Genetic modifiers of fetal hemoglobin affect the course of sickle cell disease in patients treated with hydroxyurea

Pierre Allard,[1] Nareen Alhaj,[2] Stephan Lobitz,[3,4] Holger Cario,[4,5] Andreas Jarisch,[4,6] Regine Grosse,[4,7] Lena Oevermann,[4,8] Dani Hakimeh,[4,8] Laura Tagliaferri,[1,4] Elisabeth Kohne,[5] Annette Kopp-Schneider,[2] Andreas E. Kulozik[1,4] and Joachim B. Kunz[1,4] for the German Sickle Cell Disease Study Group.[§]

[1]Department of Pediatric Oncology, Hematology and Immunology, Hopp-Children's Cancer Center (KiTZ) Heidelberg, University of Heidelberg, Heidelberg; [2]Abteilung Biostatistik, Deutsches Krebsforschungszentrum (DKFZ), Heidelberg; [3]Gemeinschaftsklinikum Mittelrhein, Kemperhof, Pädiatrische Hämatologie und Onkologie, Koblenz; [4]GPOH Konsortium Sichelzellkrankheit; [5]Universitätsklinikum Ulm, Klinik für Kinder- und Jugendmedizin, Pädiatrische Hämatologie und Onkologie, Ulm; [6]Klinikum der Johann-Wolfgang-Goethe-Universität, Zentrum für Kinder- und Jugendmedizin, Schwerpunkt Stammzelltransplantation und Immunologie, Frankfurt am Main; [7]Universitätsklinikum Hamburg-Eppendorf, Zentrum für Geburtshilfe, Kinder- und Jugendmedizin, Klinik und Poliklinik für Pädiatrische Hämatologie und Onkologie, Hamburg and [8]Charité - Universitätsmedizin Berlin, Campus Virchow-Klinikum, Klinik für Pädiatrie m.S. Onkologie/Hämatologie/KMT, Berlin, Germany

[§]A list of the members of the Sickle Cell Disease Study Group appears in the Appendix

Correspondence:

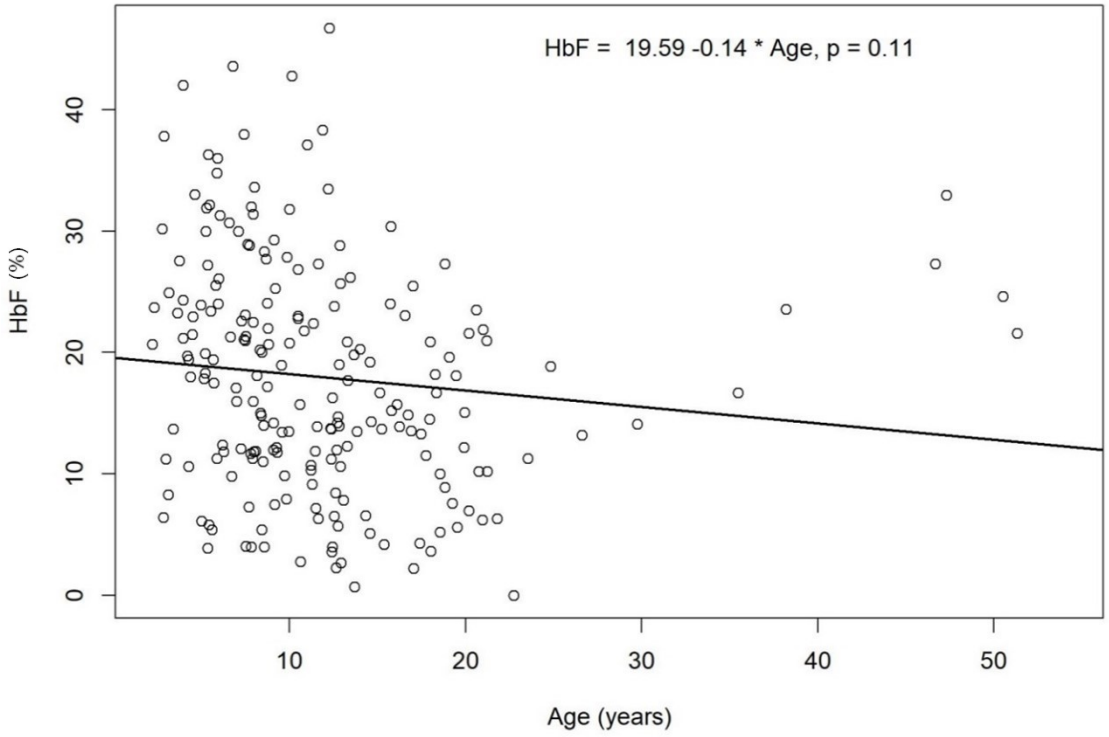
Joachim Kunz
joachim.kunz@med.uni-heidelberg.de

Received: April 11, 2021.
Accepted: July 29, 2021.
Prepublished: October 28, 2021.
https://doi.org/10.3324/haematol.2021.278952

Supplement

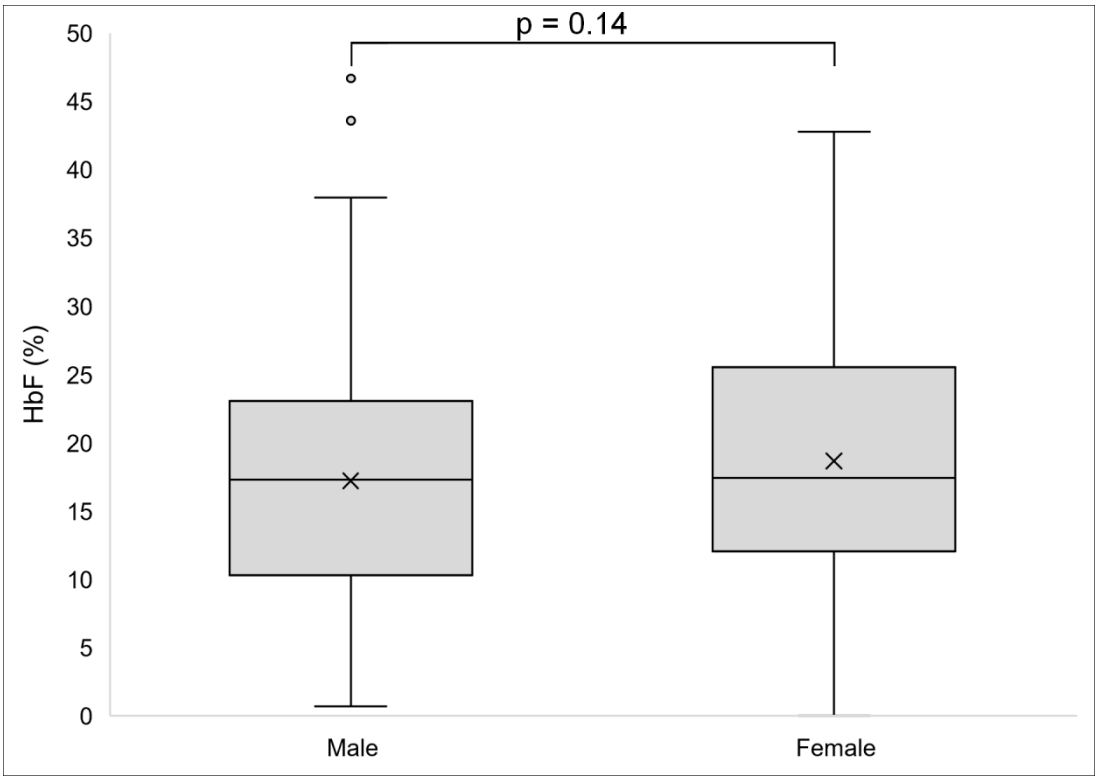
Supplemental Figure 1

Linear Regression of HbF on Age (n=208)



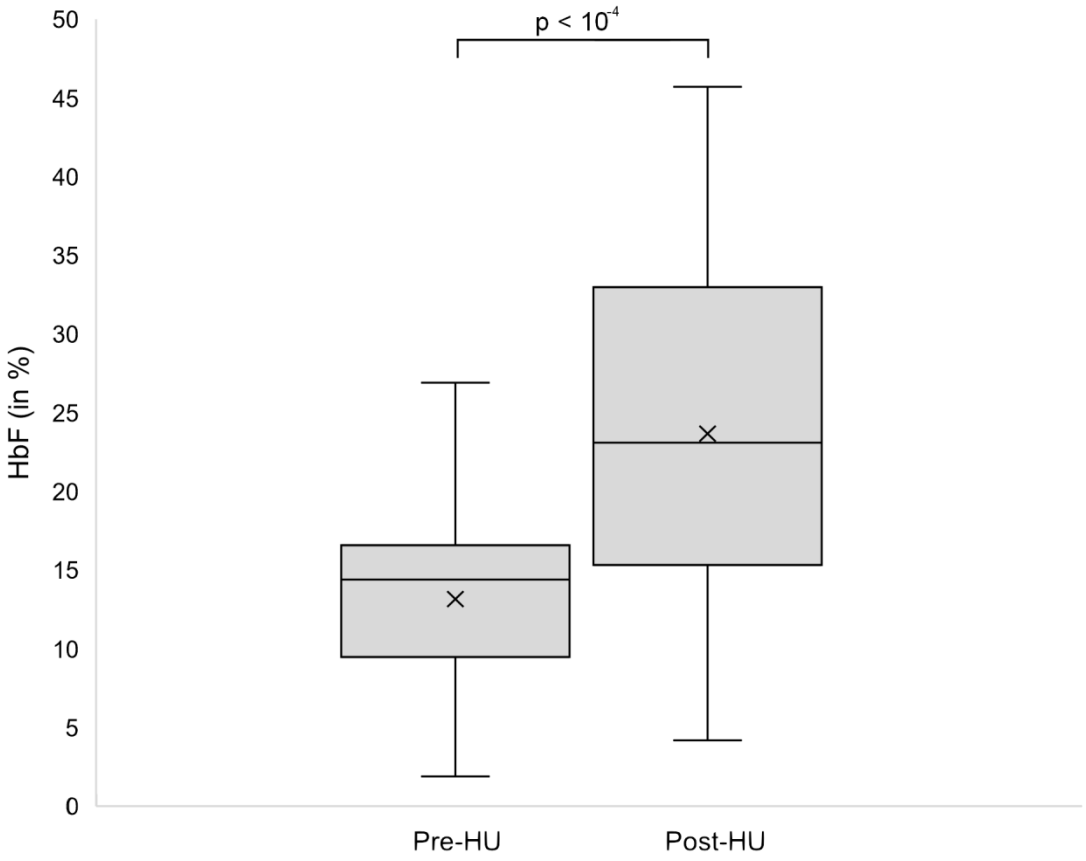
Supplemental Figure 2

HbF by Sex. Female (n=183): mean HbF 18.7 ± SD 9.3 % vs male (n=177): mean HbF 17.2 ± SD 9.7 %; T-Test p=0.14



