DETECTION OF NON-DELETIONAL TYPE OF HEREDITARY PERSISTENCE OF FETAL HEMOGLOBIN (HPFH) CONDITION ASSOCIATED WITH 619 bp β^0 -THALASSEMIA DELETION

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ABSTRACT

DNA samples from a family (parents and a son) with hereditary persistence of fetal hemoglobin (HPFH) condition were subjected to amplification of a 1.214 kbp DNA fragment from β -globin gene using polymerase chain reaction (PCR). The aim of this study was to identify the type of HPFH i.e. deletional or non deletional. Non deletional type of HPFH was identified in two samples and moreover, these samples were found to be associated with 619bp β^0 -thalassemia deletion. This is the first report on the association of non deletional HPFH with 619bp β^0 -thalassemia deletion.

KEY WORDS: Deletional and Non deletional HPFH, 619 bp deletion, Thalassemia, fetal hemoglobin.

INTRODUCTION

Low amounts of fetal hemoglobin (upto 0.8%) are present in all normal human adults (1). Hereditary persistence of fetal hemoglobin (HPFH) is a heterogeneous group of hereditary conditions characterised by increased levels of fetal hemoglobin (HbF) in adult life (2). HPFH has been classified as deletional and non-deletional based on the underlying mutation on the β -globin gene cluster (3). HPFH involves deletions of large DNA fragments on the β -globin gene cluster (4-8). Some of the non-deletional HPFH arise due to single base mutations in the promoter regions of the $G\gamma$ and $A\gamma$ globin genes (9). These HPFH determinants are associated with levels of HbF in the range of 5.25% in the heterozygotes. Another group of non-deletional HPFH which is known as Swiss type, is associated with a small increase in the levels of HbF in the range of 1-4%, which on interaction with homozygous β -thalassemia or sickle cell diseases, can nevertheless increase HbF output to levels which are clinically beneficial (10,11). Non-deletional HPFH

non linked to β -globin gene cluster has also been described (12,13).

Non-deletional and deletional types of HPFH have been identified in Indian subjects (7,12-15). This study was carried out to identify the type of HPFH in a family with high amounts of fetal hemoglobin.

MATERIALS AND METHODS

Blood samples from diagnosed cases of HPFH condition (HbF patient, son - 69.7%, father 27.9%, mother - 30%) (16,17) were collected in EDTA. Buffy coats were separated out and DNA was isolated following the standard protocol (18).

A 1.214Kbp DNA fragment from β -globin gene was amplified by PCR using 0.5 μ g template DNA 1 μ M of each oligonucleotide primer (Forward pl - 5'TGCCTCTTTGCACCATCTCA, IVS2, nt 603 to nt 622. Reverse p2-GGGCCTATGATAGGGTAAT, nt 2311 to nt 2293), 200 μ M of each dATP, dGTP, dCTP and dTTP) in a buffer containing 10 mM Tris-HCl (pH 8.3), 2mM $MgCl_2$, 50mM KCl and 0.001% gelatin 1 unit of Taq DNA polymerase (Bangalore Genei Pvt Ltd., India) in a total volume of

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50 μ L. Thermal cycling was carried out by denaturation of template DNA at 94°C for 1 min followed by primer annealing at 56°C for 1 min primer extension at 72°C for 1 min and denaturation at 94°C for 1 min for 35 cycles. In the last cycle, primer extension was prolonged for 5 min. This protocol was standardised in our laboratory following the guidelines on amplification of DNA (19).

20 μ L of the PCR product each was analysed by electrophoresis (20) using 0.8% agarose (Sigma Chemical Co., USA) gel containing ethidium bromide (Sigma Chemical Co., USA) and the gel was examined on a UV-transilluminator.

