Estimation of the difference in HbF expression due to loss of the 5' δ-globin BCL11A binding region

Elyes Slim Ghedira,[1,2] Laure Lecerf,[1] Emmanuelle Faubert,[1] Bruno Costes,[1] Kamran Moradkhani,[1] Dora Bachir,[3] Frédéric Galactéros,[3] and Serge Pissard[1,4]

[1]APHP-Molecular Genetics Department, Henri Mondor Hospital, Créteil, France; [2]Faculty of Pharmacy, Monastir University, Monastir, Tunisia; [3]APHP, Red Blood Cell Disease Unity, Henri Mondor Hospital, Créteil, France, and [4]Université Paris-Est Créteil (UPEC), Inserm U955 eq.11, France

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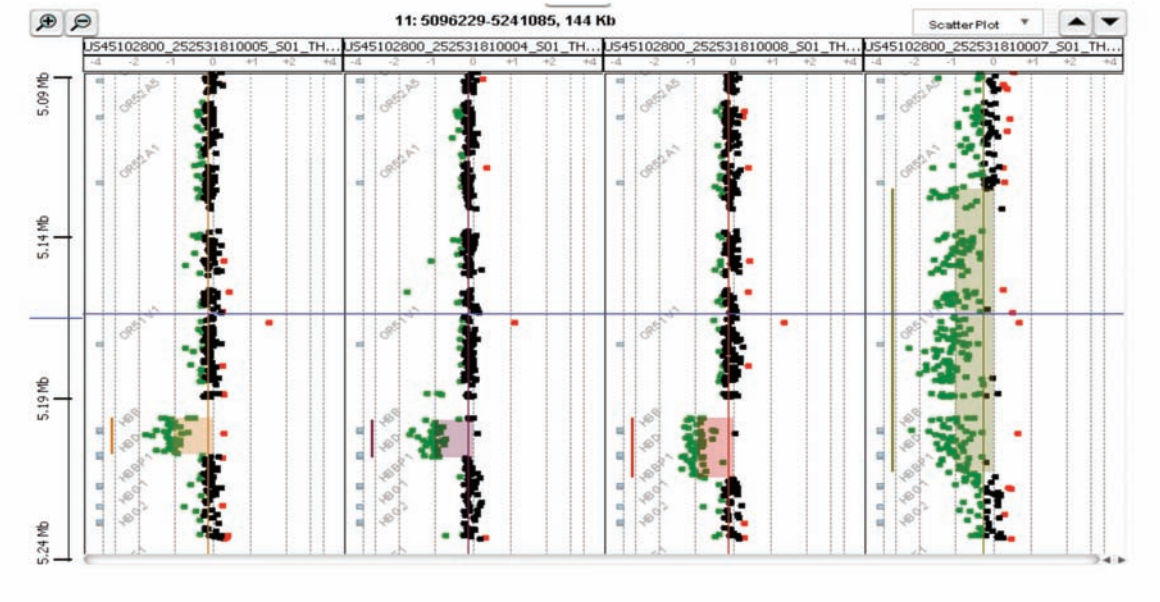
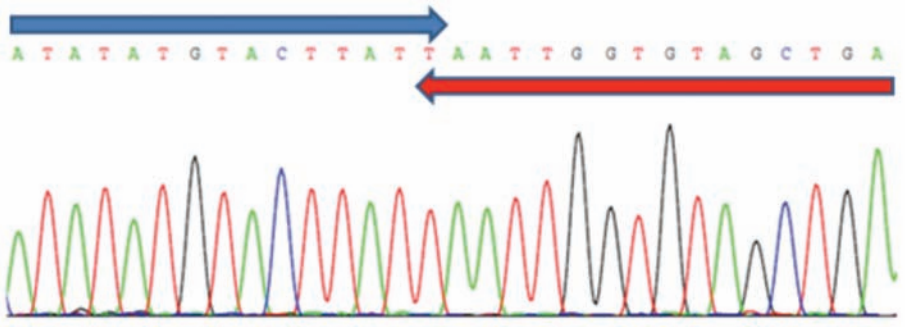
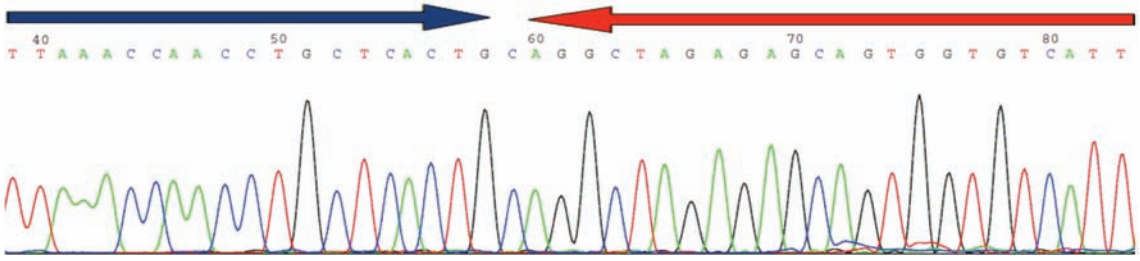


