The Genetic Basis of Asymptomatic Codon 8 Frame-Shift β^0-Thalassaemia Homozygotes

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Supplementary data

Figure S1 Nucleotide Sequences of *HBB* from Initiation Codon ATG to Codon 13

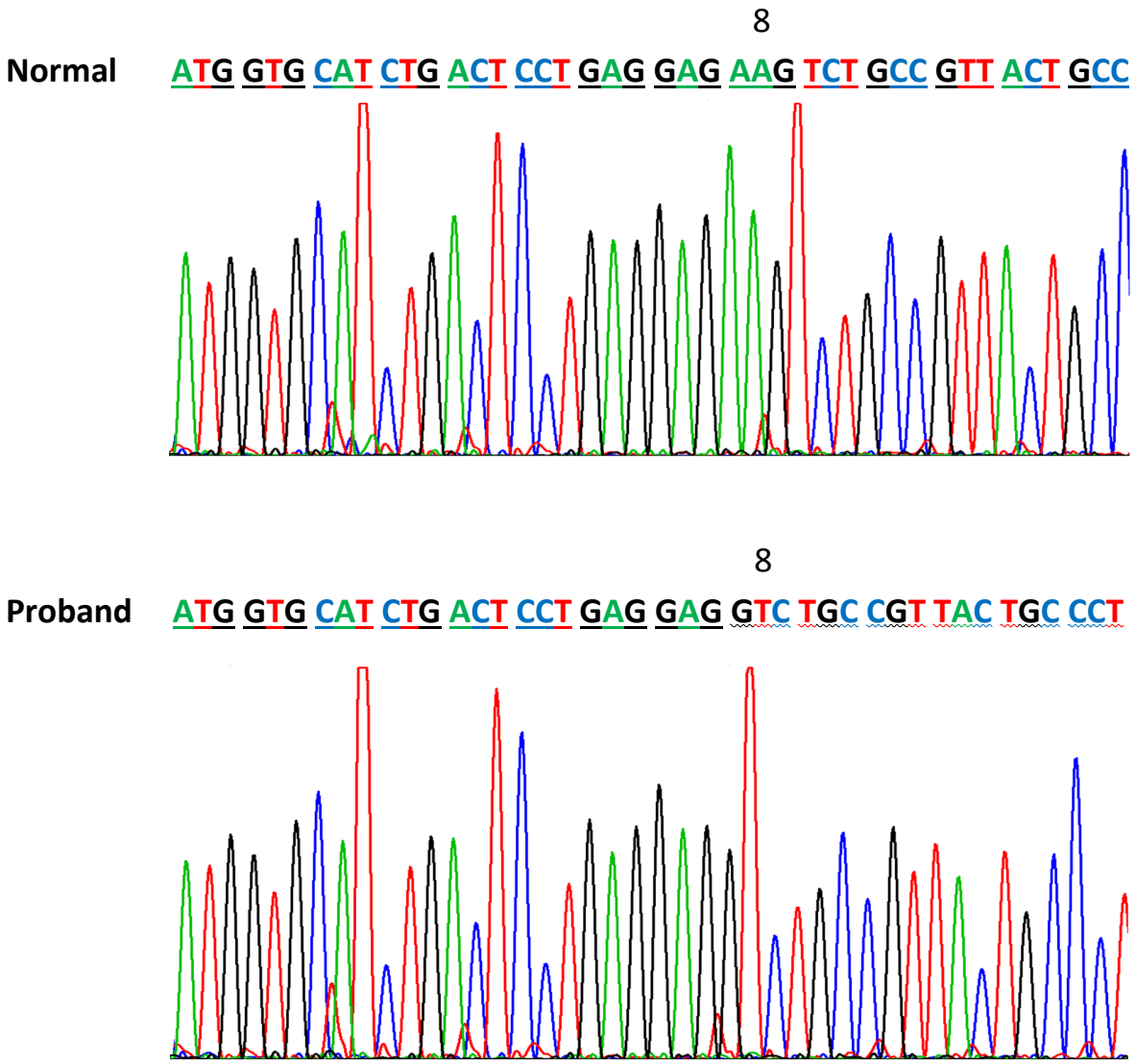

Figure Legend: The top figure shows the nucleotide sequences from a normal individual beginning from the *HBB* initiation codon ATG to codon 13. Note codon 8 is AAG. The lower figure shows the