Blood Cells, Molecules and Diseases 51 (2013) 39–47

Contents lists available at SciVerse ScienceDirect

Blood Cells, Molecules and Diseases

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

Detection of germline rearrangements in patients with α- and β-thalassemia using high resolution array CGH

Ariane Blattner [1], Saskia Brunner-Agten [1], Katja Ludin, Martin Hergersberg, Roberto Herklotz, Andreas R. Huber, Benno Röthlisberger *

Center of Laboratory Medicine, Kantonsspital Aarau, Tellstrasse, 5001 Aarau, Switzerland

ARTICLE INFO

Article history:
Submitted 13 October 2012
Revised 29 January 2013
Available online 13 March 2013

(Communicated by G. Stamatoyannopoulos, M.D., Dr. Sci., 29 January 2013)

Keywords:
α-Thalassemia
β-Thalassemia
δβ-Thalassemia
Hereditary persistence of fetal hemoglobin (HPFH)
Array CGH
Multiplex ligation-dependent probe amplification (MLPA)
KLF1-gene

ABSTRACT

Approximately 80% of α-thalassemia mutations are deletions in the α-globin cluster on chromosome 16 and about 10% of β-thalassemia mutations are deletions in the β-globin gene cluster on chromosome 11. Larger deletions involving the β-globin gene cluster lead to (δβ)-, (γδβ)-, (εγδβ)-thalassemia, or hereditary persistence of fetal hemoglobin (HPFH). Array comparative genomic hybridization (CGH) was applied to screen for deletions in the α- and β-globin gene clusters not detected by routine gap-PCR. In total, in 13 patients with hypochromia and inclusion bodies (IBs) the α-globin gene cluster was analyzed and in 13 patients with increased fetal hemoglobin levels with or without hypochromia the β-globin gene cluster was examined. All samples were subsequently investigated by multiplex ligation-dependent probe amplification (MLPA). In 9 out of 13 patients deletions of the α-globin gene cluster were identified; 5 of these deletions remove the entire α-globin cluster and extend to the telomere. Additional sequencing of the remaining 4 patients revealed polyadenylation mutation in 1 of them. 7 deletions were identified in the β-globin gene cluster in 13 patients. Additional sequencing of the remaining 6 patients revealed mutations in one of the γ-globin gene promoters in 3 of them and a *KLF1*-mutation in 1 of them.
Array CGH is a reliable method to screen for deletions in thalassemia and hemoglobinopathy. The method offers the advantage of a high resolution with the possibility to characterize breakpoints on sequence level.

Introduction

It was in the α- and β-globin gene clusters on chromosome 16 and on chromosome 11, respectively, where the structure of segmental duplications has been described for the first time [1]. Segmental duplications (also called low copy repeats, LCR) often result in an increased frequency of copy number variant (CNV) mutations by non-allelic homologous recombination (NAHR) [2]. The two genes *HBA1* and *HBA2* encoding α-globin in the α-globin gene cluster are highly homologous and closely linked. Approximately 80% of α-thalassemia results from deletions of the α-globin genes or of the control region of the α-globin gene cluster (e.g. $--^{THAI}$, $--^{FIL}$, $--(\alpha)^{20.5\ kb}$, $--^{MED}$, $--^{SEA}$). The phenotypes of α-thalassemia are diverse and related to the genotype, ranging from asymptomatic hypochromia and microcytosis to lifelong transfusion-dependent anemia and HbH disease or hydrops fetalis in the absence of functional α-globin [1,3,4].

About 90% of β-thalassemia mutations are point mutations in the β-globin gene cluster. In addition to the β-globin gene (*HBB*) this gene cluster comprises the ε-globin (*HBE*), ^{A}γ-globin (*HBG1*), ^{G}γ-globin (*HBG2*), and the δ-globin gene (*HBD*). These genes are temporarily expressed during development according to their chromosomal arrangement. A L1 repetitive element lies downstream of *HBB*, and this LCR together with the highly homologous *HBG1* and *HBG2* genes are repetitive elements in the β-globin gene cluster contributing to CNV mutations. The frequency of NAHR at the *HBG1–HBG2* gene pair has recently been determined to be 1.3×10^{-5} in human sperm cells, which is very similar to the NAHR frequency of 4×10^{-5} in human sperm at the similarly organized α-globin gene pair [5]. Several deletions of different sizes encompass the *HBD* and the *HBB* gene and lead to δβ-thalassemia or HPFH (hereditary persistence of fetal hemoglobin). In heterozygous δβ-thalassemia patients, up-regulation of γ-globin gene expression results in HbF levels of 5–20% which are accompanied by hypochromic and microcytic red blood cell indices while HPFH is characterized by increased HbF levels of 15–30% in heterozygous carriers without hypochromia or microcytosis [4,6,7]. Up-regulation of γ-globin gene expression can also be caused by point mutations in one of the γ-globin gene promoters.