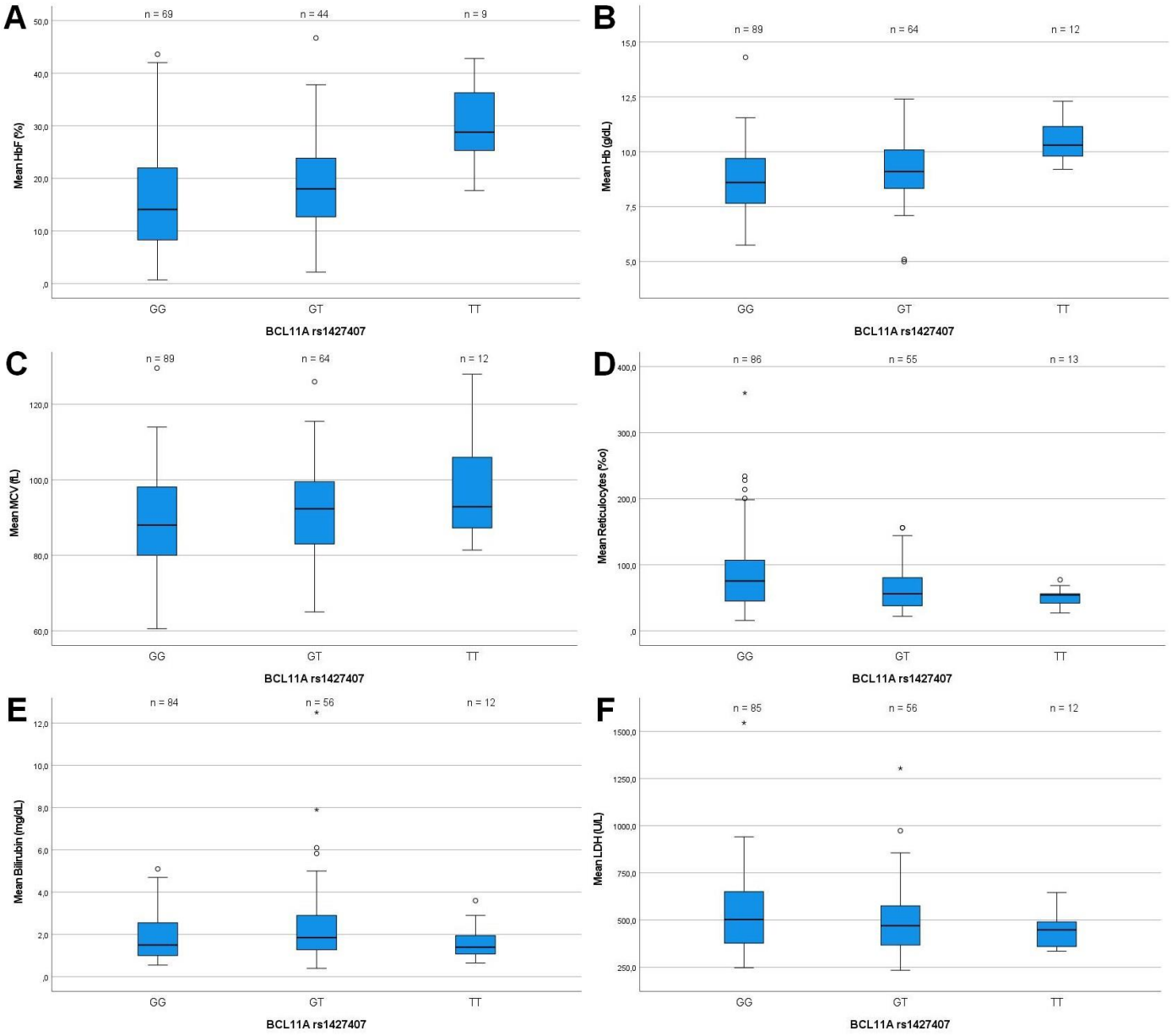
Supplemental Figure 3

HbF before and during HU-Therapy (n=25). Pre-HU mean HbF 13.2 ± SD 5.5 % vs post-HU mean HbF 23.7 ± SD 11.3 %; T-Test $p<10^{-4}$



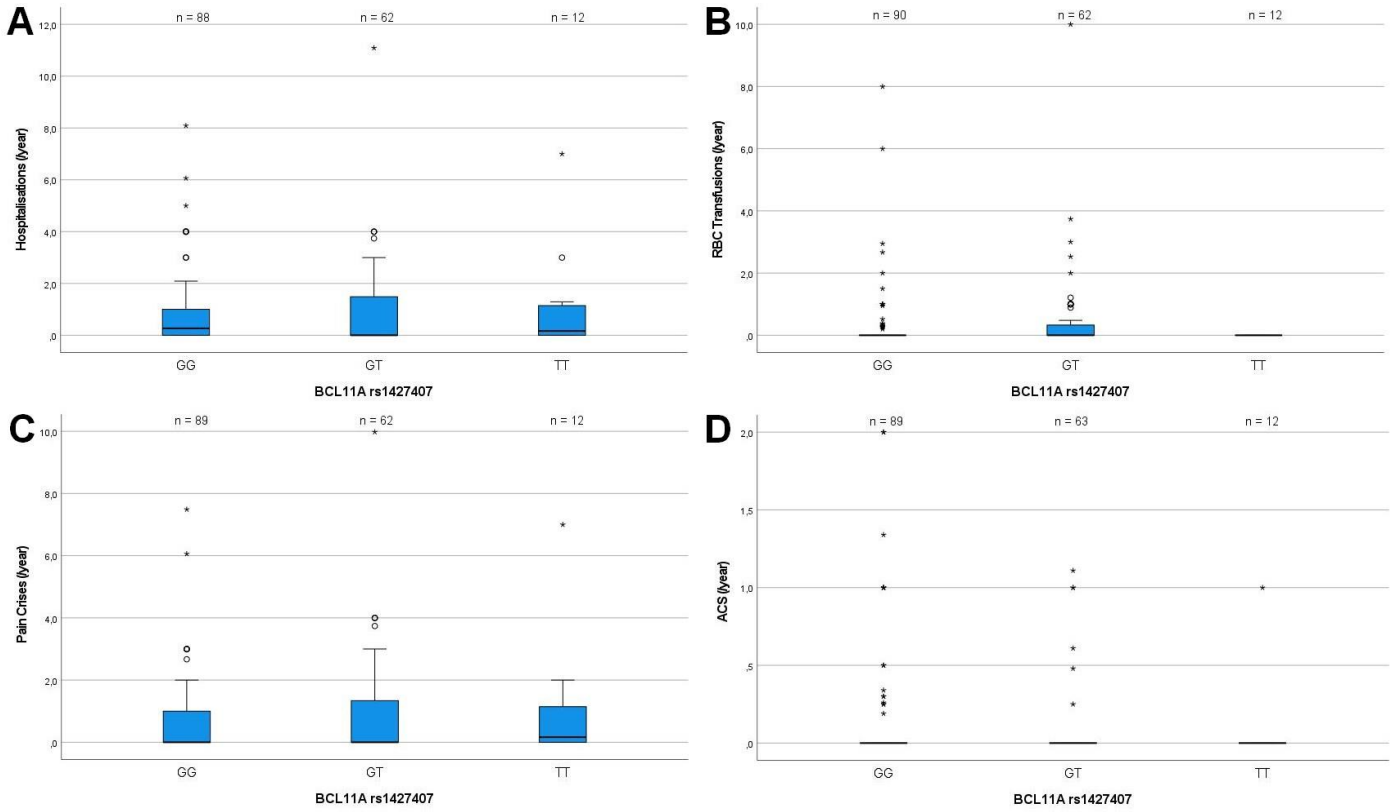
Supplemental Figure 4

Laboratory parameters of patients with *BCL11A* rs1427407 GG compared to *BCL11A* rs1427407 GT and *BCL11A* rs1427407 TT. n- number of patients available for analysis (A) GG patients (n=69) mean HbF 16.3 ± 9.8 % vs GT patients (n=44) mean HbF 18.6 ± 8.5 % vs TT patients (n=9) mean HbF 30 ± 8 %. (B) GG patients (n=89) mean Hb 8.7 ± 1.5 g/dL vs GT patients (n=64) mean Hb 9.3 ± 1.5 g/dL vs TT patients (n=12) mean Hb 10.5 ± 1 g/dL. (C) GG patients (n=89) mean MCV 88.8 ± 12.6 fL vs GT patients (n=64) mean MCV 90.9 ± 12.5 fL vs TT patients (n=12) mean MCV 98.1 ± 15.8 fL. (D) GG patients (n=86) mean reticulocytes 84.8 ± 56.2 **‰** vs GT patients (n=55) mean reticulocytes 66.2 ± 34.8 **‰** vs TT patients (n=13) mean reticulocytes 50.8 ± 13.8 **‰**. (E) GG patients (n=84) mean bilirubin 1.9 ± 1.1 mg/dL vs GT patients (n=56) mean bilirubin 2.5 ± 2 mg/dL vs TT patients (n=12) mean bilirubin 1.6 ± 0.9 mg/dL. (F) GG patients (n=85) mean LDH 525.6 ± 197.6 U/L vs GT patients (n=56) mean LDH 495 ± 185.7 U/L vs TT patients (n=12) mean LDH 442.7 ± 93.9 U/L.



