Table SI. List of amino-linked oligonucleotide probes for PCR-reverse dot blot hybridization with common and rare β-thalassemia mutations (25).

A, Common β-thalassemia mutations			
Name	Mutations	Normal ASO (5' to 3')	Mutant ASO (5' to 3')
R01	Codons 41/42 (-TCTT)	CAGAGGTTCTTTGAGTCCTT	CAAAGGACTCA/ACCTCTGG
R02	IVS I-1 (G>T)	ATACCAACCTGCCCAG	CTGGGCAGTTTGGTAT
R03	IVS I-5 (G>C)	CCTTGATACCAACCTGC	GCAGGTTGCTATCAAG
R05	Codon 19 (A>G)	GTGGGGCAAGGTGAAC	TTCATCCACGCTCACCTT
R06	Codon 26 (G>A), Hb E	CAGGGCCTCACCACCA	TTGGTGGTAAGGCCCT
R09	Codon 17 (A>T)	GTGGGGCAAGGTGAAC	GTGGGGCTAGGTGAAC
R10	Codons 71/72 (+A)	TCGGTGCCTTT/AGTGAT	GGTGCCTTTAAGTGATG
R11	Codon 35 (C>A)	GGTGGTCTACCCTTGGA	TCCAAGGTTAGACCACC
R12	IVS II-654 (C>T)	GGGTTAAGGCAATAGCAAT	ATTGCTATTACCTTAACCC
R14	-28 (A>G)	GGGCATAAAAGTCAGGG	CCCTGACTTCTATGCCC
B, Rare β-thalassemia mutations			
Name	Mutations	Normal ASO (5' to 3')	Mutant ASO (5' to 3')
R04	Codons 8/9 (+G)	AGGAGAAG/TCTGCCGTT	CGGCAGACCTTCTCCT
R07	Codons 27/28 (+C)	CAGGG/CCTCACCACCA	GGTGAGGCCCCTGG
R08	Codon 15 (G>A)	CCTGTGGGGCAAGGTGA	CCCTGTAGGGCAAGGTGA
R13	Codon 43 (G>T)	CAGAGGTTCTTTGAGTCCTT	CCCAGAGGTTCTTTTAGTC
R15	-29 (A>G)	GGGCATAAAAGTCAGGG	CCCTGACTTTCATGCCC
R16	-30 (T>C)	GGGCATAAAAGTCAGGG	CCTGACTTTTGTGCCC
R17	Codon 41 (-C)	CAGAGGTTCTTTGAGTCCTT	CCAGAGGTT/TTTGAGTCC
R18	Codon 26 (G>T)	CAGGGCCTCACCACCA	AGGGCCTAACCACCAA
R20	Codons 123-124 (-8 bp), Hb Khon Kaen	TGCACTGGTGGGGTGAA	CAGCCTGCACT/GAATTC
R21	IVS I-1 (G>A)	ATACCAACCTGCCCAG	CTGGGCAGATTGGTAT
R22	Codon 30 (G>A)	ATACCAACCTGCCCAG	ATACCAACTTGCCCAGG
R27	Cap site (A>C)	CTATTGCTTACATTTGCTT	AAATGGAAGCAATAGATGG
R28	105 bp deletion	GCATAAAAGTCAGGGCAG	GCATAAAAG/CCGTTACTG
R29	Codon 6 (G>A), Hb C	TGACTCCTGAGGAGAAGT	CTGACTCCTAAGGAGAAG

Table SII. List of primer sequences of PCR-RDB, MARMS-PCR, multiplex-gap PCR, PCR-HRM, and DNA sequencing

