Clinical and Genetic Heterogeneity in Black Patients With Homozygous β-Thalassemia From the Southeastern United States


The presence of various substitutions and deletions resulting in β-thalassemia was studied in 19 black patients with homozygous β-thalassemia and in numerous relatives; all patients were from Georgia, South Carolina, and Alabama. Methodology included gene mapping, amplification of genomic DNA with Taq polymerase, identification of known nucleotide substitutions or a single nucleotide deletion through hybridization with synthetic oligonucleotides, cloning and sequencing of a β-globin gene, and sequencing of amplified genomic DNA. Of the 38 chromosomes tested, 21 (55%) had the A→G substitution at nt −29, eight (21%) had the C→T substitution at nt −88, three (8%) had the substitution at codon 24, while one each of the following abnormalities were also detected: frameshift at codon 6, a C→T mutation at codon 61 (new), a deletion of 1.35 kilobases including the 5′ end of β, a γ(4γδ)β-thalassemia, and one thalassemia determinant that remained unidentified. The C→A mutation at nt 848 of IVS-II occurred at a position 3 nucleotides 5′ to the third exon, adjacent to the invariant AG dinucleotide of the acceptor sequence. The A→T mutation in codon 61 (AAG→TAG) resulted in the creation of a stop codon and thus in β-thalassemia. The various mutations occurred on chromosomes with different haplotypes; however, chromosomes with a specific mutation but with different haplotypes belonged to one specific framework, which suggested that crossovers were responsible for these different types. Hemoglobin (Hb) F levels were generally high (55% to 75% with 98.5% in one patient with β(β⁺); a few patients with specific haplotypes and an α-thalassemia-2 heterozygosity had a lower Hb F level. The δγ in the Hb F was consistently high when the C→T mutation occurred at nt −158 to the Cap site of the δγ-globin gene; seven patients with +/+ at this site had an average δγ of 73.8%, eight patients with +/− had 64.8%, and one patient with −/− had 34.2%. Variations in hematologic values and in Hb F, δγ, and Hb A₂ levels of relatives with a β-thalassemia heterozygosity depended to some extent on the types of mutations or deletions and on the haplotypes of the chromosomes with the β-thalassemia determinant.

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DNA from one patient (no. 15, R.B., Table I) was digested with \textit{BclI}, and the 10.5 kb fragment was cloned in the vector EMBl-3. The 4.4 kb \textit{PsrI} fragment containing the \(\beta\)-globin gene was subcloned into the plasmid pAT153 (Amersham Corp, Arlington Heights, IL) and sequenced by using the procedure of Sanger et al. Eight synthetic oligonucleotides 19 to 20 nt long and complementary to parts of the \(\beta\)-globin gene were used as sequencing primers. Because the \(\beta\)-thal substitution on one chromosome (\(A\rightarrow G\) at nt –29) was readily determined in genomic DNA by the PCR method listed earlier, the DNA of the one clone obtained was first tested for this substitution by the same procedure with a negative result. The presence of the new mutation (\(C\rightarrow A\) at nt 848 of IVS-II) was confirmed by dot-blot hybridization of amplified DNA with the following probes: the \(\beta\)-IVS-II-848 mutant TTCTCCCA and the normal \(\beta\)-IVS-II-849, TTCTCCCA AGCTCTGGG and the normal \(\beta\)-IVS-II-849, TTCTCCCA AGCTCTGGG.

**Fig 1.** Schematic drawing of the \(\beta\)-globin gene with three exons, two introns, and the Cap and poly A sites. The locations of the two sets of primers are shown at the top, and the compositions of these four synthetic oligonucleotides are given at the bottom. The 12 nucleotide substitutions causing \(\beta\)-thal that have been observed thus far in blacks are also given; the new substitution T –> C at the donor splice site of IVS-1 will be described separately.