Supplemental Figure 5

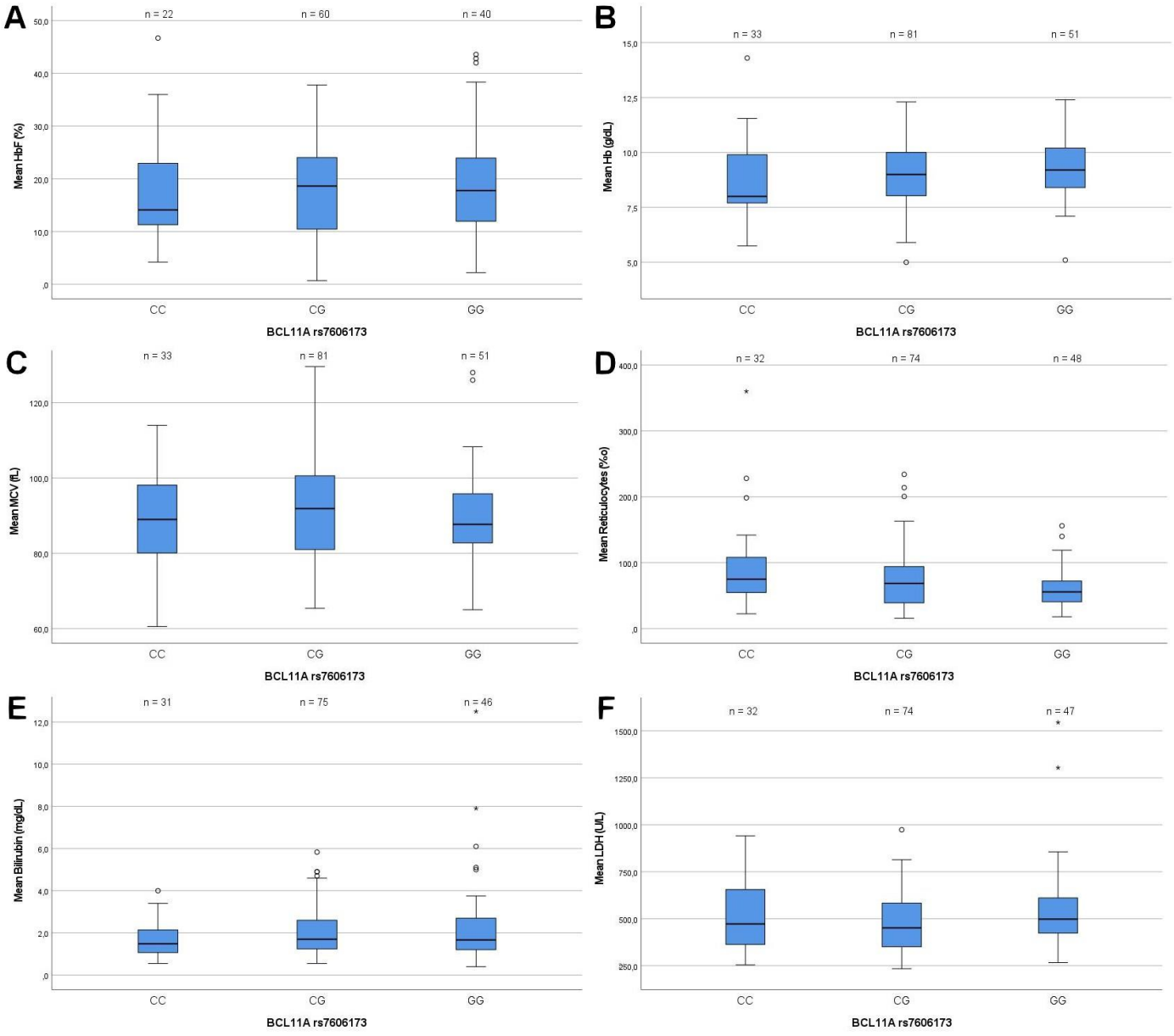
Frequency of complications in patients with *BCL11A* rs1427407 GG compared to *BCL11A* rs1427407 GT and *BCL11A* rs1427407 TT. n- number of patients available for analysis (A) GG patients (n=88) mean frequency of hospitalizations 0.9 ± 1.5 per year vs GT patients (n=62) mean frequency of hospitalizations 1 ± 1.8 per year vs TT patients (n=12) mean frequency of hospitalizations 1.1 ± 2.1 per year. (B) GG patients (n=90) mean frequency of red blood cells transfusions 0.3 ± 1.1 per year vs GT patients (n=62) mean frequency of red blood cells transfusions 0.5 ± 1.4 per year vs TT patients (n=12) mean frequency of red blood cells transfusions 0 ± 0 per year. (C) GG patients (n=89) mean frequency of pain crises 0.7 ± 1.2 per year vs GT patients (n=62) mean frequency of pain crises 0.9 ± 1.6 per year vs TT patients (n=12) mean frequency of pain crises 1.1 ± 2 per year. (D) GG patients (n=89) mean frequency of acute chest syndromes 0.1 ± 0.4 per year vs GT patients (n=63) mean frequency of acute chest syndromes 0.1 ± 0.2 per year vs TT patients (n=12) mean frequency of acute chest syndromes 0.1 ± 0.3 per year.



Supplemental Figure 6

Laboratory parameters of patients with *BCL11A* rs7606173 CC compared to *BCL11A* rs7606173 CG and *BCL11A* rs7606173 GG.

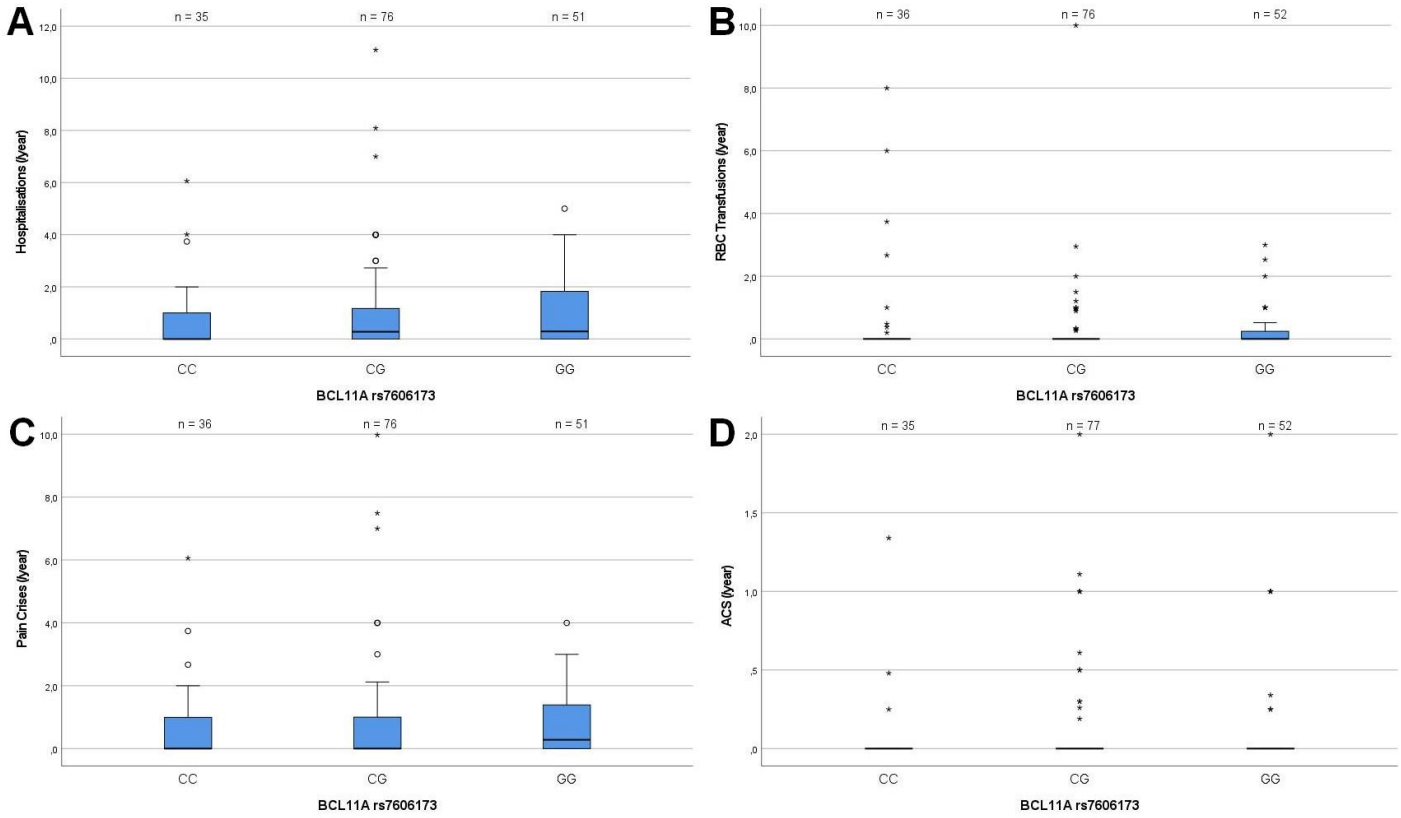
n- number of patients available for analysis (A) CC patients (n=22) mean HbF 17 ± 10.4 % vs CG patients (n=60) mean HbF 17.5 ± 9 % vs GG patients (n=40) mean HbF 19.7 ± 10.6 %. (B) CC patients (n=33) mean Hb 8.7 ± 1.8 g/dL vs CG patients (n=81) mean Hb 9 ± 1.5 g/dL vs GG patients (n=51) mean Hb 9.3 ± 1.4 g/dL. (C) CC patients (n=33) mean MCV 88.6 ± 12.8 fL vs CG patients (n=81) mean MCV 91.4 ± 13.3 fL vs GG patients (n=51) mean MCV 89.7 ± 12.5 fL. (D) CC patients (n=32) mean reticulocytes 92.1 ± 66.5 **‰** vs CG patients (n=74) mean reticulocytes 77.3 ± 46.3 **‰** vs GG patients (n=48) mean reticulocytes 61 ± 30.4 **‰**. (E) CC patients (n=31) mean bilirubin 1.7 ± 0.9 mg/dL vs CG patients (n=75) mean bilirubin 2.1 ± 1.2 mg/dL vs GG patients (n=46) mean bilirubin 2.3 ± 2.2 mg/dL. (F) CC patients (n=32) mean LDH 503.4 ± 176.9 U/L vs CG patients (n=74) mean LDH 479.8 ± 148.8 U/L vs GG patients (n=47) mean LDH 555.1 ± 238.2 U/L.



