited β-thalassaemia caused by the $\beta^{CD121(-G)}$ mutation (HBB:c.364delG), found in a Thai adult with unusually high Hbs A_2 and F.

MATERIALS AND METHODS

Subject and haematological analysis

The patient was a Thai adult who was found to have constant hypochromic microcytic anaemia at a community hospital in Ubon Ratchathani Province, Northeast Thailand. He was followed up at 24, 27 and 36 years of age. Blood specimens of the patient and his wife, who was an Hb E carrier, were transferred to the thalassaemia centre of Khon Kaen University, Thailand, for further investigation. Haematological parameters were recorded and Hb analysis was done using a capillary electrophoresis system (Capillarys 2 Flex Piercing; Sebia, Lisses, France).

DNA analysis

Screening for α-thalassaemia and β-thalassaemia mutations found in Thailand is routinely performed in our laboratory using PCR-based methods.[3] The α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$) was examined as described.[4] The entire β-globin gene of the patient was sequenced using ABI PRISM 3730xl analyser (Applied Biosystems, Foster City, California, USA) (figure 1). Examination of the −158 $^{G}\gamma$ *Xmn*I polymorphism (rs7482144) was done using PCR-restriction fragment length polymorphism (RFLP) assay. Screening for Krüppel-like factor 1 (KLF1) polymorphisms previously found in Thailand, including G176AfsX179, T334R, −154(C-T), R328H, G335R, Q217X and S270W, was done using PCR-related techniques.[5] The PCR-RFLP assay was also developed for identification of the $\beta^{CD121(-G)}$ mutation found in this study (figure 2). Amplification of β-globin gene fragment spanning exon 3 (572 base pair (bp)) was performed by PCR using primers G88 (5′-GCCTCTTTGCACCATTCTAA-3′) and F13 (5′-AATGCACTGACCTCCCACAT-3′). The PCR product was digested with *Eco*RI (New England Biolabs, Beverly, Massachusetts, USA). Normal control fragment is digested into 276 bp and 296 bp fragments, whereas the indigestible 572 bp fragment indicates the presence of the $\beta^{CD121(-G)}$ mutation.

Prediction of secondary structure

The secondary structure of an abnormal β-globin chain was predicted using the online hierarchical neural network (HNN) program (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_hnn.html).[6]

RESULTS AND DISCUSSION

Haematological parameters of the patient at 24, 27 and 36 years old are summarised in table 1. He constantly suffered from hypochromic microcytic anaemia with Hb level around 10 g/dL and haematocrit (Hct) of 30%–31%. Glucose-6-phosphate dehydrogenase (G-6-PD) was normal and anaemia did not improve after treatment with standard iron supplementation protocol. Due to limited service availability at the community hospital, thalassaemia

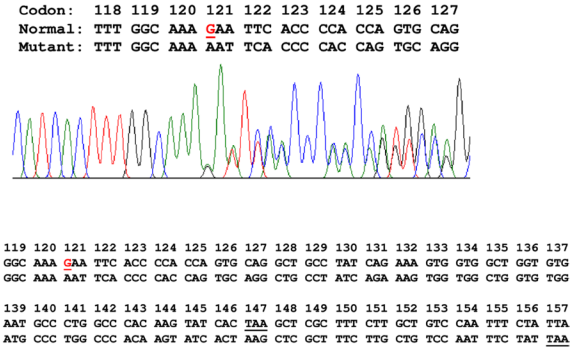

Figure 1 DNA sequencing profile of the patient demonstrating a (–G) deletion at codon 121, leading to a frameshifting translation with a new stop codon at position 157 and an elongated β-globin chain with 156 amino acid residues.

investigation was not carried out until he was 36 years old, when his blood specimen was referred to us. Examination at this visit revealed that he had fever, jaundice and anaemia. Blood examination showed reduced Hb (77 g/L), Hct (25.0%), mean cell volume (69.5 fL) and mean cell haemoglobin (23.3 pg), with an increased red cell distribution width of 31.5%. Blood film showed hypochromia, aniso-poikilocytosis with schistocyte, spherocyte and target cells. Hb analysis showed Hbs A (88.5%), A_2 (6.8%) and F (4.7%) without any abnormal Hb. These elevated Hbs A_2 and F as compared with carriers of β^0-thalassaemia (n=278) and β^+-thalassaemia (n=55) in our series[7] (table 1) prompted us to investigate α-globin, β-globin and γ-globin genes of the patient. Screening for common α-thalassaemia and β-thalassaemia mutations and α-globin gene triplication ($\alpha\alpha\alpha^{anti3.7}$) yielded negative results. The $-158\ ^{G}\gamma$ *Xmn*I polymorphism was found to be heterozygote for C-T transition