**Table 1.** Hematologic and Hb Composition Data and Mutations, Haplotypes, and Number of \(\alpha\)-Globin Genes for 19 Black Patients With Thalassemia Major

<table>
<thead>
<tr>
<th>No.</th>
<th>Patient</th>
<th>Sex-Age</th>
<th>Diagnosis*</th>
<th>Haplotypes†</th>
<th>(\alpha) Genes</th>
<th>Hb (g/dL)</th>
<th>PCV (L/L)</th>
<th>RBC (1012/L)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>(A_2) (%)</th>
<th>(F) (%)</th>
<th>(\gamma) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>I.M.</td>
<td>F-44</td>
<td>–29/–29</td>
<td>A/A</td>
<td>4</td>
<td>10.6</td>
<td>375</td>
<td>4.90</td>
<td>76</td>
<td>21.4</td>
<td>28.3</td>
<td>3.6</td>
<td>68.0</td>
<td>70.0</td>
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<tr>
<td>2.</td>
<td>F.S.</td>
<td>F-27</td>
<td>–29/–29</td>
<td>A/A</td>
<td>4</td>
<td>10.5</td>
<td>333</td>
<td>5.27</td>
<td>62</td>
<td>19.9</td>
<td>30.4</td>
<td>4.9</td>
<td>61.9</td>
<td>77.8</td>
</tr>
<tr>
<td>3.</td>
<td>D.B.</td>
<td>F-8</td>
<td>–29/–29</td>
<td>A/A</td>
<td>4</td>
<td>11.0</td>
<td>370</td>
<td>5.00</td>
<td>74</td>
<td>22.6</td>
<td>30.5</td>
<td>4.1</td>
<td>67.1</td>
<td>73.5</td>
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<td>4.</td>
<td>Jao.S.</td>
<td>F-27</td>
<td>–29/–29</td>
<td>A/B</td>
<td>3</td>
<td>11.4</td>
<td>410</td>
<td>4.30</td>
<td>95</td>
<td>26.4</td>
<td>27.8</td>
<td>6.4</td>
<td>49.0</td>
<td>58.4</td>
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<tr>
<td>5.</td>
<td>J.S.</td>
<td>M-23</td>
<td>–29/–29</td>
<td>A/B</td>
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<td>9.1</td>
<td>325</td>
<td>4.70</td>
<td>68</td>
<td>19.0</td>
<td>28.0</td>
<td>9.9</td>
<td>11.9</td>
<td>60.3</td>
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<tr>
<td>6.</td>
<td>L.G.</td>
<td>F-28</td>
<td>–29/–29</td>
<td>A/A*</td>
<td>3</td>
<td>9.1</td>
<td>278</td>
<td>4.02</td>
<td>68</td>
<td>22.6</td>
<td>33.1</td>
<td>8.6</td>
<td>43.8</td>
<td>66.9</td>
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<td>7.</td>
<td>S.Ja.</td>
<td>F-13</td>
<td>–29/–29</td>
<td>A/X</td>
<td>4</td>
<td>10.2</td>
<td>285</td>
<td>4.04</td>
<td>71</td>
<td>25.2</td>
<td>35.8</td>
<td>3.5</td>
<td>73.9</td>
<td>64.7</td>
</tr>
<tr>
<td>9.</td>
<td>C.W.</td>
<td>F-24</td>
<td>–88/–88</td>
<td>A1/A1</td>
<td>4</td>
<td>10.7</td>
<td>360</td>
<td>5.58</td>
<td>63</td>
<td>18.6</td>
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<td>5.9</td>
<td>71.7</td>
<td>72.0</td>
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<tr>
<td>10.</td>
<td>T.M.F.</td>
<td>M-23</td>
<td>–88/–88</td>
<td>A/Y</td>
<td>4</td>
<td>11.4</td>
<td>340</td>
<td>5.10</td>
<td>67</td>
<td>21.6</td>
<td>32.4</td>
<td>6.9</td>
<td>56.6</td>
<td>34.2</td>
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<tr>
<td>11.</td>
<td>M.D.</td>
<td>M-17</td>
<td>–88/–88</td>
<td>A/Y</td>
<td>4</td>
<td>13.3</td>
<td>400</td>
<td>6.00</td>
<td>63</td>
<td>20.8</td>
<td>33.3</td>
<td>7.4</td>
<td>68.6</td>
<td>51.4</td>
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<tr>
<td>12.</td>
<td>L.B.</td>
<td>M-37</td>
<td>–29/CD24</td>
<td>A/A</td>
<td>4</td>
<td>13.5</td>
<td>440</td>
<td>6.83</td>
<td>64</td>
<td>19.8</td>
<td>30.7</td>
<td>4.8</td>
<td>54.8</td>
<td>74.4</td>
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<tr>
<td>13.</td>
<td>D.R.</td>
<td>F-24</td>
<td>–29/CD24</td>
<td>A/A</td>
<td>4</td>
<td>10.7</td>
<td>300</td>
<td>4.30</td>
<td>69</td>
<td>24.7</td>
<td>35.7</td>
<td>3.1</td>
<td>66.5</td>
<td>78.6</td>
</tr>
<tr>
<td>14.</td>
<td>D.M.G.</td>
<td>M-4</td>
<td>–29/CD24</td>
<td>A/B</td>
<td>3</td>
<td>9.1</td>
<td>300</td>
<td>4.96</td>
<td>60</td>
<td>18.3</td>
<td>30.3</td>
<td>9.3</td>
<td>24.2</td>
<td>76.8</td>
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<tr>
<td>15.</td>
<td>R.B.</td>
<td>F-48</td>
<td>–29/848</td>
<td>A/C</td>
<td>4</td>
<td>5.8</td>
<td>229</td>
<td>2.36</td>
<td>97</td>
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<td>24.6</td>
<td>2.6</td>
<td>73.0</td>
<td>63.9</td>
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<td>16.</td>
<td>L.I.</td>
<td>F-45</td>
<td>–29/66</td>
<td>A/–</td>
<td>4</td>
<td>7.4</td>
<td>260</td>
<td>2.93</td>
<td>89</td>
<td>25.5</td>
<td>28.5</td>
<td>4.0</td>
<td>65.4</td>
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<td>17.</td>
<td>B.D.</td>
<td>F-49</td>
<td>–88/1.350</td>
<td>A/–</td>
<td>4</td>
<td>10.4</td>
<td>306</td>
<td>4.40</td>
<td>70</td>
<td>23.7</td>
<td>34.1</td>
<td>5.0</td>
<td>89.1</td>
<td>64.2</td>
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<tr>
<td>18.</td>
<td>S.Jo.</td>
<td>F-14</td>
<td>–27/1</td>
<td>B/F4°</td>
<td>3</td>
<td>7.8</td>
<td>275</td>
<td>2.90</td>
<td>95</td>
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<td>28.4</td>
<td>3.5</td>
<td>16.3</td>
<td>51.7</td>
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<td>19.</td>
<td>S.D.</td>
<td>F-1</td>
<td>CD6/CD61</td>
<td>A/C</td>
<td>4</td>
<td>6.9</td>
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<td>2.79</td>
<td>77</td>
<td>24.7</td>
<td>32.1</td>
<td>1.5</td>
<td>98.5</td>
<td>63.4</td>
</tr>
</tbody>
</table>