Supplemental Figure 7

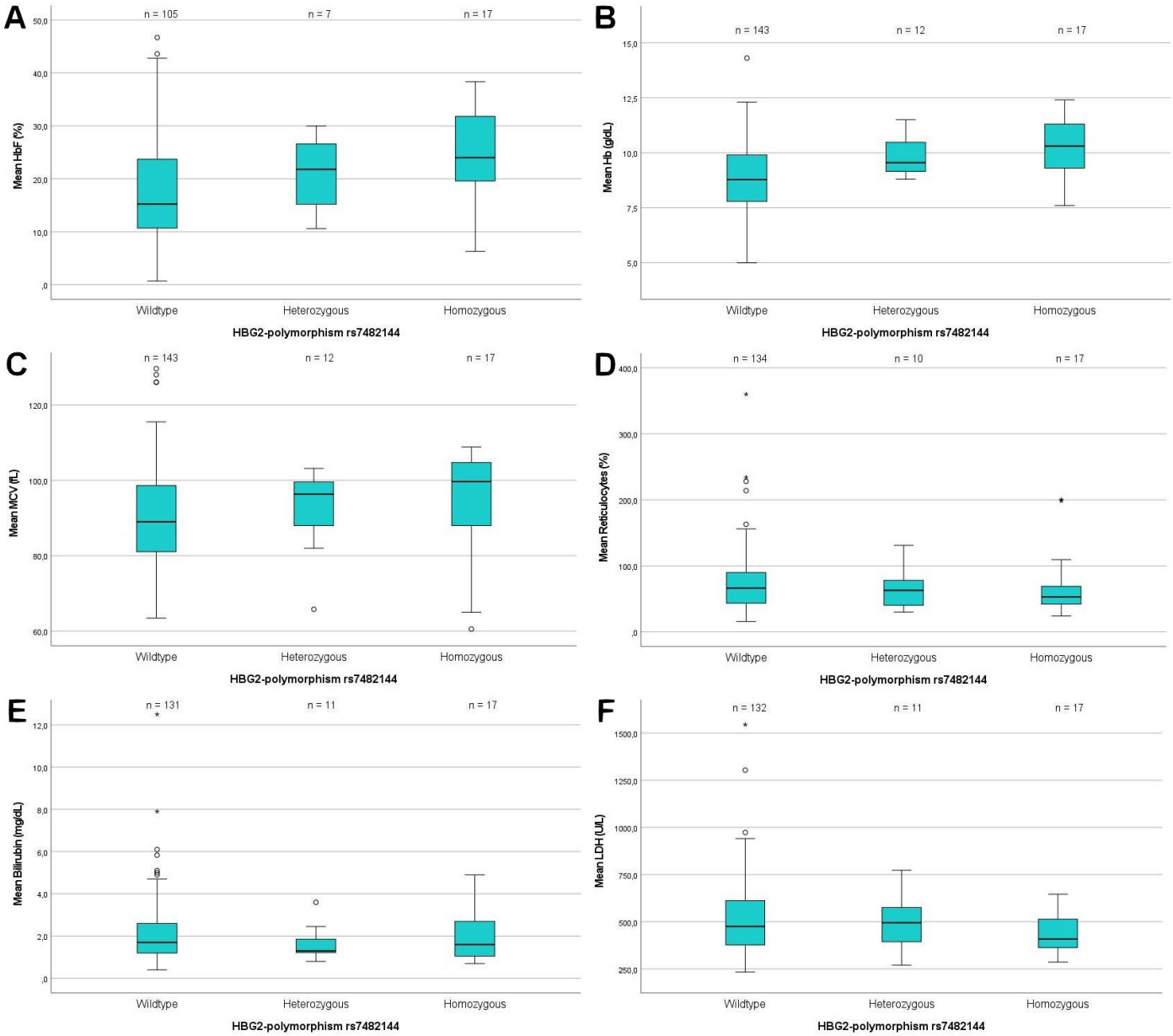
Frequency of complications in patients with *BCL11A* rs7606173 CC compared to *BCL11A* rs7606173 CG and *BCL11A* rs7606173 GG.

n- number of patients available for analysis (A) CC patients (n=35) mean frequency of hospitalizations 0.8 ± 1.4 per year vs CG patients (n=76) mean frequency of hospitalizations 1.1 ± 1.9 per year vs GG patients (n=51) mean frequency of hospitalizations 1 ± 1.3 per year. (B) CC patients (n=36) mean frequency of red blood cells transfusions 0.6 ± 1.8 per year vs CG patients (n=76) mean frequency of red blood cells transfusions 0.3 ± 1.2 per year vs GG patients (n=52) mean frequency of red blood cells transfusions 0.3 ± 0.6 per year. (C) CC patients (n=36) mean frequency of pain crises 0.7 ± 1.3 per year vs CG patients (n=76) mean frequency of pain crises 0.9 ± 1.7 per year vs GG patients (n=51) mean frequency of pain crises 0.8 ± 1.1 per year. (D) CC patients (n=35) mean frequency of acute chest syndromes 0.1 ± 0.3 per year vs CG patients (n=77) mean frequency of acute chest syndromes 0.1 ± 0.3 per year vs GG patients (n=52) mean frequency of acute chest syndromes 0.1 ± 0.4 per year.



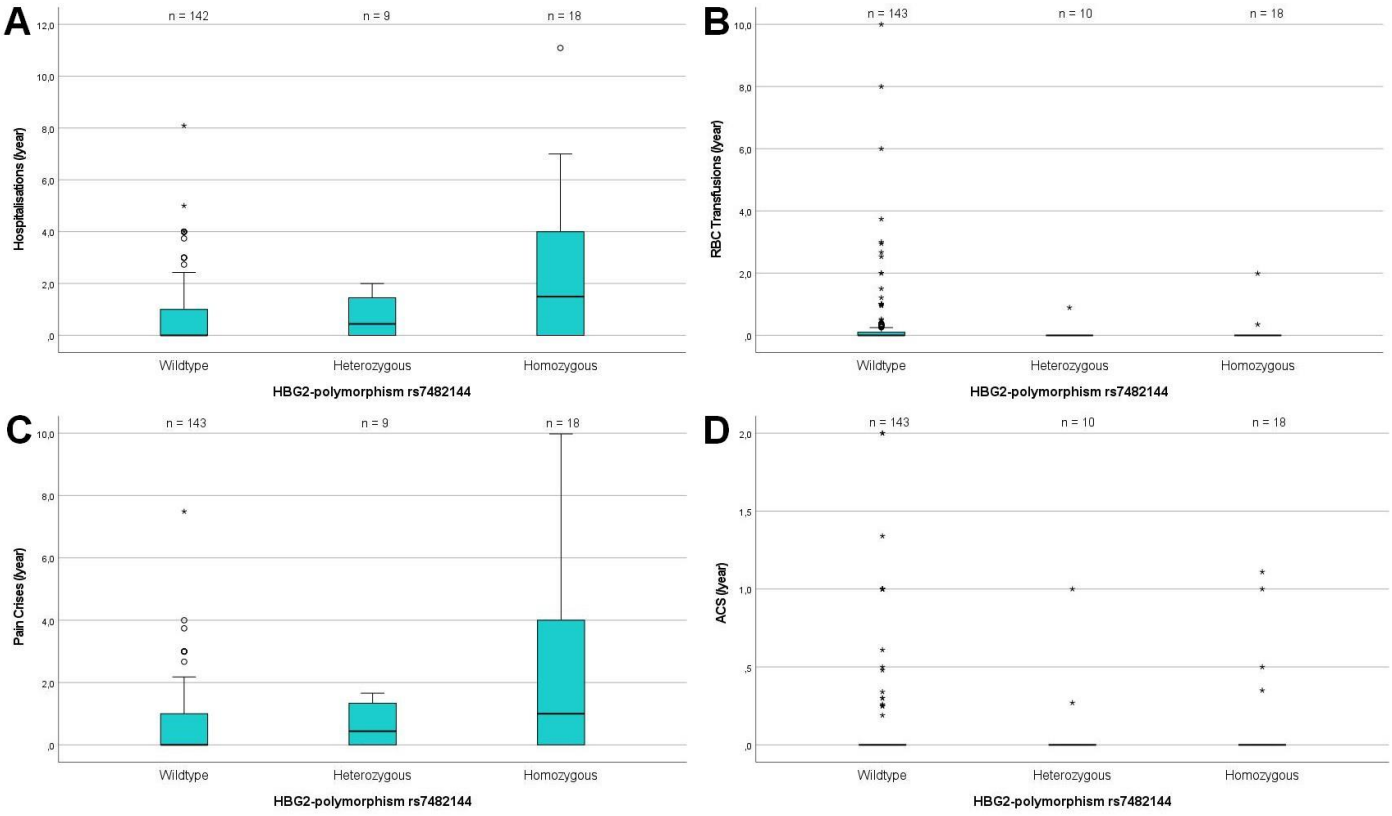
Supplemental Figure 8

Laboratory parameters of patients with *HBG2* rs7482144 wildtype (GG) compared to rs7482144 GA and rs7482144 AA. n- number of patients available for analysis (A) wildtype patients (n=105) mean HbF 17.5 ± 10 % vs heterozygous patients (n=7) mean HbF 20.9 ± 7.5 % vs homozygous patients (n=17) mean HbF 24.7 ± 9 %. (B) wildtype patients (n=143) mean Hb 8.9 ± 1.5 g/dL vs heterozygous patients (n=12) mean Hb 9.8 ± 0.9 g/dL vs homozygous patients (n=17) mean Hb 10.3 ± 1.5 g/dL. (C) wildtype patients (n=143) mean MCV 90.3 ± 13 fL vs heterozygous patients (n=12) mean MCV 92.5 ± 10.6 fL vs homozygous patients (n=17) mean MCV 94.1 ± 14.2 fL. (D) wildtype patients (n=134) mean Reticulocytes 75.6 ± 48.1 **‰** vs heterozygous patients (n=10) mean Reticulocytes 63.5 ± 30.2 **‰** vs homozygous patients (n=17) mean Reticulocytes 70.4 ± 52.4 **‰**. (E) wildtype patients (n=131) mean bilirubin 2.1 ± 1.6 mg/dL vs heterozygous patients (n=11) mean bilirubin 1.7 ± 0.8 mg/dL vs homozygous patients (n=17) mean bilirubin 2 ± 1.3 mg/dL. (F) wildtype patients (n=132) mean LDH 516.5 ± 194.2 U/L vs heterozygous patients (n=11) mean LDH 490.7 ± 145.9 U/L vs homozygous patients (n=17) mean LDH 426.3 ± 95.6 U/L.