The detailed analysis of rearrangements in the globin gene loci is of interest for three reasons: (i) Identification of as many mutations as possible is the primary aim of any genetic analysis [8]. (ii) Characterization of

* Corresponding author. Fax: +41 62 838 53 99.
E-mail addresses: saskia.brunner@ksa.ch (S. Brunner-Agten), katja.ludin@ksa.ch (K. Ludin), roberto.herklotz@ksa.ch (R. Herklotz), andreas.huber@ksa.ch (A.R. Huber), benno.roethlisberger@ksa.ch (B. Röthlisberger).
[1] These authors contributed equally to this work.

1079-9796/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bcmd.2013.02.002

Table 1
Hematological parameters of patients with criteria for α-thalassemia (group A) and of patients with criteria for δβ-thalassemia or HPFH (group B).

Patient	Sex	Age [years]	Hb [g/L]	Hk [L/L]	Ec [g/T]	MCH [pg]	MCV [fl]	MCHC [g/L]	RDW [%]	ZnPP [μmol/mol Häm]	I.B.	Hb F [%]	Hb A2 [%]	Hb variants [%]	Aberrations (app.)	Break points		Ethnie
																Left	Right	
Group A																		
A1	F	36	109*	0.357*	5.07	21.5*	70.4*	305*	13.2	70*	++++	1.5*	2.6	None	284.2 kb deletion	Telomer	283,499–284,899	evt. TR
A2	M	5	116	0.357	5.96	19.5*	59.9*	325	15.8*	49	++	0.7	2.7	None	275.7 kb deletion	Telomer	275,556–275,750	CH (+F?)
A3	M	29	105*	0.321*	5.01	21*	64.1*	327	14.3	n.d	++++	0.4	2.4	None	187.0 kb deletion	Telomer	186,491–187,497	n.a
A4	F	43	102*	0.304*	4.7	21.7*	64.7*	336	14.2	181*	++++	1.3	2.4	None	178.3 kb deletion	Telomer	165,782–167,215	RC
A5	F	36	117*	0.364	5.01	23.4*	72.7*	321	12.6	40	++++	0.7	2.3	None	170.1 kb deletion	Telomer	169,881–170,247	n.a
A6a	M	51	143	0.45	6.38*	22.4*	70.5*	318	13.5	42	+++	<0.5	2.1	None	40.7 kb deletion	77,606–78,466	116,910–120,635	CH
A6b	F	18	127	0.399	5.8*	21.9*	68.8*	318	16.1*	66*	+	1	2.2	None	40.7 kb deletion	77,606–78,466	116,910–120,635	CH
A7	M	20	142	0.444	6.6*	21.5*	67.3*	320	12.6	12	++++	1.1	2.3	None	40.7 kb deletion	77,606–78,466	116,910–120,635	CH/I
A8a	M	28	122*	0.387*	6.13*	19.9*	63.1*	315	14	51*	++++	0.9	2.3	None	31.1 kb deletion	137,053–138,051	168,624–169,326	n.a.
A8b	F	59	133	0.413	6.14*	21.7*	67.3*	322	13.6	34	++++	0.5	2.3	None	31.1 kb deletion	137,053–138,051	168,624–169,326	n.a.
A9	F	23	107*	0.349*	5.34*	20*	65.4*	307*	13.8	34	++++	0.9	2.2	None	48.3 kb deletion	159,440–160,238	206,337–209,882	CH
A10	F	28	134	0.42	5.52*	24.3*	76.1*	319	14.3	56*	++	1.4	2.3	None	het polyad. mutation			I
A11	M	69	140	0.412	5.45	25.7*	75.6*	340	14.9	28	++++	<0.5	2.9	None	–			
A12	F	62	127	0.393	5.13*	24.8*	76.6*	323	14.7	35	++++	0.6	2.5	None	–			
A13	F	80	130	0.397	5.06	25.7*	78.5*	327	20.2*	13	+++	0.7	2.8	None	ATMDS			