Abbreviations: PCV, packed cell volume; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

* A

† By cation-exchange HPLC. § By reversed-phase HPLC.

†‡ Transfused.
turer (Sequenase Sequencing Kit, USB, Cleveland) with minor modifications: the use of cold dATP in the labeling reaction (1 μL of 15 μmol/L dATP) instead of radioactive dATP and a 5'-labeled primer and a fivefold dilution of the labeling mix (dGTP). The primer (about 300 ng) was labeled with [γ-32P] ATP (>7,000 Ci/mmol; ICN Immunologicals, Lisle, IL; crude preparation) and T4 polynucleotide kinase (USB) and separated from the reaction products by column chromatography (Biogel P-4, Bio-Rad Laboratories, Richmond, CA) on a column equilibrated with 10 mmol/L triethylammoniumcarbonate, pH 7.3. The labeled primer, with a specific activity of 1 to 2 × 10^6 cpm/μg, was divided into ten aliquots and lyophilized, and one aliquot was used for each sequencing reaction. The 700-bp DNA-amplified fragment (Fig 1) was sequenced with three 19- to 20-nt-long primers: one located in the region of codon 39 and the others at the 5' end of the first intron and in the middle of the second exon, respectively; the last two primers were reverse primers. The usual autoradiography time was eight to 30 hours without using intensifying screens. Further details have been published.9-11 DNA primers and probes were synthesized by using an Applied Biosystems (Foster City, CA) 380B DNA Synthesizer and tested for purity (>95%) by HPLC.