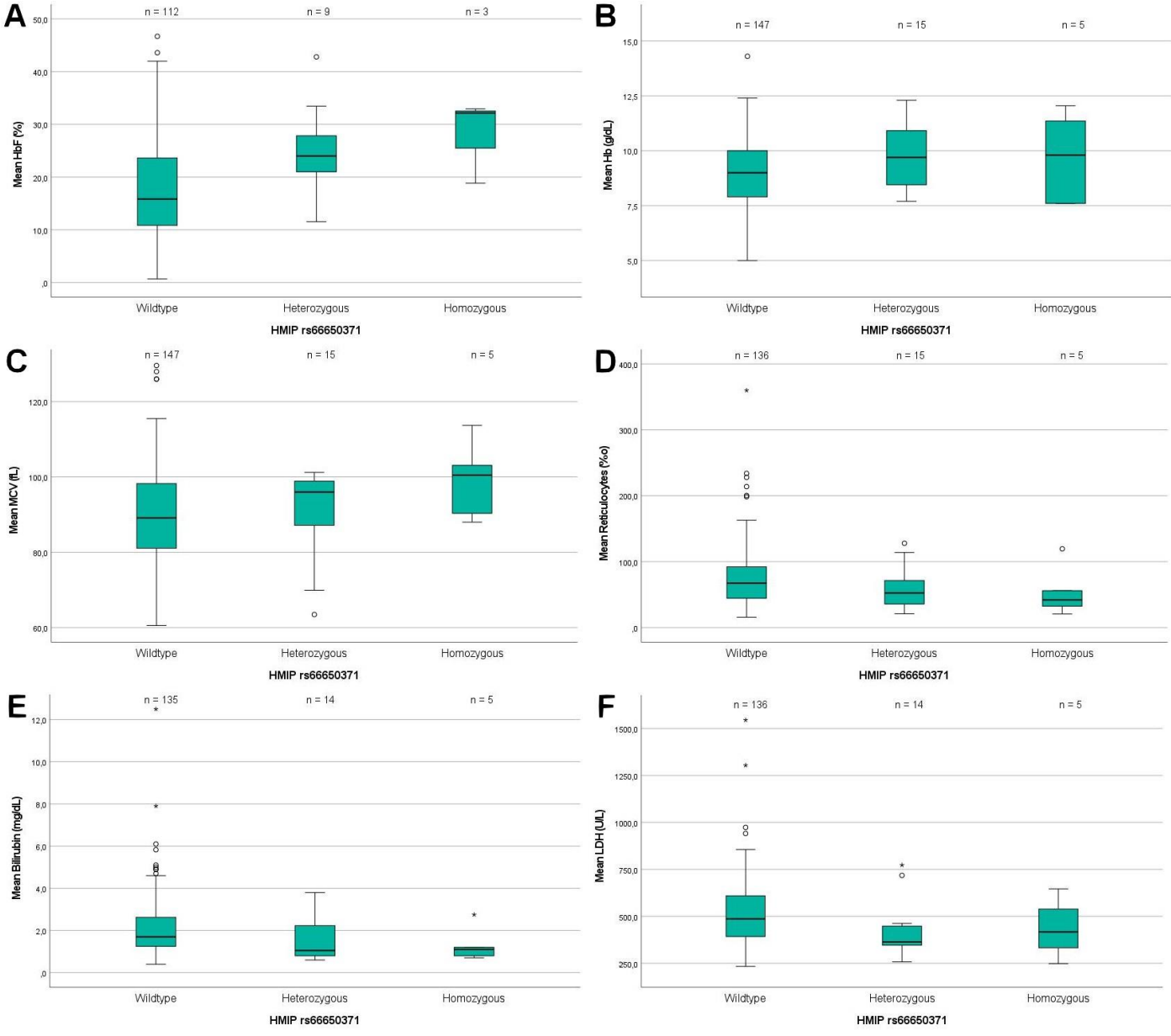
Supplemental Figure 9

Frequency of complications in patients with *HBG2* rs7482144 wildtype (GG) compared to rs7482144 GA and rs7482144 AA. n- number of patients available for analysis (A) wildtype patients (n=142) mean frequency of hospitalizations 0.8 ± 1.3 per year vs heterozygous patients (n=9) mean frequency of hospitalizations 0.8 ± 0.8 per year vs homozygous patients (n=18) mean frequency of hospitalizations 2.4 ± 3 per year. (B) wildtype patients (n=143) mean frequency of red blood cells transfusions 0.4 ± 1.3 per year vs heterozygous patients (n=10) mean frequency of red blood cells transfusions 0.1 ± 0.3 per year vs homozygous patients (n=18) mean frequency of red blood cells transfusions 0.1 ± 0.5 per year. (C) wildtype patients (n=143) mean frequency of pain crises 0.6 ± 1.1 per year vs heterozygous patients (n=9) mean frequency of pain crises 0.7 ± 0.7 per year vs homozygous patients (n=18) mean frequency of pain crises 2.2 ± 2.9 per year. (D) wildtype patients (n=143) mean frequency of acute chest syndromes 0.1 ± 0.3 per year vs heterozygous patients (n=10) mean frequency of acute chest syndromes 0.1 ± 0.3 per year vs homozygous patients (n=18) mean frequency of acute chest syndromes 0.2 ± 0.4 per year.