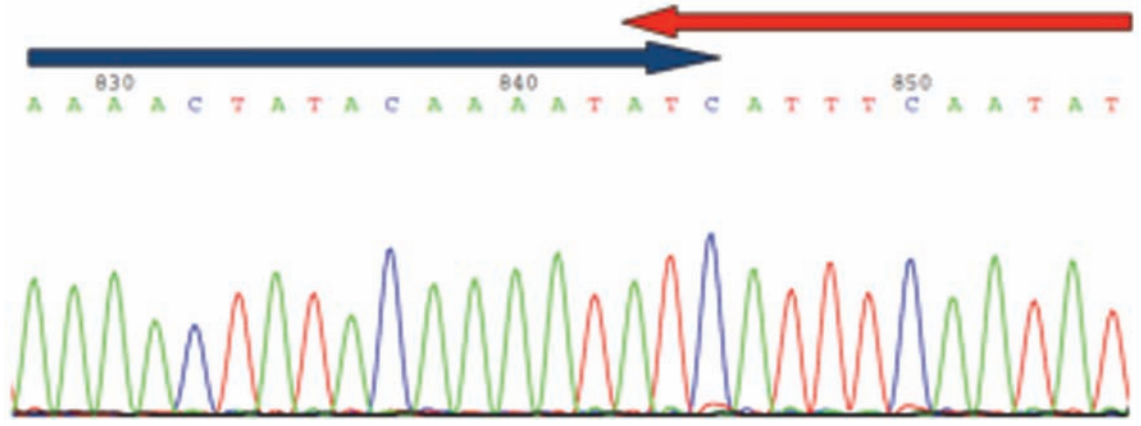
Online Supplementary Figure S1. The four deletions identified on pan-globin array. 5' side of the β-globin locus is down, and 3' is up. From left to right: 12.6 Kb, 20.7Kb, 23.7 Kb and 84.8 Kb.



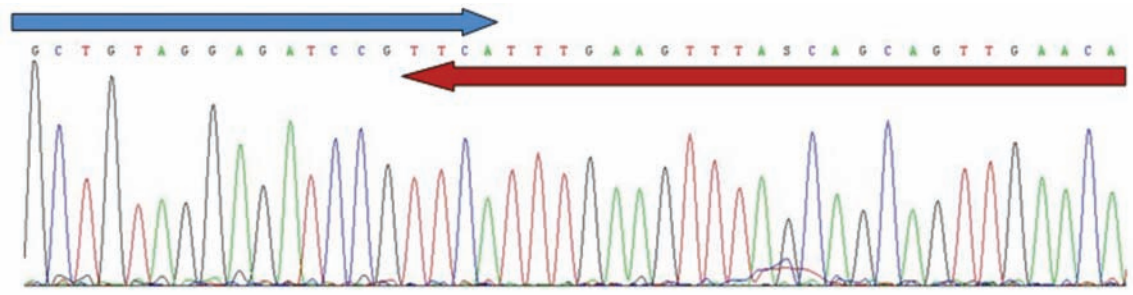
Online Supplementary Figure S2. 12.6Kb deletion primers and breakpoint sequence. F : 5'– GAGGCTCCAACTCAAAGATGA–3' R : 5'–ATGGCTCTACGGATGTGTGA–3' PCR needed the use of an Expand Long Template PCR System Taq (Roche Diagnostics GmbH) 12610bp del, GRCh37.p5, NC_000011.9:g. 5241853_5254462del First time described deletion. The deletion 5' breakpoint is located within IVS2 of the δ<-globin gene : HBD:c.316-140



