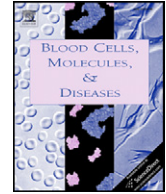
Blood Cells, Molecules, and Diseases 45 (2010) 276–279

Contents lists available at ScienceDirect

Blood Cells, Molecules, and Diseases

journal homepage: www.elsevier.com/locate/ybcmd



Molecular identification of the novel Gγ-β hybrid hemoglobin: Hb Gγ-β Ulsan (Gγ through 13; β from 19)

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ARTICLE INFO

Article history:
Submitted 24 September 2010
Revised 27 September 2010
Available online 29 October 2010

(Communicated by G. Stamatoyannopoulos, M.D., Dr. Sci., 27 September 2010)

Keywords:
Hemoglobin
Hybrid hemoglobin
Mutation
HBB
HBG2

ABSTRACT

Gene fusion is a very rare mechanism that produces hemoglobin variants. Less than ten types of β-like hybrid globins have been reported to date. Herein we identified the first hybrid hemoglobin between Gγ- and β-globins in a five-year-old Korean male who had thalassemia minor feature and triplication of the *HBA2* gene (αα/αααα). The novel globin originated from a 27,707-base pair deletion spanning from the *HBG2* to *HBB* gene (NG_000007.3: g.42947_70653del). Its protein sequence included 13 N-terminal amino acids from Gγ-globin, five common amino acids from Gγ- and β-globins, and 128 amino acids from β-globin (Gγ through 13; β from 19). Molecular genetic analyses characterized the hybrid DNA and RNA. Mass spectrometry and *de novo* protein sequencing successfully identified the fusion peptide in the hybrid hemoglobin. We named this novel hybrid Hb Gγ-β Ulsan. The novel hemoglobin constituted 37.0% of the total hemoglobin and showed reduced oxygen affinity.
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Introduction

Hemoglobinopathies and thalassemia are caused by various mutations in the globin gene complex. Fusion between different globin genes is a very rare mechanism leading to hybrid hemoglobin (Hb) variants. Only a few types of hybrid hemoglobins composed of β-like globins have been reported and characterized [1]: δ-β hybrid (Hb Lepore-Hollandia, Hb Lepore-Baltimore, Hb Lepore-Boston-Washington, and Hb Lepore-Leiden), δ-β/β-δ hybrid (Hb Parchman), β-δ hybrid (Hb Miyada, Hb P-Congo, and Hb P-India), Aγ-β hybrid (Hb Kenya), and others [2–5]. These structurally abnormal hemoglobins are mostly produced by unequal crossing over from the misaligned globin genes. Clinically, the carriers of hybrid hemoglobins can be either healthy or show thalassemic features, especially when the hybrids interact with other hemoglobin variants [6,7]. Herein we report the first hybrid hemoglobin between Gγ- and β-globins, Hb Gγ-β Ulsan, in a 5-year-old Korean male.

Materials and methods

This study was reviewed and approved by the institutional review board. Routine hematologic tests were performed by standard laboratory procedures. Genomic DNA was extracted from blood using the PureGene DNA isolation kit (Gentra Systems, Minneapolis, MN, USA). The *HBB*, *HBA1*, and *HBA2* gene sequences were analyzed by gene-specific, long-range polymerase chain reaction (PCR) and sequencing using ABI PRISM 3730xl genetic analyzer (Applied Biosystems, Foster City, CA, USA). Deletions or duplications in the β- and α-globin gene clusters were screened by multiplex ligation-dependent probe amplification (MLPA) P102-B1/P140-B2 kit (MRC-Holland, Amsterdam, The

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doi:10.1016/j.bcmd.2010.10.001

Table 1
The PCR primers for molecular genetic analyses of the Hb Gγ-β Ulsan.