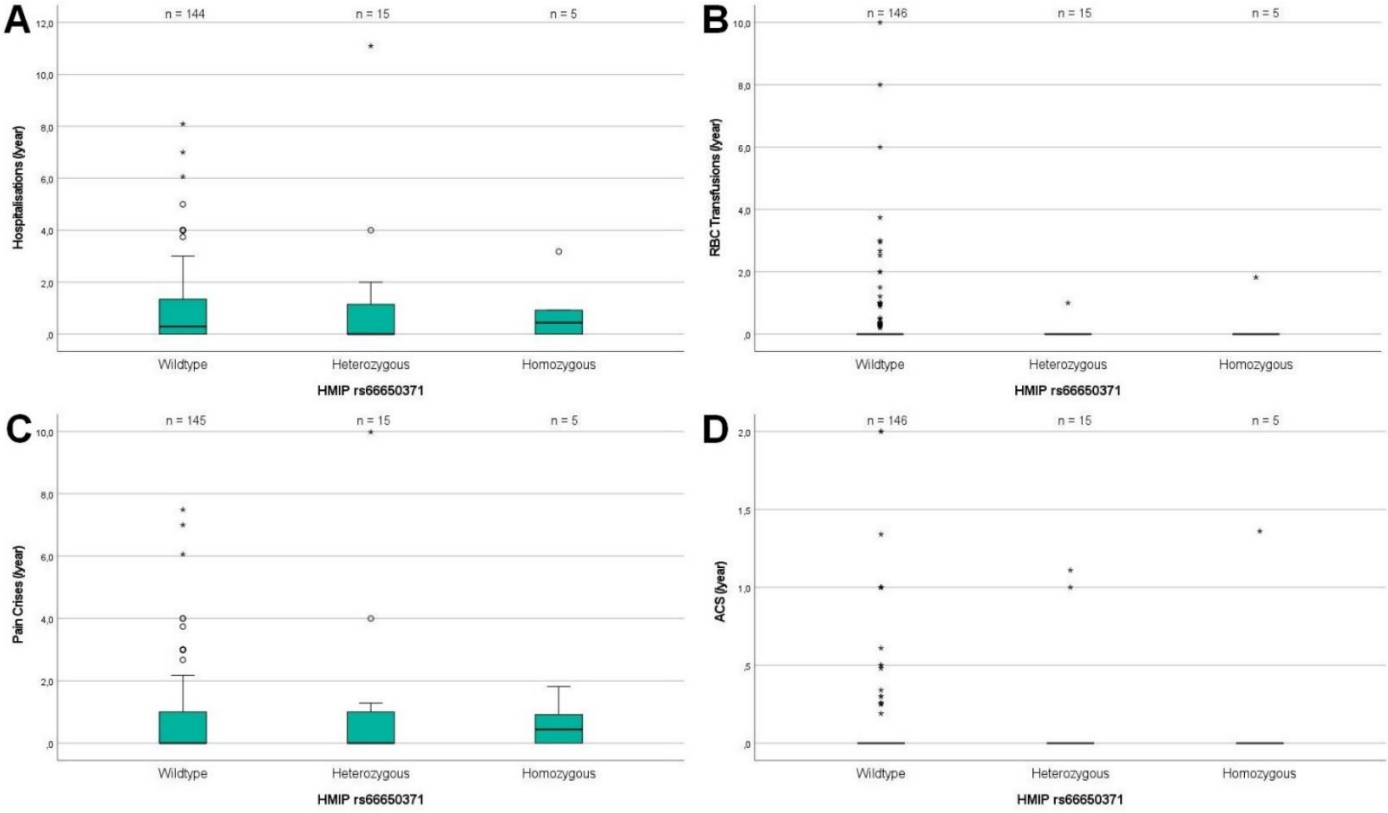
Supplemental Figure 10

Laboratory parameters of patients with *HMIP* rs66650371 wildtype compared to heterozygous *HMIP* rs66650371 delCTA and homozygous *HMIP* rs66650371 delCTA. n- number of patients available for analysis (A) wildtype patients (n=112) mean HbF 17.5 ± 9.6 % vs heterozygous patients (n=9) mean HbF 24.8 ± 9.4 % vs homozygous patients (n=3) mean HbF 28 ± 7.9 %. (B) wildtype patients (n=147) mean Hb 9 ± 1.5 g/dL vs heterozygous patients (n=15) mean Hb 9.7 ± 1.4 g/dL vs homozygous patients (n=5) mean Hb 9.7 ± 2.1 g/dL. (C) wildtype patients (n=147) mean MCV 90 ± 13.1 fL vs heterozygous patients (n=15) mean MCV 90.8 ± 11.6 fL vs homozygous patients (n=5) mean MCV 99.1 ± 10.4 fL. (D) wildtype patients (n=136) mean reticulocytes 78.2 ± 49.5 **‰** vs heterozygous patients (n=15) mean reticulocytes 57 ± 32 **‰** vs homozygous patients (n=5) mean reticulocytes 54.1 ± 38.8 **‰**. (E) wildtype patients (n=135) mean bilirubin 2.2 ± 1.5 mg/dL vs heterozygous patients (n=14) mean bilirubin 1.5 ± 1 mg/dL vs homozygous patients (n=5) mean bilirubin 1.3 ± 0.8 mg/dL. (F) wildtype patients (n=136) mean LDH 519.2 ± 188.9 U/L vs heterozygous patients (n=14) mean LDH 418.6 ± 151.4 U/L vs homozygous patients (n=5) mean LDH 436.5 ± 159 U/L



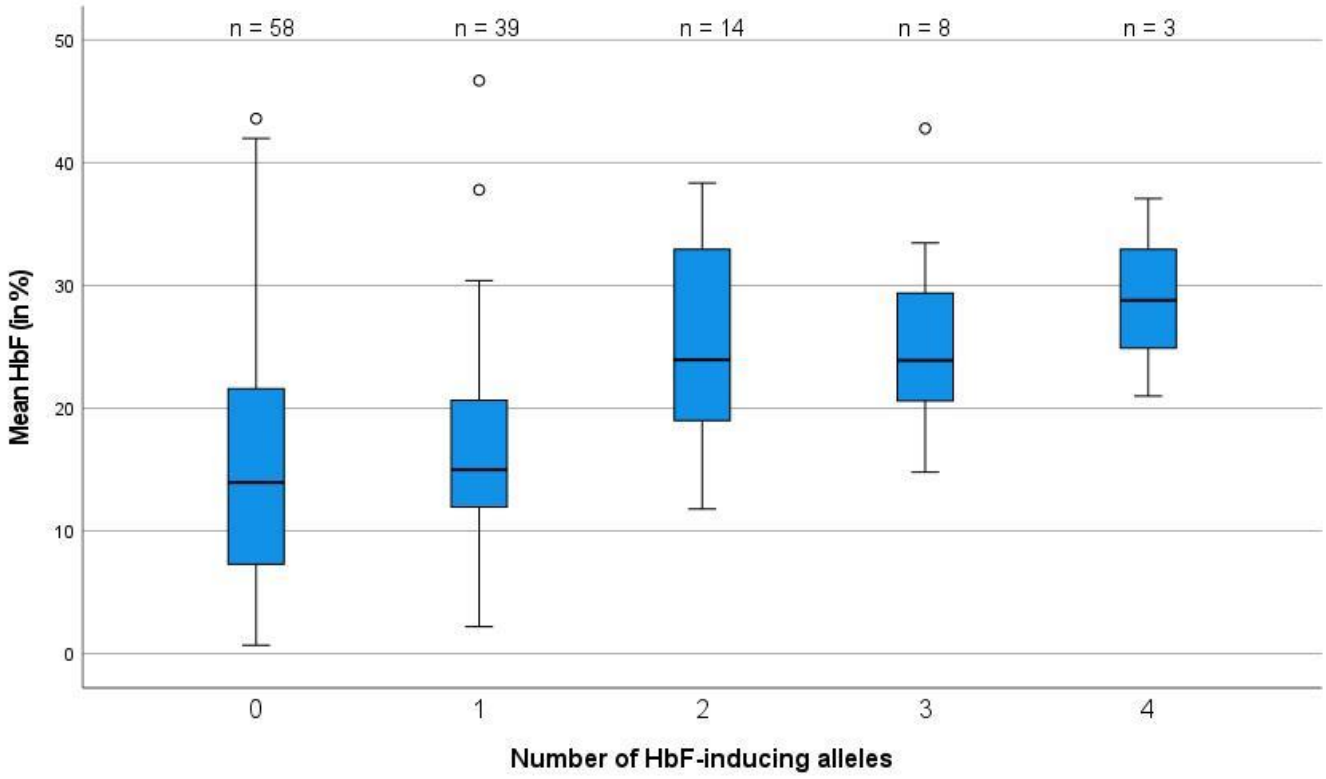
Supplemental Figure 11

Frequency of complications in patients with *HMIP* rs66650371 wildtype compared to heterozygous *HMIP* rs66650371 delCTA and homozygous *HMIP* rs66650371 delCTA. n- number of patients available for analysis (A) wildtype patients (n=144) mean frequency of hospitalizations 1 ± 1.5 per year vs heterozygous patients (n=15) mean frequency of hospitalizations 1.3 ± 2.9 per year vs homozygous patients (n=5) mean frequency of hospitalizations 0.9 ± 1.3 per year. (B) wildtype patients (n=146) mean frequency of red blood cells transfusions 0.4 ± 1.3 per year vs heterozygous patients (n=15) mean frequency of red blood cells transfusions 0.1 ± 0.3 per year vs homozygous patients (n=5) mean frequency of red blood cells transfusions 0.4 ± 0.8 per year. (C) wildtype patients (n=145) mean frequency of pain crises 0.8 ± 1.3 per year vs heterozygous patients (n=15) mean frequency of pain crises 1.2 ± 2.7 per year vs homozygous patients (n=5) mean frequency of pain crises 0.6 ± 0.8 per year. (D) wildtype patients (n=146) mean frequency of acute chest syndromes 0.1 ± 0.3 per year vs heterozygous patients (n=15) mean frequency of acute chest syndromes 0.1 ± 0.4 per year vs homozygous patients (n=5) mean frequency of acute chest syndromes 0.3 ± 0.6 per year.



Supplemental Figure 12

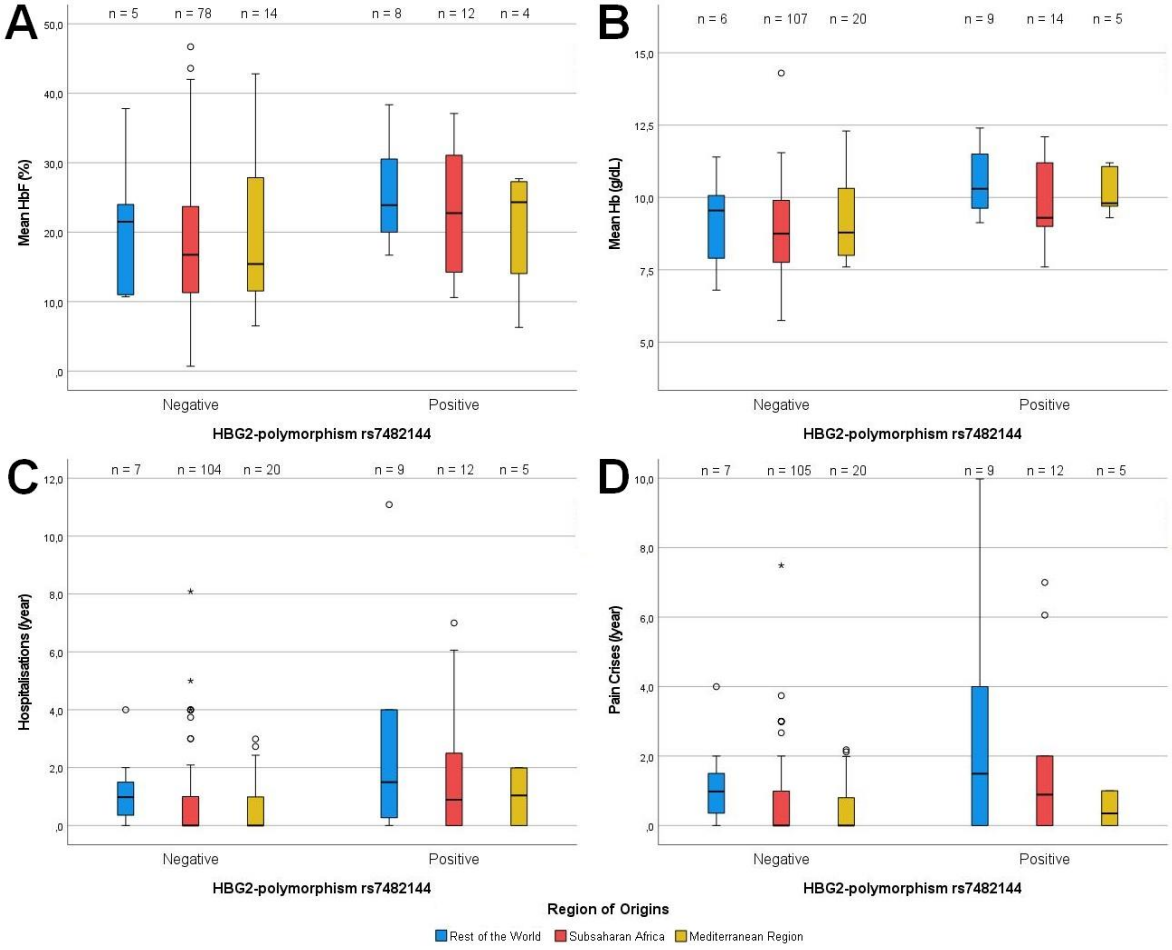
Fetal hemoglobin while on hydroxyurea by number of HbF-inducing alleles. n- number of patients available for analysis; HbF-inducing alleles BCL11A rs1427407 T, HMIP rs66650371 delCTA and HBG2 rs7482144 A