RESULTS

Patients. All patients (except 14-year-old S.Jo., no. 18, and 1-year-old S.D., no. 19) were mildly affected by their disease and were active as students, carpenters, construction workers, ministers, and housewives. The oldest patient was 80 years, and four were between 40 and 50 years old. Disease was accidentally detected in many during testing surveys of high school students, in a testing program conducted by the army, or by an attentive family physician who consulted us because of a mild anemia in his patient. None of the 17 patients was transfusion dependent, but the adults complained of fatigue (particularly during pregnancies), and cardiac complications were present in all older patients. Patient 15 (R.B.) had hemosiderosis and diabetes and was splenectomized. Splenomegaly was noted in several subjects. Table 1 lists the latest hematologic data showing a relatively mild, microcytic anemia (except for patients 15 and 16 who had a more severe anemia and clinical complications). Patient 18 (S.Jo.) is a 14-year-old student with moderate anemia who is regularly transfused once every 6 weeks, and patient 19 (S.D.) is a young child with severe disease who at present is transfusion dependent (packed red cells once in 4 to 5 weeks); both children are being treated with deferoxamine mesylate (Desferal).

The β-thal mutations. Figure 2 shows some of the results obtained with the PCR method; heterozygotes and homozygotes for the A → G substitution at nt −29 and those for the C → T mutation at nt −88 were readily detected. Seven patients were homozygous for the A → G mutation at nt −29 and three for the C → T mutation at nt −88 (Table 1). Thirty-two of the 38 β-thal chromosomes were readily identified: 21 (55%) had A → G at nt −29; eight (21%) had C → T at nt −88, and three (8%) had the mutation at codon 24. Two of the remaining six chromosomes had a deletional type of β-thal: subject 16 (L.J.) had a 3.7- (46Δβ)thal described before,10 while patient 17 (B.D.) had the 1.35-kb deletion involving the 5' end of the β-globin gene.11

Identification of the C → A mutation at nt 848 of IVS-II. This mutation was readily identified by sequencing (Fig 3) and confirmed by PCR and hybridization with specific synthetic probes (bottom film of Fig 2). The C → A mutation occurs at a position 5' to the acceptor consensus sequence TCTTCTTCCA(C → A) AGCTTCT (the last five nucleotides are the first five nucleotides of exon 3), with the invariant AG nucleotide (positions 849 and 850) underlined.

Identification of the A → T mutation at codon 61 by sequence analysis of amplified DNA. DNA from patient 19 (S.D.) was amplified as described earlier by using primer set 1 (Fig 1). The quantity of DNA was increased from 1 to 2 μg and the number of amplification cycles from 30 to 35; 2.5 units of the Taq polymerase was added at the start, while the same quantity was added after 15 cycles. Four amplifications were combined, and the amplified DNA (~700 bp) was
suggests that the amplified purified and sequenced as described earlier. Figure 3 at the first position of codon 61 (AAG TAG), which changes the codon for lysine to a terminating codon.

purified and sequenced as described earlier. Figure 3 at the right illustrates part of the sequencing gel showing that the first nucleotide of codon 61 is an A as well as a T. This suggests that the amplified DNA fraction contained two species, one with an AAG as codon 61 (for lysine) and a second with a TAG as codon 61 (a terminating codon).

To confirm this observation amplified DNA samples from patient 19, his parents (pedigree 4 in Fig 5), and some other individuals were hybridized with synthetic probes; one set of probes was designed to detect the GAG G-G deletion in codon 6 (this defect was observed on the second chromosome of patient 19; see earlier) and the other to identify the A TAG substitution at codon 61. The data given in Fig 4 nicely illustrate the presence of the codon 6 abnormality in the patient and her mother (and in the Greek control) and that of codon 61 in the patient and her father.