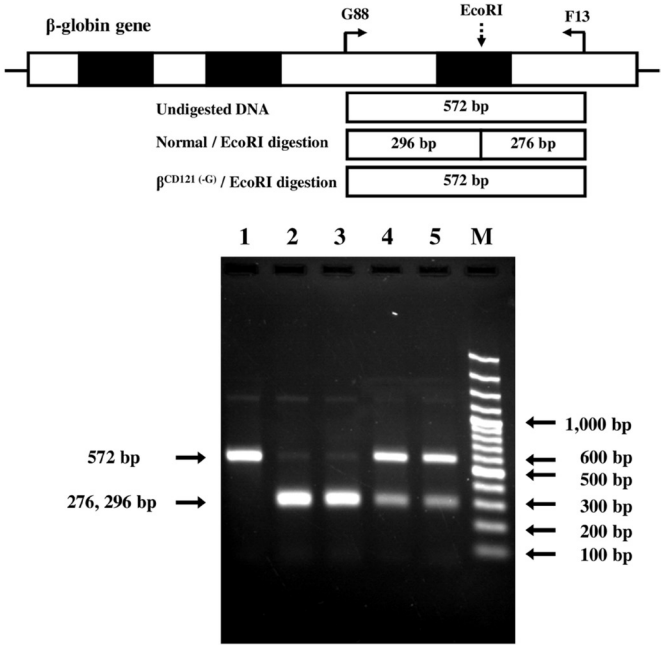

Figure 2 Identification of the $\beta^{CD121(-G)}$ mutation by PCR-RFLP assay using *Eco*RI digestion. M represents the GeneRuler 100 bp Plus DNA Ladder. Lane 1: undigested amplified DNA; lanes 2 and 3: *Eco*RI-digested amplified DNA of normal controls; lanes 4 and 5: *Eco*RI-digested amplified DNA of the patient with $\beta^{CD121(-G)}$ mutation. bp, base pair; RFLP, restriction fragment length polymorphism.

Table 1 Haematological parameters and Hb analysis of the patient at various ages as compared with β^0-thalassaemia and β^+-thalassaemia carriers in our series[7]

	The patient			β^0-thalassaemia	β^+-thalassaemia
Age (years)	24	27	36*	trait	trait
n		1		278	55
α-genotype		αα/αα		αα/αα	αα/αα
β-genotype		β^A/β^{th}		β^A/β^0	β^A/β^+
RBC (10^{12}/L)	4.3	4.1	3.6	5.5±0.9	5.5±0.8
Hb (g/L)	100	100	77	111.5±17.0	126.0±17.7
Hct (%)	31.0	30.0	25.0	34.9±5.2	38.6±5.2
MCV (fL)	73.0	73.0	69.5	63.1±3.8	70.7±4.1
MCH (pg)	23.0	24.0	23.3	20.2±1.6	23.3±2.1
MCHC (g/L)	32.0	33.0	33.5	32.1±2.7	32.6±1.8
RDW (%)	na	na	31.5	17.0±1.8	16.7±1.9
Hb analysis	na	na	A_2A	A_2A	A_2A
% Hb A_2	na	na	6.8	5.7±0.6	5.9±0.6
% Hb F	na	na	4.7	1.3±1.1	1.5±0.9

*Blood film: anisocytosis 1+, microcytosis 1+, poikilocytosis 2+, schistocyte 1+, few spherocytes and target cells, and few hypochromias.
Hb, haemoglobin; Hct, haematocrit; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; MCV, mean cell volume; na, not available; RBC, red blood cells; RDW, red cell distribution width.

(i.e. *Xmn*I ±), unlikely to be responsible for the high Hb F phenotype. Screening for the seven KLF1 SNPs (single nucleotide polymorphisms) known to be associated with high Hb F phenotype in Thai subjects also yielded negative results. Further DNA sequencing of the whole β-globin gene revealed a (−G) deletion at codon 121 (GAA to -AA), hitherto undescribed in other populations (figure 1). This frameshift mutation leads to a new termination codon at codon 157 within the 3' untranslated region instead of 147. The mutation eliminates an *Eco*RI restriction site at codon 121 of β-globin gene; it could be confirmed by PCR-RFLP assay as shown in figure 2. He was finally diagnosed with severely affected β-thalassaemia heterozygote.

Many mutations in exon 3 of β-globin gene leading to elongated variants with abnormal carboxy terminal and instability have been described. Synthesised β-globin chain variants cannot form αβ dimers because most of the $\alpha_1\beta_1$ contacts have been removed. These frameshift mutations, leading to dominant β-thalassaemia, are summarised in figure 3. It is noteworthy that the majority of them are located between codons 109 and 141,

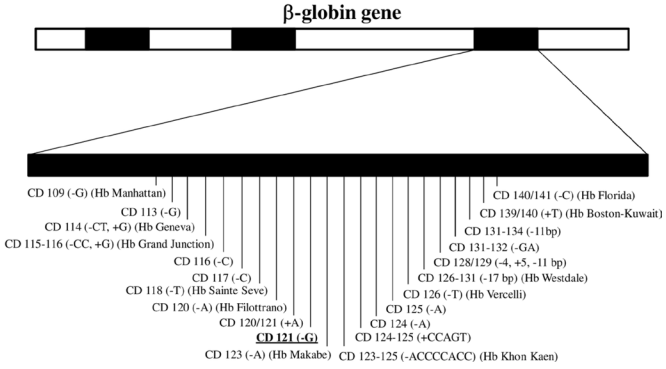

Figure 3 The frameshift mutations and their positions in exon 3 of β-globin gene associated with the dominant form of β-thalassaemia reported worldwide based on IthaGenes database,[2] including the CD121 (−G) found in this study. bp, base pair; Hb, haemoglobin.