Supplemental Figure 13

Mean values of laboratory parameters and complications of γ-globin promoter polymorphism rs7482144-negative patients compared to the γ-globin promoter polymorphism rs7482144-positive patients by region of origins. (A) Negative patients from Rest of the World (n=5): mean HbF 21 ± SD 11.2 %, negative patients from Subsaharan Africa (n= 78): mean HbF 17.7 ± SD 9.8 %, negative patients from Middle Sea (n=14): mean HbF 19.6 ± SD 11.1 %, ANOVA p=0.654 vs positive patients from Rest of the World (n=8): mean HbF 25.5 ± SD 7.2 %, positive patients from Subsaharan Africa (n= 12): mean HbF 23.3 ± SD 9.4 %, positive patients from Middle Sea (n=4): mean HbF 20.7 ± SD 9.9 %, ANOVA p=0.669. (B) Negative patients from Rest of the World (n=6): mean Hb 9.2 ± SD 1.7 g/dL, negative patients from Subsaharan Africa (p=107): mean Hb 8.9 ± SD 1.4 g/dL, negative patients from Middle Sea (n=20): mean Hb 9.3 ± SD 1.5 g/dL, ANOVA p=0.425 vs positive patients from Rest of the World (n=9): mean Hb 10.6 ± SD 1.2 g/dL, positive patients from Subsaharan Africa (p=14): mean Hb 9.8 ± SD 1.4 g/dL, positive patients from Middle Sea (n=5): mean Hb 10.2 ± SD 0.9 g/dL, ANOVA p=0.439 (C) Negative patients from Rest of the World (n=7): mean frequency of hospitalizations per year 1.2 ± SD 1.4, negative patients from Subsaharan Africa (n=104): mean frequency of hospitalizations per year 0.7 ± SD 1.3, negative patients from Middle Sea (n=20): mean frequency of hospitalizations per year 0.7 ± SD 1, ANOVA p=0.558 vs positive patients from Rest of the World (n=9): mean frequency of hospitalizations per year 2.7 ± SD 3.5, positive patients from Subsaharan Africa (n=12): mean frequency of hospitalizations per year 1.8 ± SD 2.4, positive patients from Middle Sea (n=5): mean frequency of hospitalizations per year 1.0 ±

SD 1.0, ANOVA p=0.533. (D) Negative patients from Rest of the World (n=7): mean frequency of pain crises per year 1.2 ± SD 1.4, negative patients from Subsaharan Africa (n=105): mean frequency of pain crises per year 0.6 ± SD 1.1, negative patients from Middle Sea (n=20): mean frequency of pain crises per year 0.5 ± SD 0.8, ANOVA p=0.238 vs positive patients from Rest of the World (n=9): mean frequency of pain crises per year 2.5 ± SD 3.2, positive patients from Subsaharan Africa (n=12): mean frequency of pain crises per year 1.7 ± SD 2.4, positive patients from Middle Sea (n=5): mean frequency of pain crises per year 0.5 ± SD 0.5, ANOVA p=0.384.
Hb – total hemoglobin; HbF – fetal hemoglobin; SD – standard deviation



Supplemental Table 1

Distribution of the polymorphisms and minor allele frequency (MAF) by regions of origin

Polymorphism		**$n_{Sub\text{-}Saharan\ Africa}$**	**$\%_{Sub\text{-}Saharan\ Africa}$**	**n_{RoW*}**	**$\%_{RoW*}$**	**$n_{Mediterranean\ Sea}$**	**$\%_{Mediterranean\text{-}Sea}$**	**n_{All**}**	**$\%_{All**}$**
α-Globin-Deletions	0	150	57.7	17	77.3	51	91.1	243	65.2
	1	89	34.2	5	22.7	4	7.1	107	28.7
	2	21	8.1	0	0	1	1.8	23	6.2
	all	260	100	22	100	56	100	373	100
BCL11A rs1427407	GG	102	61.1	4	26.7	16	55.2	135	56.7
	GT	56	33.5	11	73.3	10	34.5	88	37
	TT	9	5.4	0	0	3	10.3	15	6.3
	all	167	100	15	100	29	100	238	100
	MAF***	0.22		0.37		0.28		0.25	
BCL11A rs7606173	CC	36	21.6	0	0	7	24.1	47	19.8
	CG	84	50.3	9	60	11	37.9	117	49.2
	GG	47	28.1	6	40	11	37.9	74	31.1
	all	167	100	15	100	29	100	238	100
	MAF***	0.53		0.7		0.57		0.56	
γ-Globin *XmnI* rs7482144	Wildtype	149	88.2	7	41.2	27	84.4	207	84.5
	Heterozygous	7	4.1	4	23.5	2	6.2	14	5.7
	Homozygous	13	7.7	6	35.3	3	9.4	24	9.8
	all	169	100	17	100	32	100	245	100
	MAF***	0.1		0.47		0.13		0.13	
HMIP rs66650371 3bp del	Wildtype	158	94	12	80	16	53.3	211	87.9
	Heterozygous	8	4.8	3	20	11	36.7	23	9.6
	Homozygous	2	1.2	0	0	3	10	6	2.5
	all	168	100	15	100	30	100	240	100
	MAF***	0.04		0.1		0.28		0.07	

* RoW: rest of the world (Iraq n=13, all others n≤2); ** includes patients with unknown region of origin; *** Minor allele frequency

Supplemental Table 2

Patient characteristics by region of origin. A Welch-ANOVA test pointed at significant differences in the mean values of fetal hemoglobin, of total hemoglobin and of MCV in patients from different regions of origin. The Games-Howell post-hoc analysis that corrects for multiple testing confirmed a significant difference for fetal hemoglobin and MCV, but did not reach significance for total hemoglobin (p=0.057).

		Sub-Saharan Africa	Mediterranean Region	Rest of the World*
Sex (female/male)		145 / 140	33 / 29	12 / 13
Age at last observation under treatment (years)	**Mean +/-SD**	11.8 ± 8.1	13.9 ± 9.1	14.3 ± 10.9
	10. percentile	4.3	5.5	4.8
	Median	10.5	11.8	11.9
	90. percentile	19.5	21.5	24
	n	226	52	23
	Welch-ANOVA	1.639		
	p	0.205		
Daily HU dose (mg/kg)	**Mean +/-SD**	23.4 ± 5.5	22.9 ± 6.7	20.4 ± 6.5
	10. percentile	16.5	13.9	11.7
	Median	22.9	21.6	20.9
	90. percentile	31	32.1	27.8
	n	217	50	21
	Welch-ANOVA	2.011		
	p	0.146		
HU therapy duration (months)**	**Mean +/-SD**	35.9 ± 29	44.9 ± 42.8	28.1 ± 22.2
	10. percentile	6.4	10.1	11
	Median	28	32	23
	90. percentile	80.2	97.5	52.5
	n	209	47	21
	Welch-ANOVA	2.263		
	p	0.115		
Fetal hemoglobin in patients on HU (%)	**Mean +/-SD**	17.7 ± 9.5	19.4 ± 8.9	23.8 ± 7.9
	10. percentile	5.5	7.2	13.9
	Median	16.2	20.7	23.5
	90. percentile	30.3	27.9	34.8
	n	144	31	16
	Welch-ANOVA	**4.227**		
	p	**0.023**		
Hemoglobin in patients on HU (g/dL)	**Mean +/-SD**	8.9 ± 1.4	9.5 ± 1.5	9.4 ± 1.8
	10. percentile	7.1	7.7	7.1
	Median	8.9	9.5	9.5
	90. percentile	10.8	11.3	11.6
	n	207	46	20
	Welch-ANOVA	**3.257**		
	p	**0.049**		
MCV in patients on HU (fL)	**Mean +/-SD**	90.9 ± 13.9	97.9 ± 12.3	95.4 ± 9.6
	10. percentile	73.9	84.5	84.4
	Median	89.1	98.4	96.6

	90. percentile	108.4	112.5	103.6
	n	207	46	20
	Welch-ANOVA	**6.478**		
	p	**0.003**		
Reticulocytes in patients on HU (‰)	**Mean +/-SD**	77.1 ± 44.6	64.7 ± 38.7	64.6 ± 54.9
	10. percentile	31.7	25.7	26
	Median	68.2	60	48.1
	90. percentile	127.7	110.7	140.5
	n	194	43	16
	Welch-ANOVA	1.920		
	p	0.162		
Bilirubin in patients on HU (mg/dL)	**Mean +/-SD**	1.9 ± 1	1.9 ± 1.4	3.2 ± 2.6
	10. percentile	0.8	0.7	1.2
	Median	1.6	1.5	2.7
	90. percentile	3.2	3.7	5
	n	193	43	19
	Welch-ANOVA	2.528		
	p	0.094		
LDH in patients on HU (U/L)	**Mean +/-SD**	517.9 ± 172.1	470.5 ± 220.2	498.2 ± 205.5
	10. percentile	318.8	284	326.7
	Median	498	434	421
	90. percentile	731.2	598.8	740.3
	n	193	43	18
	Welch-ANOVA	0.9		
	p	0.415		
Hospitalizations /year* **	**Mean +/-SD**	0.8 ± 1.3	0.7 ± 1.1	2 ± 2.5
	10. percentile	0	0	0
	Median	0	0	1.2
	90. percentile	2	2.6	4
	n	204	45	22
	Welch-ANOVA	2.593		
	p	0.086		
Red blood cell transfusions /year* **	**Mean +/-SD**	0.3 ± 0.9	0.4 ± 0.8	1.1 ± 2.2
	10. percentile	0	0	0
	Median	0	0	0
	90. percentile	1	1.9	2.9
	n	205	44	23
	Welch-ANOVA	1.799		
	p	0.177		
Pain crises /year* **	**Mean +/-SD**	0.6 ± 1.1	0.5 ± 0.8	1.8 ± 2.3
	10. percentile	0	0	0
	Median	0	0	1
	90. percentile	2	2	4
	n	205	45	22
	Welch-ANOVA	2.952		
	p	0.062		
ACS /year* **	**Mean +/-SD**	0.1 ± 0.4	0.2 ± 0.4	0.2 ± 0.4
	10. percentile	0	0	0
	Median	0	0	0

	90. percentile	0.3	0.8	0.9
	n	205	45	23
	Welch-ANOVA	0.713		
	p	0.495		

SD- standard deviation, n- number of patients available for analysis, HU- Hydroxyurea; ACS- acute chest syndrome

* Iraq n=13, all others n≤2

** The duration of HU therapy was calculated from the first dose until laboratory controls. Data points are missing if no laboratory control or weight or date of the first dose was documented.