Family studies. Family members were included in the evaluation of most patients (excluded are patients 2, 3, 15, and 18), and some pedigrees are shown in Fig 5. The pedigree of patient 10 is of interest because the mother and sister both had S-thal (with ~20% Hb A) whereas the father and one brother had simple thal traits. Identification of the thalassemia in these subjects was readily made by the dot-blot procedure. Two of the children of patient 17 (second pedigree) inherited the chromosome with the C A substitution at nt 88, while the third was heterozygous for β-thal due to the 1.35-kb deletion. The severe microcytosis and high Hb A2 level, earlier noted for a different individual,11 were again observed. Patient 14 (third pedigree) is also of interest because this child had a relatively low level of Hb F (24.2%) and an unusual haplotype (see the next section). His paternal β-thal relatives (with the A G mutation at nt 29) had Hb F levels of 2.3%, while his mother (with the T A mutation at codon 24) had a low Hb F level of 0.5%. The pedigree of patient 19 indicates that the parents had the two different types of β-thal, both having a marked microcytosis.

Fig 3. (Left) Patient 15 (R.B.). The sequencing gel of the cloned gene shows the C → A substitution at the nt 848 position of the second intron, ie, at the position adjacent to the invariant AG dinucleotide of the βIVS-II acceptor splice site. Codons 105 through 117 are also listed. (Right) Patient 19 (S.D.). The sequencing gel of the amplified DNA shows two nucleotides (A and T) at the first position of codon 61 (AAG TAG), which changes the codon for lysine to a terminating codon.

Fig 4. Hybridization of amplified DNA from patient 19, her parents, a Greek β-thal heterozygote, and four controls. (Top) Identification of the GAG G-G deletion at codon 6. (Bottom) Identification of the AAG TAG substitution at codon 61. Sample 7, patient 19 (S.D.); sample 8, father D; sample 3, mother D (cases II-2 and II-1 of the fourth pedigree of Fig 5); sample 2, a Greek β-thal heterozygote with the GAG G-G deletion at codon 6; samples 1, 4, 5, and 6, negative controls. The same membrane was used for both experiments; hybridization with probes to detect the deletion at codon 6 was followed by hybridization to identify the substitution at codon 61.

Fig 5. Pedigrees of four families with the stated β-thal abnormalities. The dot-blot at the top (family of patient 10) illustrates the sensitivity of the detection method. Some hematologic and Hb composition data are shown for members of the other three families (see the text for additional details).
with low levels of Hb F, while the propositus had a severe anemia with 98.5% Hb F, 1.5% Hb A2, and no Hb A.

**Haplotypes and Hb F data.** Haplotypes are listed in Table 1 and detailed in the footnote to this table. Nomenclature was that used in earlier publications for haplotypes in blacks; some of these might be the same as haplotypes in Mediterraneans for which Orkin et al introduced the I X nomenclature.35 The A G mutation at nt -29 occurred on chromosomes with four different haplotypes: 16 (of 21) had haplotype B or [- - - - - - - + + + + ], three (of 21) had haplotype B or [- - - - - - - + + + + ], while two chromosomes had haplotype A or [- - - - - - - + + + + ] and type X or [- + + + + + + + + + ] and type X or [- + + + + + + + + + ]. Hb F levels in homozygotes varied between 15% and 74%; patients with the haplotype combination A/B or A/A had the lowest levels, while the Hb A2 levels were higher (6.4% to 9.9% v 3.5% to 4.9% in the patients with A/A or A/X). The three patients with lower Hb F and higher Hb A2 levels also had an -thal-2 heterozygosity (+ -3)/a (Table 1). The 0 values in Hb F were high, but the highest values (70% to 78%) were found in the three homozygotes with haplotypes A/A as compared with 58% to 65% in the other four.

Two of the three patients with a homozygosity for the C T mutation at nt -88 had haplotype A or [- + + + + + + ] for both chromosomes. Their Hb F values were high (65% to 72%) with high 0 values (69% to 72%). The third patient had two chromosomes with haplotype Y or [- + + + + + + ] (Table 1, Fig 4); his Hb F was somewhat lower at 57% with a 0 value of only 34%.

Five patients had compound heterozygotes. Patient 11 with the A G at nt -29 and the C T at nt -88 had the haplotype A/Y combination with an Hb F level of 57% (0, 61%). Two of the three patients with the A G substitution at nt -29 and the T A substitution at codon 24 had haplotypes A/A (Hb F levels, 55% to 67%; 0, 74% to 79%), but the third had the T A mutation at codon 24 on a chromosome with haplotype B, or [- + + + + + + + + + ]. This healthy 4-year-old child, who also had an -thal-2 heterozygosity, had a low Hb F level of 24% with a 0 value of 77% (Table 1, Fig 4). Patient 15 with the compound heterozygosity for A G at nt -29 and the newly discovered C A mutation at nt 848 of the IVS-II had haplotype A for the chromosome with the mutation at nt -29 and haplotype C or [ + - + - - - - - - - ] for that with the nt 848 mutation. Haplotype C is identical to haplotype I of Mediterranean thalassemia patients. Her Hb F level was a high 73% with a 0 value of 64%. Unfortunately, family studies could not be extended.