KA1	M	21	131*	0.43	6.39*	20.5*	67.3*	305*	13.3	49	++++	0.8	2.5	None	$--^{FIL}$ het			
KA2	F	14	103*	0.330*	5.30*	19.4*	62.8*	309*	15.9*	86*	++	0.5	2.3	None	$--(\alpha)^{20.5\ kb}$ het			
KA3	M	62	128*	0.409	5.97*	21.4*	68.5*	313	13.6	21	++++	1	2.5	None	$--^{MED}$ het			
KA4	M	53	125	0.387	5.05	24.8*	76.6*	323	13.5	97*	n.d	<0.5	2.1	None	$-\alpha^{3.7}$ het			
KA5	M	5	122	0.354	4.62	26.4	76.6*	345	13.9	58*	n.d	1	2.5	None	$-\alpha^{3.7}$ het			
KA6	M	41	133	0.412	6.14*	21.7*	67.1*	323	13.7	34	++	<0.5	2.8	None	$-\alpha^{3.7}$ homo			
Group B																		
B1	M	31	141	0.444	6.08*	23.2*	73*	318*	21.2*	61*	n.d	17.1*	2.4	None	1093 kb deletion	4,127,761–4,129,744	5,221,705–5,222,265	D/NL/RI/RC
B2	F	22	97*	0.294*	3.93	24.7*	74.8*	330	12.9	37	n.d	24.6*	1.8	None	84.4 kb deletion	5,135,687–5,136,813	5,220,459–5,220,799	n.a
B3	M	51	141	0.438	6.47*	21.8*	67.7*	322	21.1*	118*	n.d	9.7*	2.6	None	48.4 kb deletion	5,178,944–5,179,086	5,226,525–5,228,371	CL
B4	F	8	107*	0.335*	5.48*	19.5*	61.1*	319	18.3*	66*	n.d	8.3*	2.3	None	32.3 kb deletion	5,193,770–5,202,068	5,229,956–5,230,564	TR
B5	F	47	112*	0.348*	5.01	22.4*	69.5*	322	19.7*	144*	n.d	10.6*	2.6	None	13.7 kb deletion	5,193,770–5,202,068	5,210,736–5,212,446	n.a
B6	F	73	121	0.392	5.44*	22.2*	72.1*	309*	21.6*	104*	n.d	11.6*	2.3	None	13.7 kb deletion	5,193,770–5,202,068	5,210,736–5,212,446	
B7	F	51	117*	0.37	5.13*	22.8*	72.1*	316	19.2*	152*	n.d	9.4*	2.2	None	13.5 kb deletion	5,204,482–5,204,662	5,211,512–5,211,980	
B8	F	36	132	0.383	4.26	31	89.9	345	11.2	83*	n.d.	5.9*	2.2	None	–			
B9	F	9	120	0.417	5.53*	21.7*	75.4*	288*	15.1*	183*	++	7.8*	2.3	None	KLF1:c.519-520insCGCgCCC			
B10	M	39	147	0.431	5.01	29.3	86	341	10.6	n.d.	n.d.	16*	1.8	None	HBG1:c.-170 G>A			CH/I
B11	M	1	88*	0.253*	3.88	22.7*	65.2*	348	23.3*	119*	n.d	27.9*	2.7	None	–			
B12	M	48	158	0.451	5.03	31.4	89.7	350	11.2	29	n.d.	14.6*	1.8	None	HBG1:c.-249C>T			I
B13	F	41	89*	0.267*	3.46*	25.7*	77.2*	333	17.3*	313*	n.d	20.6*	0.9	None	HBG2:c.-255C>G; $-\alpha^{3.7}$ het			n.a.
KB1	M	43	94*	0.298*	3.59*	26.2*	83.0	315	14.8	112*	n.d	38.2*	2.1	HbC (hemi)	HPFH-1 het			
KB2	M	23	130*	0.399*	5.73	22.7*	69.6*	326	20*	131*	n.d	10.7*	2.6	None	Sicilian het			
KB3	F	22	117*	0.356*	5.35*	21.9*	66.5*	329	18.8*	62*	n.d	10.6*	2.2	None	Sicilian het			
KB4	F	31	103*	0.344*	4.55	22.6*	75.6*	299*	19.9*	154*	n.d	18.3*	2.3	None	Sicilian het			
KB5	F	27	114*	0.356*	4.91	23.2*	72.5*	320	21.5*	76*	n.d	18.3*	2.3	None	Sicilian het			
KB6	F	41	117*	0.363	4.71	24.8*	77.1*	322	14.3	74*	n.d	4.5*		Hb Lepore	Lepore het			
KB7	F	0	82*	0.305*	3.7*	22.2*	82.4*	269*	27.2*	232*	n.d	n.d.	0.6	None	132.2 kb			