*** Clinical events were only evaluated in patients with > 6 months of follow up while on hydroxyurea, age >2 years.

Supplemental Table 3

Patient characteristics of patients with severe SCD while on hydroxyurea (HU) compared to patients with non-severe SCD while on hydroxyurea. n- number of patients available for analysis; SD- standard deviation; t-score of unpaired t-Test or Welch-Test; p- t-Test or Welch-Test; 95% Confidence Intervals

		n[1]	**Mean**	**SD**	**Mean difference**	**t-Score**	**p**	**95%-CI**
Age (years)	Non-severe	131	11.3	6.9	**-2.8**	**-2.044**[2]	**0.043**	**[-5.5, -0.1]**
	Severe	37	14	8.6				
Number of HbF-inducing alleles[4]	Non-severe	74	0.7	0.9	**-0.7**	**-2.317**[3]	**0.028**	**[-1.3, -0.1]**
	Severe	23	1.4	1.3				
HU Dose (mg/kg)	Non-severe	131	23	5	-0.6	-0.589[2]	0.557	[-2.5, 1.3]
	Severe	37	23.5	5.9				
HU Duration (months)	Non-severe	131	34.8	26.6	-8.7	-1.184[3]	0.243	[-23.6, 6.1]
	Severe	37	43.5	42.6				
HbF (%)	Non-severe	131	17.9	9.3	1.2	0.701[2]	0.484	[-2.2, 4-6]
	Severe	37	16.7	8.7				
Hemoglobin (g/dL)	Non-severe	131	8.9	1.3	-0.1	0.275[3]	0.785	[-0.6, 0.4]
	Severe	37	9	1.8				
MCV (fL)	Non-severe	131	91.4	12.7	0.7	0.300[2]	0.765	[-3.8, 5.2]
	Severe	37	90.7	10.7				
Reticulocytes (‰)	Non-severe	131	71.7	37.8	-12.6	-1.739[2]	0.084	[-26.9, 1.7]
	Severe	37	84.3	43				
Bilirubin (mg/dL)	Non-severe	131	2	1.1	-12.6	-1.603[3]	0.116	[-1.1, 0.1]
	Severe	37	2.5	1.7				
LDH (U/L)	Non-severe	131	503.3	150	-11.5	-0.282[3]	0.779	[-93.9, 70.9]
	Severe	37	514.8	235.				

				6				
Hospitalizations (/year)	Non-severe	131	0.3	0.5	**-2.3**	**-8.022**[3]	**<0.001**	**[-2.6, -2]**
	Severe	37	2.6	1.7				
Transfusions (/year)	Non-severe	131	0.2	0.5	**-0.4**	**-2.796**[3]	**0.007**	**[-0.6, -0.1]**
	Severe	37	0.6	0.7				
Pain crises (/year)	Non-severe	131	0.3	0.4	**-2**	**-6.709**[3]	**<0.001**	**[-2.6, -1.4]**
	Severe	37	2.2	1.8				
ACS (/year)	Non-severe	131	0	0.1	**-0.3**	**-4.191**[3]	**<0.001**	**[-0.4, -0.1]**
	Severe	37	0.3	0.4				

[1]only patients with complete data set were considered; [2]Unpaired t-Test; [3]Welch-Test;
[4] *BCL11A* rs1427407 T, *HMIP* rs66650371 delCTA, *HBG2* rs7482144 A

Supplemental Table 4

Mean values of fetal hemoglobin, daily hydroxyurea (HU) dose and duration of HU-therapy from the *BCL11A* rs1427407 T -negative population compared to the BCL11A rs1427407 T -positive population. n- number of patients available for analysis; SD- standard deviation; t-score of unpaired t-Test; p- t-Test; 95% Confidence Intervals

	BCL11A rs1427407 T	**n***	**Mean**	**SD**	**Mean difference**	**t-score****	**p****	**95%-CI**
HbF (%)	Negative	67	16.6	9.8	**-3.8**	**-2.116**	**0.037**	**[-7.3, -0.2]**
	Positive	49	20.4	9				
HU dose (mg/kg)	Negative	67	23.5	5.1	**1.9**	**2.053**	**0.042**	**[1.9, 3.7]**
	Positive	49	21.6	4.7				
HU duration (months)	Negative	67	29.1	21.3	3.4	0.888	0.377	[-4.2, 11.1]
	Positive	49	25.6	19.5				

*only patients with complete data set were considered; **Unpaired t-test

Supplemental Table 5

Mean values of fetal hemoglobin, daily Hydroxyurea (HU) dose and duration of HU-therapy from the *BCL11A* rs7606173 -negative population compared to the BCL11A rs7606173 -positive population. n- number of patients available for analysis; SD- standard deviation; t-score of unpaired t-Test; p- t-Test; 95% Confidence Intervals

	BCL11A rs7606173 G	**n***	**Mean**	**SD**	**Mean difference**	**t-score****	**p****	**95%-CI**
HbF (%)	Negative	22	17	10.4	-1.5	-0.648	0.518	[-6, 3]
	Positive	94	18.5	9.4				
HU Dose (mg/kg)	Negative	22	23.8	4.2	1.4	1.145	0.255	[-1, 3.7]
	Positive	94	22.5	5.2				
HU duration (months)	Negative	22	27.8	18.1	0.3	0.055	0.956	[-9.4, 9.9]
	Positive	94	27.6	21.1				

*only patients with complete data set were considered; **Unpaired t-test

Supplemental Table 6

Mean values of fetal hemoglobin, daily Hydroxyurea (HU) dose and duration of HU-therapy from the *HMIP* rs66650371 delCTA -negative population compared to the HMIP rs66650371 delCTA -positive population. n- number of patients available for analysis; SD- standard deviation; t-score of unpaired t-Test or Welch-Test; p- t-Test or Welch-Test; 95% Confidence Intervals

	HMIP rs66650371 delCTA	**n***	**Mean**	**SD**	**Mean difference**	**t-score**	**p**	**95%-CI**
HbF (%)	Negative	107	17.8	9.6	**-6.2**	**-2.085****	**0.039****	**[-12.2, -0.3]**
	Positive	11	24	7.3				
HU Dose (mg/kg)	Negative	107	22.9	5.1	**3**	**3.439*****	**0.002*****	**[1.2, 4.8]**
	Positive	11	19.9	2.4				
HU duration (months)	Negative	107	26.8	18.9	-23.2	-1,373***	0.199***	[-60.8, 14.4]
	Positive	11	50.1	55.8				

*only patients with complete data set were considered; **Unpaired t-Test; ***Welch-Test

Supplemental Table 7

Mean values of fetal hemoglobin, daily Hydroxyurea (HU) dose and duration of HU-therapy from the *HBG2*-polymorphism rs7482144 A -negative population compared to the *HBG2*-polymorphism rs7482144 A -positive population. n- number of patients available for analysis; SD- standard deviation; t-score of unpaired t-Test; p- t-Test; 95% Confidence Intervals

	HBG2 rs7482144 A	**n***	**Mean**	**SD**	**Mean difference**	**t-score****	**p****	**95%-CI**
HbF (%)	Negative	98	17.4	9.6	**-6.3**	**-2.0927**	**0.004**	**[-10.5, -2]**
	Positive	24	23.6	8.6				
HU dose (mg/kg)	Negative	98	22.9	5	0.3	0.260	0.795	[-2, 2.6]
	Positive	24	22.6	5.5				
HU duration (months)	Negative	98	28.1	19.3	0.5	0.118	0.906	[-8.6, 9.7]
	Positive	24	27.5	24				

*only patients with complete data set were considered; **Unpaired t-Test

Supplemental Table 8

Poisson Regression between HbF and frequency of hospitalization, within the γ-globin *XmnI* rs7482144 -negative population. Exp-transformed coefficient of Poisson-regression. p- Poisson regression; n- number of patients available for analysis.

	Frequency of hospitalization (per year)
exp(coef)	0.943
95%-CI	0.923 – 0.963
p	$<10^{-4}$
n	97

Supplemental Table 9

Poisson Regression between total hemoglobin and frequency of hospitalization, within the γ-globin *XmnI* rs7482144 -negative population. Exp-transformed coefficient of Poisson-regression. p- Poisson regression; n- number of patients available for analysis.

	Frequency of hospitalization (per year)
exp(coef)	0.870
95%-CI	0.783 – 0.968
p	0.01
n	130

Supplemental Table 10

Poisson Regression between HbF and frequency of hospitalization, within the γ-globin *XmnI* rs7482144 -positive population. Exp-transformed coefficient of Poisson-regression. p- Poisson regression; n- number of patients available for analysis.

	Frequency of hospitalization (per year)
exp(coef)	0.990
95%-CI	0.961 – 1.021
p	0.5217
n	21

Supplemental Table 11

Poisson Regression between total hemoglobin and frequency of hospitalization, within the γ-globin *XmnI* rs7482144 -positive population. Exp-transformed coefficient of Poisson-regression. p- Poisson regression; n- number of patients available for analysis.

	Frequency of hospitalization (per year)
exp(coef)	0.963
95%-CI	0.782 – 1.188
p	0.7259
n	26