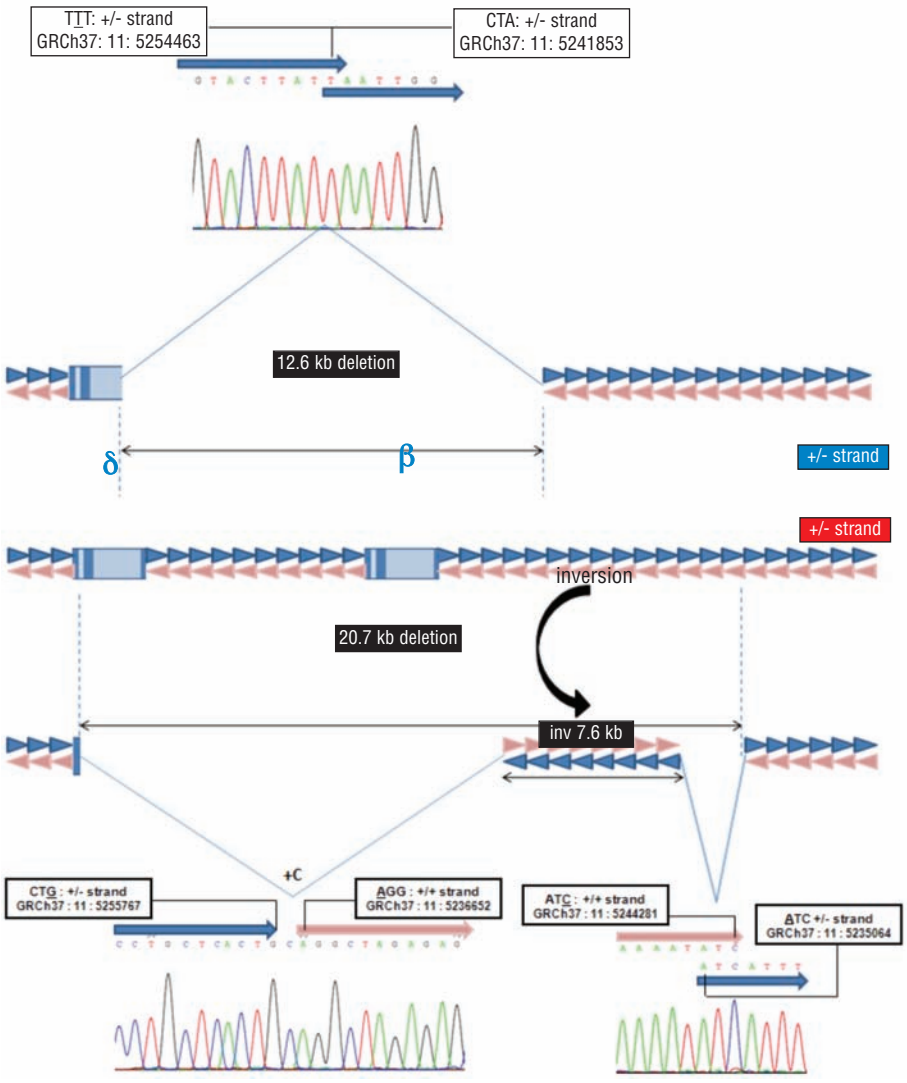
Online Supplementary Figure S3. 20.7 Kb deletion primers and breakpoints sequence (Up : 5' breakpoint. Down : 3' breakpoin) F 5'– GGCAAGTTAAGGGAATAGTGGA –3' R 5'– CAGGTTTTGCCCATTCTGTA –3' 20702bp del, GRCh37.p5, NC_000011.9:g.[5235065_5255766delins(invNC_000011.9:g.5236652_5244281);5255766_5255767insG] not reported yet in the globin gene server HbVar database (*globin.cse.psu.edu/hbvar*)The deletion 5' breakpoint is located within 5'UTR of the δ-globin gene : HBD:c.-103.