nucleotide sequences from the proband. Note codon 8 is GTC, as a result of homozygous deletion of dinucleotides AA from the normal codon 8 sequences.

Figure S2 MLPA on Proband's *HBB* Gene Cluster

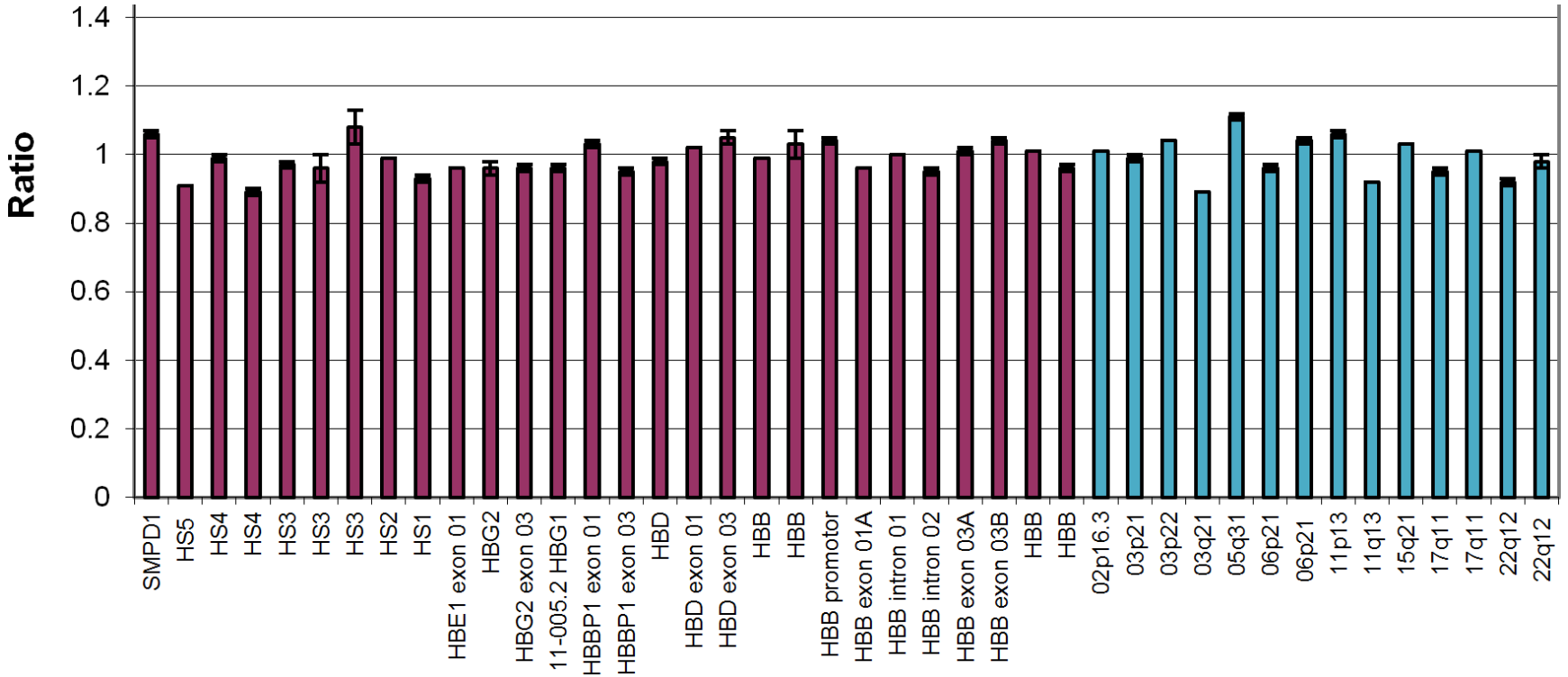

Figure Legend: MLPA on proband's *HBB* gene cluster showing equal signal strength of probes from LCR to 9 kb downstream from *HBB*. The blue-colored bars represent probes for genes on other chromosomes which serve as control.