The two patients with either the A G at nt -29 or the C T mutation at nt -88 in combination with chromosomes having a large deletion (patient 16) or a smaller deletion (patient 17) had rather severe disease with high Hb F levels and high 0 values (the high 0 value in patient 15 is due to the only gene [0] remaining on the chromosome with the deletion). The haplotypes of the two -thal chromosomes of patient 19 were type A (frameshift at codon 6) and type C (mutation at codon 61). The two heterozygotes with haplotype A had high 0 values, while the one patient with the mutation at codon 61 on the -thal chromosome with haplotype C had low Hb F and low 0 values (see the pedigree in Fig 5).

The heterozygous -thal relatives. Table 2 lists hematologic data for 40 -thal trait relatives with specific nucleotide substitutions and haplotypes. The data are comparable except for the more severe microcytosis seen in the two subjects with the deletional -thal (deletion of 1.35 kb). Average levels of Hb A2, Hb F, and 0 in Hb F are also listed, while the individual values for Hb A2 and Hb F (as percent 0) are shown in Fig 6. Hb A2 levels averaged around 5% in all individuals except the two with the 1.35-kb deletion who had the higher values of about 7%. Hb F data showed great variation, with the lowest values in heterozygotes for the A G mutation at nt -29 (haplotype B) and the T A mutation at codon 24 (haplotypes A and B,). 0 values were low except for heterozygotes with the -thal mutation on a chromosome with haplotype A or A.

**DISCUSSION**

The detailed analyses of the -thal determinants in the 19 black patients with -thal major resulted in the detection of six types caused by a single nucleotide substitution or a single nucleotide deletion and by two types caused by a deletion of part of or the total -globin gene. Twenty-one of the 38

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**Table 2.** Hematologic and Hb Composition Data for Relatives With a -thal Trait (Average Values Only)

<table>
<thead>
<tr>
<th>Condition*</th>
<th>Haplotypes</th>
<th>n</th>
<th>Age (yr)</th>
<th>Hb (g/dL)</th>
<th>PCV (L/L)</th>
<th>RBC (10^12/L)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC (g/dL)</th>
<th>A2</th>
<th>F (%)</th>
<th>0 (%)</th>
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<tr>
<td>29/N</td>
<td>A/Z</td>
<td>15</td>
<td>2-59</td>
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<td>.380</td>
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<td>30.8</td>
<td>4.75</td>
<td>3.3</td>
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<td>29/N</td>
<td>B/Z</td>
<td>4</td>
<td>24-57</td>
<td>10.9</td>
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<td>5.05</td>
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<td>50</td>
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*See Table 1; Z indicates various haplotypes for the normal chromosomes.
†By microcolumn chromatography.‡
‡By alkali denaturation.
The DNA amplification–synthetic probe methodology played a major role in identifying these abnormalities. The use of the Taq polymerase improved and simplified the method considerably. Moreover, direct sequencing analysis of enzymatically amplified genomic DNA by using a limited number of primers was most useful in detecting (new) mutations in our β-thal patients.

Haplotype analyses showed that the most frequently observed substitution occurred on chromosomes with different haplotypes. The A → G substitution at nt −29, for instance, was found on chromosomes with four different haplotypes (type A, 16 chromosomes; type B, three chromosomes; types A, T, and X, one chromosome each; Table 1). However, all four haplotypes can be classified under framework 1 (AvaiI+; Bam HI+), and it seems reasonable to assume that this substitution occurred on a chromosome with haplotype A (which corresponds to type IX of Orkin et al35) and that the other three types are the result of crossovers 5′ to the β-globin gene.2 The situation for the C → T substitution at nt −88 appears comparable. Haplotype A, [− + + + + + + + + − +], is classified under framework 1 (AvaiI+, Bam HI+), and so is haplotype Y [+ − − − − − − − +], which was found in two families (subjects 10 and 11). Haplotypes A and B, of the chromosomes with the codon 24 T → A substitution are of framework 1; the occurrence of type B can again be explained by a crossover at a position 5′ to the β-globin gene. The two rare mutations (C → A at nt 848 of the IVS-II and A → T at codon 61) occurred on a chromosome with haplotype C, which is the same or similar to the common haplotype I of the Mediterranean patients35 and is relatively uncommon in blacks. Thus, it is quite possible that some of these β-thal substitutions are “imports” rather than specific “black” mutations.