Online Supplementary Figure S4. 23.7 Kb (French West-indies and Algerian) deletion primers. F 5'– TGAAGTTGATGGAAACAAT-GAAG–3' R 5'– ATGGCTCTACGGATGTGTGA –3'



Online Supplementary Figure S5. The primers engineered to PCR-sequence the deletion breakpoints. 84.8 Kb deletion primers. F 5'– AAACCTCTTCCGCCATTAGG –3' R 5'– TGAGGAAAGCACTATGAATAACCA –3' 84837bp del, GRCh37.p5. NC_000011.9:g.5175177_5260013del. Deletion described for the first time.



Online Supplementary Figure S6. Breakpoints of the new 12.6kb deletion and the Turkish deletion-inversion with breakpoints sequences images and nucleotides coordinates. 5' breakpoint of the 12.6kb deletion lays in the IVS2 of δ-globin gene. 5' breakpoint of the Turkish deletion-inversion lays in the 5UTR of δ-globin gene.

Online Supplementary Table S1. HbF rate correlated SNPs analysis : Primers and technical conditions.

BCL11A rs4671393

F : 5'-CCC.CAT.TAG.CAG.CAT.GGA.GAG.TCA-3' ;
R : 5'-GGT.CCT.TCT.GCT.TCC.TGT.TCA.CCT.C-3'

BCL11Ars11886868

F : 5'-CTA.CCA.CCA.CAG.TGT.TGA.GA-3' ;
R : 5'-TGG.CAA.CCA.ATA.GGT.TGA.ATG.GA-3'

HSB1L-MYB rs9399137

F : 5'-TCA.CTT.TAA.AAG.GCG.GTA.TTG-3'
R : 5'-AGA.AGC.ACT.TTG.GCA.AGC.AT-3'

PCR Amplification : vol dNTP (2mM): 5 µL ; vol primer F (20 µM): 1 µL ; vol primer R (20 µM): 1 µL ; vol Taq sigma Aldrich (5UI/µL): 0,25 µL ; vol total PCR: 50 µL. Hybridization at 55°C. Labeling : Big Dye Big Dye Terminator v3.1 cycle sequencing kit protocol (Applied biosystems, #4336919). Purification : Sephadex resine G50. separation : capillary electrophoresis on 16 capillaries Sequencer 3130 xL Genetic Analyzer (ABL prism, Applied Biosystems). reading : manual (Chromas v1.4.5).

Online Supplementary Table S2. BCL11A sequencing : Primers and technical conditions. The sequence of exons, flanking regions and promoter was achieved (BCL11A XL and L form), looking for any mutation into BCL11A which may have decreased the native protein. Neither coding sequence variation nor promoter sequence variation was found (*data not shown*).

	primer F (5'>3') Tm° (C°)	primer R (5'>3') Tm° (C°)	amplicon length	Taq	vol (µl) MgCl2 (Gold 25mM) / MgSO4 (plat 50 mM)	Hybridation Temp° (C°)	DMSO (µl)
CACCC boxes	CGGTGCACTCTCGCTCTATT 60.6 °	CGTCAGGAGTCTGGATGGAC 60.7 °	424 pb	Platinium High fidelity Invitrogen (Mg free Buffer)	3	57	5
exon 1	TTGGATGTCAAAAGGCACTG 59.3	TAAAAATGCATGCACACACC 57.6	296 pb	Platinium High fidelity Invitrogen (Mg free Buffer)	3	56	5
exon 2	TTGTATGCTCACATTTTCTGGG 59.6	TGTTCTGGACGTAAGCAACAG 58.6	649 pb	Ampli-Taq Gold Applied Biosystems (Mg free buffer)	4	58	
exon 3	TCTGCAAGGCTGTTGTAATG 57.1	GGCTGCCAAGTGAGTAATGG 60.2	366 pb	Ampli-Taq Gold Applied Biosystems (Mg free buffer)	3	58	
exon4-1	CTTAGGCCTTGGGGTTGAG 59.2	TTCCCTGCCAGCTCTCTAAG 59.3	660 pb	Ampli-Taq Gold Applied Biosystems (Mg free buffer)	3	58	
exon4-2	CACCCGAGTGCCTTTGAC 59.8	CTCTTGAACTTGGCCACCAC 60.3	540 pb	Platinium High fidelity Invitrogen (Mg free Buffer)	2	60	
exon4-3	AAGACGCACATGCACAAATC 59.3	TTGTCTGGAGTCTCCGAAGC 60.1	774 pb	Platinium High fidelity Invitrogen (Mg free Buffer)	3	60	
exon4-4	CCCAACACGGAGAACGTGTA 61.5	AAGGGGAGTGGTGAAAAAGG 59.9	622 pb	Ampli-Taq Gold Applied Biosystems (Mg free buffer)	4	58	
exon5	AAATCATTGGGCCAAGTCAC 59.4	ACGGCTTCTTGGAGGCTACT 60.0	253 pb	Taq polymerase Sigma Aldrich	56		