Figure S3 SNPs Present in Proband's *HBD* to *HBG1* Intergenic Region

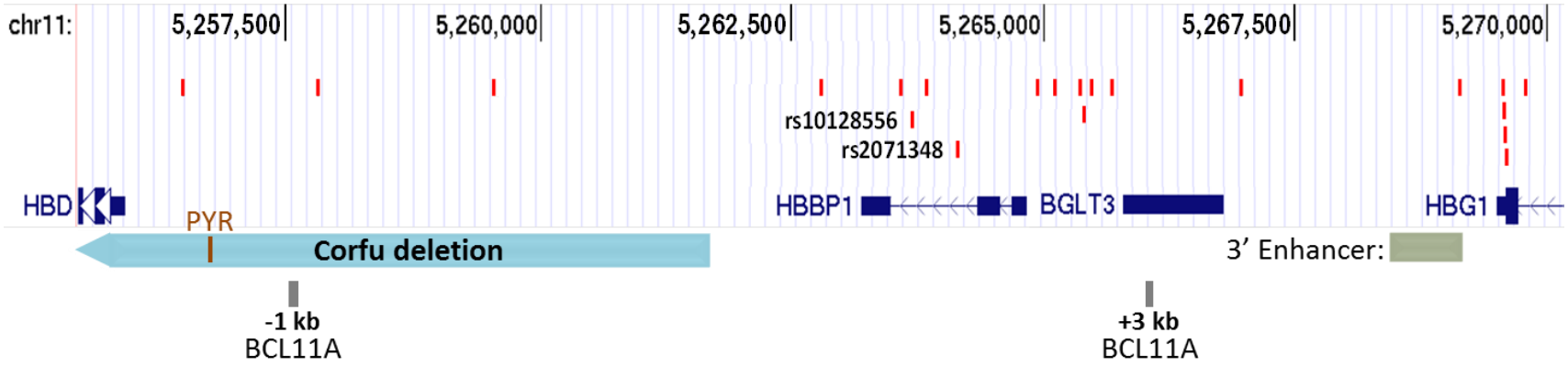

Figure Legend: The figure shows ~14.5 kb *HBG1* – *HBD* intergenic region. The numbers on the first row represent hg19 Chr11 positions. Each perpendicular red bar represents a single SNP found in the proband, all homozygous for the alternate alleles (Supplementary Table 2). The light green bar represents the enhancer 3' to *HBG1* (Bodine, Ley, EMBO J 1987; 6:2997-3004). BGLT3 is the template for a long non-coding RNA (Kiefer et al, Blood 2011; 118:6200-6208). The light blue bar represents the Corfu deletion (Kulozik et al, Blood 1988; 71:457-462). The brown strip represents PYR complex DNA-binding site (Bank, Blood 2006; 107:435-443). The grey strips represent two purported BCL11A binding sites, at 3 kb downstream of *HBG1*, and 1 kb upstream of *HBD* (Sankaran et al, N Engl J Med 2011; 365:807-814). SNP rs10128556 is associated with elevated HbF in sickle cell anemia patients (Galarneau et al, Nat Genet 2010; 42:1049-1051). SNP rs2071348 is associated with elevated HbF and milder disease severity in patients with HbE/β^0-thalassemia (Nuinoon et al, Hum Genet 2010; 127:303-314).