Most patients had high levels of Hb F of 55% to 75%. Higher levels were seen in patient 17 (B.D.), who had a deletional type of β-thal, and in patient 19 (S.D.), who had 98.5% Hb F prior to her first blood transfusion. Some patients, however, had lower levels such as 49% (patient 4), 12% to 15% (patient 5), 43.8% (patient 6), and 24.2% (patient 14) (data are given in Table 1; patient 18 with 16.3% Hb F is excluded because the Hb F level was determined in a blood sample that was collected between two blood transfusions). Interesting, all four patients have one β-thal chromosome lacking the Xmn I site (haplotype B in patients 4 and 5, type αγ in patient 6, type Bγ in patient 14). Moreover, they are also the only four patients with an α-thal-2 heterozygosity. It may well be that more than one factor is important in determining the level of in vivo γ chain synthesis, and further studies will be needed to evaluate these possibilities.

The Gγ level in the Hb F was high when the Xmn I restriction site was present 5′ to the Gγ gene, ie, a C → T substitution is present at nt −158 5′ to this gene.37 Seven of 16 patients (patients 16, 17, and 18 are excluded because of the presence of specific deletions in patients 16 and 17, and an uncertainty in patient 18) were +/+ at this site with an average Gγ of 73.8% ± 2.8%, eight were +/− with an average Gγ of 64.8% ± 3.2% (the difference is significant at the P < .05 level), while the one patient (no. 10) with −/− had a Gγ value of only 34.2%. These results confirm earlier studies that show that the Gγ level is determined mainly by the presence of the α-thal-2 locus.
observations suggesting a direct relationship between this mutation at nt −158 and the $\delta$G level.\(^{13,16,37,38}\)

The study of the numerous heterozygous $\beta$-thal relatives made it possible to compare hematologic and Hb composition data for such subjects with specific $\beta$-thal mutations. The data summarized in Table 2 and Fig 6 show a modest microcytic, hypochromic anemia for all subjects with a specific type of $\beta$-thal; as expected, the microcytosis was more severe when the $\beta$-thal was of the $\beta^+$-type (the 1.35-kb deletion type, the frameshift at codon 6, the nonsense mutation at codon 61). Hb $\alpha_2$ levels were around 4% to 5% except for the two subjects with the 1.35-kb deletion that involved the 5' segment of the $\beta$-globin gene and a segment of DNA $\delta'$ to this gene; they had an average Hb $\alpha_2$ level of 7.1%, an increase that has been noticed before.\(^{11}\) Hb F levels varied greatly from 0.4% to 6.9%; this variation was also observed for groups of $\beta$-thal heterozygotes with a well-defined $\beta$-thal mutation (Fig 6). There can be several reasons: a more severe anemia may stimulate $\gamma$ chain synthesis, particularly in patients having $\beta$-thal chromosomes with specific mutations at the $\delta$G promoter such as the C → T substitution at nt −158 (haplotypes A and A1, Fig 6); the contribution by the normal chromosome may differ; and the possible presence of an $\alpha$-thal might result in a decrease in Hb F formation. Some of these possibilities need to be explored in more detail. However, the average Hb F level (2.4%) in $\beta$-thal heterozygotes with T at position −158 of the $\beta$-thal chromosome is significantly higher than the 1.2% found in similar subjects but with C at that position.

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Clinical and genetic heterogeneity in black patients with homozygous beta-thalassemia from the southeastern United States

JM Gonzalez-Redondo, TA Stoming, KD Lanclos, YC Gu, A Kutlar, F Kutlar, T Nakatsuji, B Deng, IS Han and VC McKie