PCR Amplification : vol dNTP (2 mM): 5 µL; vol primer F (20 µM): 1 µL ; vol primer R (20 µM): 1 µL; vol Taq (5UI/ µL): 0,25 µL ; vol total PCR: 50 µL; Labeling : Big Dye Big Dye Terminator v3.1 cycle sequencing kit protocol (Applied biosystems, #4336919). Purification : Sephadex resine G50. Separation : capillary electrophoresis on 16 capillaries Sequencer 3130 xL Genetic Analyzer (ABL prism, Applied Biosystems). Reading : manual (Chromas v1.4.5) and software assisted (Seqscape v2.5).

Online Supplementary Table S3. BCL11A deletion screening using Quantitative Multiplex-PCR : Primers and technical conditions. No deletions was found (*data not shown*).

external control primers
MLH1 gene 163pb F : ATTTTTTGTTTTGCAGTTCTCC R : GTAGTAGCTCTGCTTGTTCACACA
primers intron 2 (snp 868)
200pb F : CTACCACCACAGTGTTGAGA R : TGGCAACCAATAGGTTGAATGGA
primers exon 4 finc finger
246pb F : CTCCCTCCTCTGCAATCC R : ACCGTCGTCGGACTTGAC
FANCL gene (in 3' – telomeric – of the BCL11A gene) primers
333 bp F : atgtgaagcagggagagtcg R : aaacccttaatcctccttgtcc
PAPOLG gene (in 5' – centromeric – of the BCL11A gene) primers
428pb F : tgaatagccccttcataaaca R : ggtattcaatatgttcgcagaat

PCR Amplification: vol dNTP (2 mM): 8 µL ; vol primer F (20 µM): 1 µL; vol primer R (20 µM): 1 µL; vol Taq platinium (5UI/µL): 1 µL; vol MgSO4 (50 mM): 5 µL; vol platinium buffer: 5 µL; total volume PCR: 50 µL; Separation Amplicons by dHPLC (WAVE system, Transgenomic); non denaturant mode (column temp°: 50°C); column: DNAsep 3500A (4,6mm x 50 mm).

Online Supplementary Table S4. BCL11A binding sites screening by HRM (High resolution melting analysis, Roche 480 Light cycler Real-Time PCR System): Primers and technical conditions.

HRM 5' δ-globin gene BCL11A biding site
256 bp F: ATCACCGTCATGGTCCAAGT R: GAGAGGAGCCATGAATGACC
HRM HS3-LCR BCL11A biding site
279pb F: TAGGTCAGGTTGGTGGTGCT R: TGGTCCAGAGAGTGTGCATC
HRM 3' A-γ-globin BCL11A biding site
184pb F: TAGATCAAGCCTGTGCCAGA R: AAGGAGATTTCCTCTGAATCATTG
Master Mix LC 480 HRM 2x: 10 µL
MgCl2 (25 mM): 2,4 µL F: 0,2 µL R: 0,2 µL DNA (10 ng/v): 2 µL total volume: 20 µL
Analysis Phases
Taq activation : 95°C 5mn 10 Touch down cycles : 95°C 10sec ; 65°C 10sec (touch down to 60°C, 0,5°C / cycle) ; 72°C 10sec 50 amplification cycles : 95°C 10sec ; 60°C 10sec ; 72°C 10sec Melting curves analysis : 65°C to 95°C, 0,02°C/sec

The three main BCL11A binding sites described within -globin cluster was studied, more precisely in 5' of δ-globin gene, 3' of Aγ-globin gene and in HS3-LCR in order to check for the absence of sequence variation. No sequence variation was found (data not shown).