Table SI ARMS tests to determine HbF QTL genotypes

HbF QTL	Primer name	Primer sequences 5' to 3'	PCR amplicons	SNP genotype	Final concentrations of primers picomoles/µL	PCR conditions (Temperature / Time)			
						Denaturing	Annealing	Extension	PCR cycles
rs9399137 Chr.6q23	F-9399137	GTTCTGCTTCTACAATAGACATTTTTCATG			0.1	98^{0}C / 40sec	56^{0}C / 40sec	72^{0}C / 2min	35
	R-9399137	TAAAAACCTGGCAAGATACCATTTAAAGTA			0.1				
	F-9399137-C	TAATGTAATTAACTGAACATATGGTTACTC	250 bp	C	0.2				
	R-9399137-T	AGCAGGGTTGCTTGTGAAAAAACTTTA	141 bp	T	0.1				
rs66650371 3-bp deletion Chr.6q23	TAC-1F:	TCACTCTGGACAGCAGATGTTACTAT			0.2	98^{0}C / 20sec	58^{0}C / 40sec	72^{0}C / 40sec	30
	TAC-1R	CTCAGTGATGGTATTTCTGGAGAC	206 bp	no del	0.8				
	TAC-0F	AGCCCGTCCAGACACTCATTGTT			0.1				
	TAC-0R	GCCCTGATAACATTTTGTGGTTTTCATTTAACAT	276 bp	3-bp del	0.8				
rs766432 Chr.2p15	F-766432	ACAGCGAGACCCTGTCTCAAAAACAA			0.1	98^{0}C / 40sec	56^{0}C / 40sec	72^{0}C / 2min	35
	R-766432	TTCCTTAGTGTAGTGGAGAGGTTTCTA			0.1				
	F-766432-C	GTTTCGCTTTAGCTTTATTAAGGTACAC	726 bp	C	0.1				
	R-766432-A	CTTAAAATGAATGACTTTTGTTGTATGTACAT	521 bp	A	0.2				

Genomic DNA was extracted from peripheral blood leukocytes by BioRobot EZ1 (Qiagen). PCR was performed using a thermocycler (ABI). In 10 ul reaction mixture, there were 100 ng of genomic DNA, 5 ul Multiplex mix (x2, Qiagen), 2 ul of Q-solution (x5, Qiagen) and the primer mix as shown in the table. The PCR reaction began with denaturation at 95^{0}C for 15 minutes, followed by amplification cycles as summarized in the table. After PCR, 5 ul of reaction mixture was run on 8% polyacryamide gel at 200V for 40 minutes. Note that genotyping for rs9399137 and rs766432 can be done in a multiplex reaction within a single tube. The alternate alleles are colored red in the table.

Table SII Non-identical SNP Genotypes at 7 Loci Found in Two Dizygotic Twins

Gene locus	SNP ID	hg19 position	Reference allele	Alternate allele	Proband	Twin brother
LFNG	rs61564232	Chr7:2,552,881	–	**GATG**	**GATG** / **GATG**	– / **GATG**
TFDP1	rs750015004	Chr13:114,290,958	G	**A**	G / **A**	G / G
CES3	rs145544825	Chr16:66,998,390	G	**A**	**A** / **A**	G / **A**
EXOX3L1	rs150417999	Chr16:67,222,999	G	**A**	**A** / **A**	G / **A**
CTRL	rs774504385	Chr16:67,963,944	C	**T**	**T** / **T**	C / **T**
ILF3	rs200928937	Chr19:10,798,066	G	**A**	G / **A**	G / G
ZBTB32	rs202219173	Chr19:36,207,494	C	**T**	**T** / **T**	C / **T**