Target	Name	PCR primer sequence	Usage[a]
DNA	HBB-F	AGTAGCAATTTGTACTGATGGTATGG	*HBB* gene-specific PCR
	HBB-R	TTTCCCAAGGTTTGAACTAGCTCTT	
	HBA1/2-F	GTGCGGGCTGACTTTCTC	*HBA1* gene-specific PCR
	HBA1-SR	TGCTGGAGTGGGACTTCTCT	
	HBA1/2-F	GTGCGGGCTGACTTTCTC	*HBA2* gene-specific PCR
	HBA2-SR	GGAAGGGGTGGGAATGAGAG	
	G2-F	GGAACCCAACCAGACTCTCA	Gap-PCR for deletion breakpoint in the β-globin gene cluster
	B-R	CAGCCTAAGGGTGGGAAAAT	
RNA	mG2-F1	ACTATCACAAGCCTGTGGGG	RT-PCR and mRNA sequence analysis for the novel hybrid hemoglobin
	mB-R1	AGTGATACTTGTGGGCCAGG	
	mG2-F2	TCACAGAGGAGGACAAGGCT	
	mB-R2	AGTGATACTTGTGGGCCAGG	
	mG2-F3	TTCACAGAGGAGGACAAGGC	
	mB-R3	AGTGATACTTGTGGGCCAGG	

[a] The reference sequences of the β-globin region and each gene were as follows: β-globin region, NG_000007.3; *HBA1*, NM_000558.3; *HBA2*, NM_000517.3; *HBB*, NM_000518.4; *HBG2*, NM_000184.2 in the National Center for Biotechnology Information, Entrez Gene. http://www.ncbi.nlm.nih.gov/gene?term.

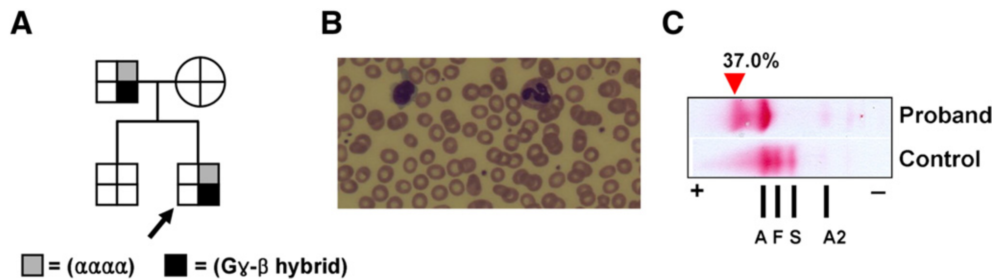

Fig. 1. Laboratory features of the Hb Gγ-β Ulsan. (A) The genotype of the proband and his family members. The proband is marked by an arrow. In each symbol, the upper quadrants represent alleles of α-globin gene cluster, and the lower quadrants represent those of β-globin gene cluster: grey quadrant, allele of genotype (αααα); black, (Gγ-β hybrid). Both of the genetic aberrations, triplication of the *HBA2* gene and the novel Gγ-β hybrid, were segregated from the father to the proband. (B) The peripheral blood smear of the proband shows microcytic hypochromic red blood cells with anisocytosis (Wright-Giemsa stain, ×400). (C) Cellulose acetate electrophoresis at alkaline pH revealed 37.0% of a novel, fast-moving component in the hemolysate (arrowhead).

Netherlands). The deletion range and the exact breakpoint in the genomic DNA were identified by gap-PCR and sequencing. Total RNA was isolated from blood using a MagnaPure LC instrument and the RNA Isolation kit (Roche Diagnostics, Mannheim, Germany). The presence of novel hybrid globin mRNA was confirmed by reverse transcription-PCR (RT-PCR) and sequencing. Primers were listed in Table 1.