RESULTS

Fig. 1a, schematic representation of the physical map on the normal human β -globin gene cluster showing Sfi I restriction sites (21) and Indian HPFH deletion (5,7,14,15). Schematic representation of the amplification of a 1.214Kbp and a 595bp DNA fragment from β -globin gene is given in Fig. 1b. Fig. 1c shows the 1.214Kbp amplified DNA product from the β -globin gene in test as well as the control samples (lanes 1-5). It also shows a 595bp DNA product in the patient's and the father's samples (lanes, 2 and 3) indicating the presence of non-deletional HPFH associated with 619bp β -thalassemia deletion.

FIGURE - 1

Fig. 1a : Schematic representation of the physical map of the normal β -globin gene cluster showing Sfi I restriction sites and Indian HPFH deletion.

Fig. 1b : Schematic representation of the human β -globin gene showing 619bp deletion. Filled boxes indicate exons 1,2 and 3 and open boxes the introns 1 and 2 respectively from 5' to 3' end. Arrows show the sites of the primers used for amplification of a 1.214Kb DNA fragment by polymerase chain reaction.

Fig. 1c : Detection of non-deletional type of HPFH associated with 619bp β -thalassemia deletion: DNA samples from HPFH cases (a family) were subjected to PCR for the amplification of a 1.21Kb DNA fragment from globin gene to identify the type of HPFH (deletional or non-deletional). The samples were analyzed by electrophoresis using agarose gel. Lanes 1-3 show the 1.214Kb amplified DNA fragment indicating the non-deletional type of HPFH; lanes 2 & 3 also show the 595bp amplified DNA fragment suggesting the association of 619bp β -thalassemia deletion with this condition. Lanes 4 & 5, 1.214Kb amplified DNA samples from normal controls. Lane 6 shows the 1.631Kb, 517bp and 506bp DNA fragments of pBR-322/Hinf 1 DNA molecular weight marker.

DISCUSSION

Heterocellular hereditary persistence of fetal hemoglobin (HPFH) is a term used to describe the genetically determined persistence of fetal hemoglobin (HbF) production in adult life, in the absence of any related hematological disorder. Some of these forms are caused by mutations in the β -globin gene cluster on chromosome 11, whereas others segregate independently. Linkage analyses in samples from a large Indian family showed that the genetic determinant for HPFH segregates independently from the $\gamma\delta\beta$ globin gene complex (12,13). Deletional type of Indian HPFH has its 5'breakpoint within the Alu repeat 3 of the A γ globin gene (14,15) and its 3' breakpoint 3' downstream of the β -globin gene (7). Therefore, to identify the type of HPFH, a 1.214 Kbp DNA from the test and the normal samples with the view that the difference in the intensity of the amplification signal will reveal the underlying deletion if any. Unfortunately, spectrophotometric quantitation of the DNA (20) was found to be inaccurate as exhibited by the variation in the intensities of the amplification signals generated by PCR (Fig. 1c); but the amplification of a 1.214Kbp and a 595bp DNA fragment from β -globin gene in both the father's and the patient's samples indicated the presence of non-deletional HPFH associated with 619bp β^0 -thalassemia deletion (Fig. 1c lane 2 and 3). The non-deletional HPFH trait in these samples could be associated with the same chromosome carrying the 619bp β^0 -thalassemia dele-

tion or it could have been segregated independently. Father, at the age of 43 years, having HPFH (HbF 27.9%) associated with 619bp β^0 -thalassemia deletion didn't have any history of anemia, whereas the patient (son) with the same condition and with high amount of HbF (69.7%) had mild anemia at the age of 12 years is difficult to explain.

This is the first report on the association of non-deletional HPFH (not characterised) associated with 619bp β^0 -thalassemia deletion. A non-deletional HPFH associated with β^0 -thalassemia mutation in an Indian patient has earlier been reported, but the underlying β^0 -thalassemia mutation was IVS-1-1 (G->T) (12). Non-deletional HPFH associated with nonsense codon 39 β^0 -thalassemia has also been reported (22).

Non-deletional HPFH in all the test samples could be due to a point mutation in the promoter region of the G γ - or A γ -globin gene or it could be due to other genetic determinants segregated independently from the $\gamma\delta\beta$ -globin gene complex as demonstrated earlier in Indian subjects (12,13).

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