Table SIII 21 SNPs Present within Proband's *HBD* to *HBG1* Intergenic Region

Chr11 position (hg19)	SNP ID	Reference allele	Alternate allele	Alternate allele frequency, according to 1,000 Genome Project	Proband's Genotype
5,256,431	rs7948416	A	**C**	C = 0.84	**C / C**
5,257,778	rs3759074	G	**A**	A = 0.19	**A / A**
5,259,534	rs6578590	T	**C**	C = 0.86	**C / C**
5,262,782	rs11036415	A	**C**	C = 0.84	**C / C**
5,263,577	rs10128555	C	**G**	G = 0.89	**G / G**
5,263,683	**rs10128556**	C	**T**	T = 0.20	**T / T**
5,263,831 - 5,263,828	rs59215941	TGTA	**–**	N.A.	**– / –**
5,264,146	**rs2071348**	T	**G**	G = 0.21	**G / G**
5,264,929	rs7934275	A	**G**	G = 0.89	**G / G**
5,265,106	rs12417960	C	**A**	A = 0.24	**A / A**
5,265,366	rs4910739	G	**C**	C = 0.83	**C / C**
5,265,396 - 5,265,394	rs143325232	AAG	**–**	N.A.	**– / –**
5,265,475	rs4910545	C	**T**	T = 0.75	**T / T**
5,265,680	rs11036431	G	**A**	A = 0.28	**A / A**
5,266,961	rs6578591	T	**C**	C = 0.88	**C / C**
5,269,140	rs6578592	C	**A**	A = 0.86	**A / A**
5,269,583	rs5009539	G	**A**	A = 0.48	**A / A**
5,269,584	rs1065686	T	**G**	G = 0.72	**G / G**
5,269,585	rs61893083	G	**A**	A = 0.72	**A / A**
5,269,586	rs62755960	A	**G**	G = 0.72	**G / G**
5,269,799	rs28440105	A	**C**	C = 0.84	**C / C**

Table SIV FSC8 Homozygotes, with No or Rare Transfusion

Ethnic background	Patient	Age - Gender	Age at dx	Hb (g/dL)	Splenec-tomy	Transfusions	α-Globin genotype	Chr11p15	Chr6q23		Chr2p16	*HBB* cluster haplotype
								rs7482144 *(Xmn I)*	rs9399137 (HMIP)	rs66650371 (3-bp deletion)	rs766432 *(BCL11A)*	
Turkish	**3**	**32 – F**	**3 yrs**	**10 at dx. Now 8.6**	**At age 7 yrs**	**Yes before splenectomy; none since**	**α α / α α**	**A** / **A**	T / **C**	WT / **del**	A / **C**	**IV** / **IV**
Turkish	**4**	**30 – F**	**2 yrs**	**4.2 at dx. Now 9.3**	**At age 11 yrs**	**Yes before splenectomy; none since**	**α α / α α**	**A** / **A**	T / **C**	WT / **del**	A / A	**IV** / **IV**
Turkish	**5**	**7 – M**	**---**	**7.7**	**No**	**Rarely**	**α α / α α**	**A** / **A**	T / T	WT / WT	A / A	**IV** / **IV**
Turkish	**6**	**13 – M**	**---**	**9.8**	**No**	**No**	**α α / α α**	**A** / **A**	T / **C**	WT / **del**	A / **C**	**IV** / **IV**

Patients #3 and #4 were sisters. They were originally reported in 1989 by Gurgey et al.

Patients #6 and #16 (Table 4) were siblings.

SNP alternate alleles are colored red.