For peptide detection and *de novo* sequencing, the hemolysate was digested with the restriction endoproteinase Glu-C (Sigma, St. Louis, MO, USA), which selectively cleaves peptide bonds C-terminal to glutamic acid residues. Cleaved peptides were analyzed by nanoscale liquid chromatography-quadrupole-time of flight mass spectrometer (NanoLC-Q-TOF, Waters, Manchester, UK) using a data-independent analysis method. Data were analyzed with the ProteinLynx Global server 2.3 platform. Oxygen equilibrium curves of the novel Hb Gγ-β Ulsan and normal hemoglobin were obtained using the Hemox analyzer (TCS, Southampton, PA, USA).

Results

Case history and laboratory findings

The proband was a 5-year-old Korean male child. His anemia was detected at seven months of age, but oral iron supplementation made no improvement in his hematologic parameters. The 35-year-old father of this proband stated that he had experienced vague symptoms of suspected anemia during his childhood, but they had resolved in adulthood

Fig. 2. The DNA, RNA, and protein analyses of the Hb Gγ-β Ulsan. (A) The genomic deletion of 27,707 bases (NG_000007.3: g.42947_70653del, grey box) and the resultant genomic fusion are schematically represented in the β-globin gene cluster. From this Gγ-β hybrid DNA, novel hybrid mRNA and protein productions were predicted and their existence was confirmed in this study. (B) Genomic multiplication and deletion in the α- and β-globin gene clusters of the proband were detected by MLPA. The blue peaks in the electropherogram are the amplified products obtained from the proband, and the red peaks are those from the normal control. The x-axis shows the size of each base pair of the separated products in the electropherogram, and the y-axis shows the peak height of each product. In the α-globin gene cluster (left), increased peak ratios (asterisks) of ~2.0 are observed for seven *HBA2*-specific probes located from *HBA2* intron 2 to the region between *HBA2* and *HBA1*. Increased peak ratios of ~1.5 are observed for *HBA1*/*HBA2* common probes located from the region between *HBA1P* and *HBA2* to *HBA* exon 3. These findings indicate triplication of the *HBA2* gene (a total of six copies of α-globin) in the proband. In the β-globin gene cluster (right), decreased peak ratios of ~0.5 (arrowhead) are observed for 11 probes located from *HBG2* exon 3 to *HBB* exon 1. These findings indicate a heterozygous deletion approximately from the *HBG2* to *HBB* gene. (C) Gap-PCR for the β-globin gene cluster shows a shortened amplicon in the proband (lane P). No amplicon was detected in the normal control (lane NC). The RT-PCR using primers in the *HBG2* and *HBB* genes detected the mRNA in the proband (lane P) but not in two normal controls (lanes NC1 and NC2). (D) Subsequent sequencing of the gap-PCR and RT-PCR products showed the fused margin of the *HBG2* exon 1 and the *HBB* exon 1 in the hybrid DNA and mRNA. Translated amino acids are shown in the middle, with background colors indicating their genomic origin (blue, *HBG2*; yellow, *HBB*; white, common sequence). (E) The mass spectrometry (MS) chromatogram (upper) and MS/MS spectrum (lower) show the unique hybrid peptide of Hb Gγ-β Ulsan. The x-axes show the retention time (upper) and *m*/*z* of the product ion (lower). The y-axes show relative abundance. The unique peptide fragment from the novel hybrid globin, single-charged mass 1775.922 [peak (a)], is shown in the chromatogram which was produced by digestion of the hemolysate with a restriction endoproteinase Glu-C. The *de novo* amino acid sequencing (MS/MS) revealed that the peak (a) was a fused peptide composed of Gγ- and β-globins, the amino acid sequence of which was DKATITSLWGKVNVDE. (F) Comparison of the amino acid sequences between the novel hybrid globin (middle) and naturally occurring Gγ- and β-globins (upper and lower lanes) shows that the novel hybrid globin has 13 amino acids of Gγ-globin in its N-terminus. The amino acids that differ between Gγ- and β-globins are marked in red. The Gγ-β fusion fragment detected by MS is shown in the red box. (G) The proband's oxygen equilibrium curve (blue line indicated by an arrow) is shifted to the right compared with the mother's as a normal control (green line).

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