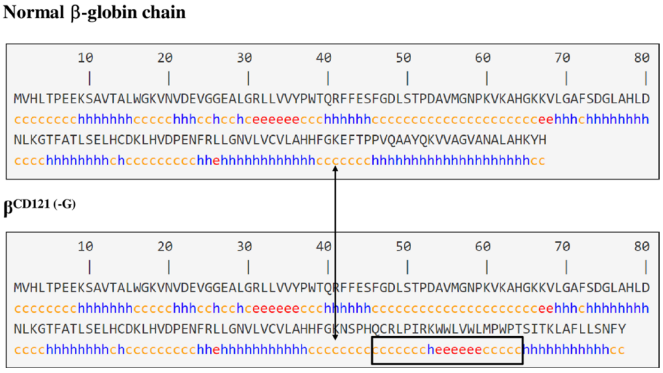

Figure 4 Secondary structure prediction for the abnormal β-globin chain associated with the $\beta^{CD121(-G)}$ using the online hierarchical neural network program. The 'h', 'e' and 'c' indicate α-helix, β-sheet and random coil, respectively.

and no corresponding Hb variants could be detected in peripheral blood of the patients. In fact, the dominant form of β-thalassaemia intermedia could be related to messenger RNA (mRNA) stability mechanisms.[8] If the premature termination is located so early, for example, within exon 2, the corresponding transcript is targeted for nonsense-mediated mRNA decay (NMD), no protein is synthesised and heterozygotes are asymptomatic. However, when termination codon is located further downstream of exon 3 that does not induce NMD, the amount of synthesised non-functional β-globin chain may overwhelm the cellular proteolytic system and cause toxic precipitation of insoluble globin chains or inclusion bodies. Heterozygotes with these mutations are affected with dominantly inherited β-thalassaemia intermedia.[9] Alternatively, this could be due to the change of the secondary structure of the Hb molecule. For the $\beta^{CD121(-G)}$ mutation found in this study, prediction by the HNN method showed that the α-helix at the carboxy terminal was replaced dramatically by the β-sheet and the random coil, as shown in figure 4. This elongated β-globin chain might be less susceptible to proteolytic degradation and cause dominant β-thalassaemia phenotype. This is in contrast to those of the frameshift mutations located close to the C-terminal, in which the viable Hb tetramers could still be formed.[2]

We have noted that the $\beta^{CD121(-G)}$ mutation described here has just recently been found in a 2-year-old Thai girl from Nakhon Si Thammarat in the south of Thailand in association with Hb E and α-thalassaemia 2 (3.7 kb deletion). The clinical presentation showed pallor, jaundice and spleen enlargement, requiring regular blood transfusion. A low number of inclusion bodies (<1:1000) were seen. Unfortunately, the phenotype of a plain heterozygote was not documented.[10] In that report, a small peak of abnormal Hb was observed (2.8%) at the C-window on Hb-high-performance liquid chromatography (HPLC) analysis. This is in contrast to the results obtained on the capillary electrophoresis of our patient in which no abnormal Hb peak was detected. Unfortunately, we were unable to obtain fresh blood specimen from the patient for Hb-HPLC analysis. We believe that this form of β-thalassaemia intermedia caused by $\beta^{CD121(-G)}$ may not be uncommon in the region. The mutation should be included in the screening profiles of β-thalassaemia mutations, which could easily be identified using PCR-RFLP assay.

Take home messages

- A dominant form of β-thalassaemia was found to be associated with the $\beta^{CD121(-G)}$ mutation in a Thai patient.
- It is associated with unusually high expression of haemoglobin (Hb) A_2 and Hb F.
- The mutation could be screened using PCR-restriction fragment length polymorphism assay.

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Contributors KS performed the experiment, analysed the data and developed the initial manuscript. RK helped in obtaining the patient's specimen and collecting initial haematological and clinical information, and read the final manuscript. GF helped to design the study, supervised the research and interpreted the data, and wrote the manuscript. SF designed the experiment, facilitated the study, acquired research grant, analysed the data, and critically revised and approved the final manuscript.

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Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval Ethical approval of the study protocol was obtained from the IRB of Khon Kaen University, Thailand (HE612242).

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