Method	Name	Sequence, 5' to 3'	Length, bp	Reference
Multiplex-gap PCR for β-thalassemia	G9	TCCCCAGTTAACCTCCTATT	20	(14)
	N1	CACATATGAGCAAGGTTGTG	20	
	G7	GATACAATGTATCATGCCTC	20	
	G10	AGACTAGCACTGCAGATTCC	20	
PCR-RDB	China 1[a]	GTACGGCTGTCATCACTTAGACCTCA	26	(25)
	China 2[a]	TGCAGCTTGTCACAGTGCAGCTCACT	26	
	China 3[a]	GTGTACACATATTGACCAAA	20	
	China 4[a]	AGCACACAGACCAGCACGTT	20	
MARMS-PCR	1C	ACCTCACCCTGTGGAGCCAC	20	(26)
	CDs 41/42	GAGTGACAGATCCCCAAAGGACTCAACCT	29	
	G	ACCTCACCCTGTGGAGCCAC	20	
	IVS I-5	CTCCTTAAACCTGTCTTGTAACCTTGTTAG	30	
PCR-HRM	Hb E (CD26-F)	CTGACTCCTGAGGAGAAGTC	20	(27)
	Hb E (CD26-R)	GCCCAGTTTCTATTGGTCTC	20	
DNA sequencing	*HBB*_F	TTGAAGTCCAACTCCTAAGC	20	(28)
	*HBB*_R	CAGAATCCAGATGCTCAAG	19	
	105*HBB*_F	CGGCTGTCATCACTTAGACC	20	This study
	105*HBB*_R	GCAGCTTGTCACAGTGCAG	19	
Multiplex-gap PCR for deletional α-thalassemia	α2/3.7-F	CCCCTCGCCAAGTCCACCC	19	(29)
	3.7-R	AAAGCACTCTAGGGTCCAGCG	21	
	α2-R	AGACCAGGAAGGGCCGGTG	19	
	4.2-F	GGTTTACCCATGTGGTGCCTC	21	
	4.2-R	CCCGTTGGATCTTCTCATTTCCC	23	
	SEA-F	CGATCTGGGCTCTGTGTTCTC	21	
	SEA-R	AGCCCACGTTGTGTTCATGGC	21	
	THAI-F	GACCATTCCTCAGCGTGGGTG	21	
	THAI-R	CAAGTGGGCTGAGCCCTTGAG	21	
Allele-specific PCR	αG17 (normal)	AGATGGCGCCTTCCTCTCAGG	21	(30)
	αG2 (Hb CS)	GCTGACCTCCAAATACCGTC	20	
	C3	CCATTGTTGGCACATTCCGG	20	

[a]5'-Biotinylated primers. HRM; high-resolution melting curve analysis, MARMS; multiplex amplification refractory mutation system, RDB; reverse dot blot hybridization.

Table SIII. Clinical and hematological findings of the 135 southern Thai β^0-thalassemia/Hb E patients without α-thalassemia interactions.

Patient characteristics	Disease severity			P-value
	Mild, n=18	Moderate, n=76	Severe, n=41	
Sex, n (%)				
Males	7 (39)	34 (45)	28 (68)	0.0279[a,d]
Females	11 (61)	42 (55)	13 (32)	
Age, years, mean ± SD	36±22.85	19±15.69	19±11.07	0.0050[b,e]
Baseline Hb, g/dl, mean ± SD	8.4±0.86	7.3±0.97	6.5±1.03	<0.0001[c,e]
Age at presentation, years, mean ± SD	22±21.46	6±7.79	2±2.25	<0.0001[c,e]
Age at first transfusion, years, mean ± SD	22±18.38	9±13.39	2±2.38	<0.0001[c,e]
Requirement for regular blood transfusion, n (%)	2 (11)	64 (84)	40 (98)	<0.0001[c,d]
Spleen size, cm, mean ± SD	4±4.38	4±4.43	8±4.54	0.0170[a,e]
Splenectomy, n (%)	2 (11)	17 (22)	28 (68)	<0.0001[c,d]
Growth development: Height, n (%)				
≤P3-10	3 (17)	18 (24)	26 (63)	<0.0001[c,d]
≥P10-25	15 (83)	57 (76)	15 (37)	
Growth development: Weight, n (%)				
≤P3-10	0 (0)	15 (20)	25 (61)	<0.0001[c,d]
≥P10-25	18 (100)	61 (80)	16 (39)	

[a]P<0.05, [b]P<0.01, [c]P<0.001. [d]Pearson's χ^2-test. [e]Independent-Sample Kruskal-Wallis Test. P, percentile.

Table SIV. Association of 4 SNPs in 3 independent regions with disease severity in southern Thai β^0-thalassemia/Hb E patients without α-thalassemia interactions.