Table SV FSC8 Homozygotes, Transfusion Dependent

Ethnic background	Patient	Age - Gender	Age at dx	Hb (g/dL)	Splenec-tomy	Transfusions	α-Globin genotype	Chr11p15	Chr6q23		Chr2p16	*HBB* cluster haplotype
								rs7482144 *(Xmn I)*	rs9399137 (HMIP)	rs66650371 (3-bp deletion)	rs766432 *(BCL11A)*	
Iraqi	**7**	**19 – F**	**7 yrs**		**At age 18 yrs**	**Yes, since age 7 yrs**	**α α / α α**	**A / A**	T / T	WT / WT	**C / C**	**IV / IV**
Iraqi	**8**	**21 – M**	**8 yrs**	**7.1 at dx**	**At age 14 yrs**	**Yes, since age 8 yrs**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / **C**	**IV / IV**
Iraqi	**9**	**15 - M**	**3 yrs**	**7.5**	**At age 6 yrs**	**Yes**	**α α / α α**	**A / A**	T / T	WT / WT	A / A	**IV / IV**
Iraqi	**10**	**14 - F**	**1 yr**	**7.9**	**At age 10 yrs**	**Yes**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / A	**IV / IV**
Iraqi	**11**	**14 - M**	**4 yrs**	**6.8**	**At age 5 yrs**	**Yes**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / **C**	**IV / IV**
Iraqi	**12**	**18 - M**	**1 yr**		**At age 12 yrs**	**Yes**	**– α $^{3.7}$ / α α**	**A / A**	T / T	WT / WT	A / A	**IV / IV**
Turkish	**13**	**16 – M**	**18-month**	**6.5 at dx**	**At age 8 yrs**	**Yes, since age 18-month**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / A	**IV / IV**
Turkish	**14**	**21 – F**	**4 yrs**	**7.6 at dx**	**No**	**Yes, since age 6 yrs**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / **C**	**IV / IV**
Turkish	**15**	**20 – M**	**9-month**	**8.2 at dx**	**At age 6 yrs**	**Yes, since age 1 yr**	**α α / α α**	**A / A**	T / T	WT / WT	A / **C**	**IV / IV**
Turkish	**16**	**7 – F**		**8.0 when transfusion began**	**No**	**Yes, since age 4 yrs**	**α α / α α**	**A / A**	T / **C**	WT / **del**	A / A	**IV / IV**
Turkish	**17**	**25 – F**			**No**	**Yes**	**α α / α α**	**A / A**	T / T	WT / WT	A / A	**IV / IV**

Table SV FSC8 Homozygotes, Transfusion Dependent (continued)

Ethnic background	Patient	Age - Gender	Age at dx	Hb (g/dL)	Splenec-tomy	Transfusions	α-Globin genotype	Chr11p15	Chr6q23		Chr2p16	*HBB* cluster haplotype
								rs7482144 *(Xmn I)*	rs9399137 (HMIP)	rs66650371 (3-bp deletion)	rs766432 *(BCL11A)*	
Moroccan	**18**	**F**				**Yes**	**α α / α α**	**A** / **A**	T / T	WT / WT	A / **C**	**IV** / **IV**
Moroccan	**19**	**15 – F**				**Yes**	**α α / α α**	**A** / **A**	T / T	WT / WT	A / A	**IV** / **IV**
North African	**20**	**DOB 1993 – F**			**Yes**	**Yes, since age 3 years**	**α α / α α**	**A** / **A**	T / T	WT / WT	A / **C**	**IV** / **IV**
North African	**21**	**DOB 1989 – F**			**At age 20 yrs**	**Yes, since age 2 years**		**A** / **A**	T / **C**	WT / **del**	A / A	**IV** / **IV**
Turkish	**22**	**4 – F**	**6 month**	**9.8 at dx**	**No**	**Yes, since age 3**	**α α / α α**	G / G	T / **C**	WT / **del**	**C** / **C**	**VII** / **VII**
North African	**23**	**DOB 2008 – F**			**No**	**Yes, since age 3 months**	**α α / α α**	G / G	T / T	WT / WT	A / **C**	**VII** / **VII**
North African	**24**	**DOB 2005 – M**			**No**	**Yes, since age 4 months**	**α α / α α**	G / G	T / T	WT / WT	A / A	**VII** / **VII**

Patients #13 and #14 were siblings.

Patients #16 and #6 (Table 3) were siblings.

Patients #23 and #24 were siblings.

Patient #23 underwent bone marrow transplantation in 2011 at age 3.

SNP alternate alleles are colored red.