SNP info	Genotype[c] / Allele	Disease Severity Status			OR (95%CI)	Risk Genotype/Allele	P value[d]
		Mild, n=18	Moderate, n=76	Severe, n=41			
rs7482144 (C/T), *HBG2*							
Genotype	CC	1 (0.056)	19 (0.250)	15 (0.366)	9.81 (1.18-81.27)	CC	0.011[a]
	CT+TT	17 (0.944)	57 (0.750)	26 (0.634)			
Allele	C	14 (0.389)	89 (0.586)	55 (0.671)	3.20 (1.42-7.22)	C	0.004[b]
	T	22 (0.611)	63 (0.414)	27 (0.329)			
rs2071348 (A/C), *HBBP1*							
Genotype	AA	1 (0.056)	19 (0.250)	12 (0.293)	7.03 (0.84-58.96)	AA	0.039[a]
	AC+CC	17 (0.944)	57 (0.750)	29 (0.707)			
Allele	A	14 (0.389)	89 (0.586)	51 (0.622)	2.59 (1.16-5.78)	A	0.019[a]
	C	22 (0.611)	63 (0.414)	31 (0.378)			
rs766432 (C/A), *BCL11A*							
Genotype	AA	9 (0.500)	56 (0.737)	24 (0.585)	1.41 (0.46-4.30)	AA	0.543
	AC+CC	9 (0.500)	20 (0.263)	17 (0.415)			
Allele	A	27 (0.750)	131 (0.862)	64 (0.780)	1.19 (0.47-2.97)	A	0.718
	C	9 (0.250)	21 (0.138)	18 (0.220)			
rs9376074 (T/C), *HBS1L*							
Genotype	TT	7 (0.389)	33 (0.434)	14 (0.341)	0.81 (0.26-2.56)	TT	0.474
	TC+CC	11 (0.611)	43 (0.566)	27 (0.659)			
Allele	T	22 (0.611)	95 (0.625)	50 (0.610)	0.99 (0.44-2.22)	T	0.578
	C	14 (0.389)	57 (0.375)	32 (0.390)			

[a]P<0.05, [b]P<0.01. [c]The recessive model was used to analyze the case-control association study. [d]The mild group (control) vs. severe group (case) were analyzed. CI; confidence interval, OR; odds ratio; SNP, single nucleotide polymorphism.

Table SV. Association of 4 SNPs in 3 independent regions with the age at onset in 135 southern Thai β^0-thalassemia/Hb E patients without α-thalassemia interactions.

SNP info	Genotype/ allele	Age at onset		P-value[b], OR (95%CI)	Risk Genotype/Allele[a]
		≤2-years-old, n=61	>2-years-old, n=74		
rs7482144 (C/T), *HBG2*					
Genotype	CC	23 (0.377)	12 (0.162)	P=0.004, OR=3.13 (1.40-7.00)	CC
	CT+TT	38 (0.623)	62 (0.838)		
Allele	C	83 (0.680)	75 (0.507)	P=0.004, OR=2.07 (1.26-3.41)	C
	T	39 (0.320)	73 (0.493)		
rs2071348 (A/C), *HBBP1*					
Genotype	AA	20 (0.328)	12 (0.162)	P=0.024, OR=2.52 (1.11-5.70)	AA
	AC+CC	41 (0.672)	62 (0.838)		
Allele	A	78 (0.639)	76 (0.514)	P=0.038, OR=1.68 (1.03-2.74)	A
	C	44 (0.361)	72 (0.486)		
rs766432 (C/A), *BCL11A*					
Genotype	AA	41 (0.672)	48 (0.649)	P=0.777, OR=1.11 (0.54-2.27)	AA
	AC+CC	20 (0.328)	26 (0.351)		
Allele	A	101 (0.828)	121 (0.818)	P=0.823, OR=1.07 (0.57-2.01)	A
	C	21 (0.172)	27 (0.182)		
rs9376074 (T/C), *HBS1L*					
Genotype	TT	29 (0.475)	25 (0.338)	P=0.104, OR=1.78 (0.89-3.56)	TT
	TC+CC	32 (0.525)	49 (0.662)		
Allele	T	81 (0.664)	86 (0.581)	P=0.163, OR=1.42 (0.87-2.34)	T
	C	41 (0.336)	62 (0.419)		

[b]Risk genotypes/alleles were set as reference genotypes/alleles and the recessive model was used to analyze the case-control association study. [b]Age at onset ≤ 2-year-old (case group) vs. age at onset > 2-year-old (control group) were analyzed. CI; confidence interval, OR; odds ratio; SNP, single nucleotide polymorphism.