Phenotypic Effect of Heterozygous α and β\(^{-}\)-Thalassemia Interaction

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In this study, we carried out restriction endonuclease mapping in order to characterize the α-globin genotype of 10 Sardinian β\(^{0}\)-thalassemia heterozygotes, all of whom presented with normal red blood cell indices and increased HbA\(_2\) levels. In 8 of these subjects, we found the deletion of two α-globin genes (−α/−α), and in the remaining two, the deletion of a single α-globin gene (−α/αα). In three of these carriers, with the (−α/−α) α-globin genotype and in one with the (−α/αα) genotype, we also found the glucose-6-phosphate dehydrogenase (G6PD) defect of the Mediterranean type. On the basis of these findings, we may conclude that the interaction of heterozygous β\(^{0}\)-thalassemia with α-thalassemia, due to the deletion of either one or two α-globin genes, may lead to the production of red blood cells with normal indices. The association of the G6PD defect with this thalassemia gene complex may eventually contribute to this effect. We suggest, therefore, that screening programs for heterozygous β-thalassemia in populations where α-thalassemia is also prevalent, should incorporate the determination of HbA\(_2\) in the first set of tests.

β\(^{-}\)-THALASSEMIA heterozygotes show remarkable phenotypic heterogeneity, with hematologic manifestations ranging from an isolated increase of HbA\(_2\) levels to a condition of moderately severe thalassemia.\(^1\) Genetic studies and globin chain synthesis analysis have provided evidence that the coinheritance of α-thalassemia may be a cause of the less severe phenotypic manifestations.\(^2\) This suggestion has been recently confirmed by α-globin gene analysis with restriction endonuclease mapping in both β\(^{0}\) and β\(^{+}\)-thalassemia of different racial backgrounds.\(^3\)

Another already known ameliorating factor is the coinheritance of the glucose-6-phosphate dehydrogenase (G6PD) defect of the Mediterranean type, which has also been seen to produce a hematologic picture of larger and more completely hemoglobinized red blood cells than seen in the majority of β-thalassemia heterozygotes.\(^4\)

In this article, we report the results of α-globin gene analysis carried out by restriction endonuclease mapping in a group of 10 Sardinian β\(^{0}\)-thalassemia heterozygotes, all of whom showed normal hematologic characteristics and whose only distinguishing feature was an increase of HbA\(_2\) levels. In 8 of these carriers, we found the deletion of two α-globin structural genes (−α/−α), and in 2, the deletion of a single α-globin gene (−α/αα). In three with the (−α/−α) α-globin genotype and in one with the (−α/αα) genotype, we also found the homozygous or the hemizygous state of the G6PD defect of the Mediterranean type.

On the basis of these findings, we may conclude that the association of α-thalassemia may be the cause of the normal red blood cell indices that have been seen in several β-thalassemia heterozygotes. From our data, it seems that an additional factor contributing to the production of this phenotypic effect could be the association of the G6PD defect of the Mediterranean type.

MATERIALS AND METHODS

Subjects

The 10 Sardinian β\(^{0}\)-thalassemia heterozygotes included in this study were selected on the basis of normal red blood cell indices. All of them showed balanced or reduced α/β globin chain synthesis ratios (Table 1). Seven are obligatory carriers of a β\(^{0}\)-thalassemia gene, as they are parents of thalassemia major/intermedia children with homozygous β\(^{0}\)-thalassemia. The remainder, detected by increased HbA\(_2\) values, can also be assumed to be carriers of β\(^{0}\)-thalassemia, since β-thalassemia is almost exclusively of the β\(^{0}\) type in the Southern Sardinian population.\(^5\)

Methods

Red blood cell indices were measured with the Coulter Counter model S regularly calibrated with a commercial standard (4C, Coulter Counter Cell Control, Coulter Diagnostic). Hemoglobin-A\(_2\) was quantitated by DE-52 microchromatography\(^7\) and hemoglobin F by an alkali denaturation method.\(^8\) G6PD activity was determined according to WHO.\(^9\) Globin chain synthesis in peripheral blood reticulocytes was performed according to Kan et al.\(^10\)

DNA was prepared from white blood cells by phenolchloroform isomyl alcohol extraction and ethanol precipitation.\(^11\) Ten micrograms of DNA was digested with 30 U of restriction endonucleases BgI II and Bam HI (Boehringer, Mannheim, West Germany) for 24 hr under conditions recommended by the manufacturers. The restricted DNA was electrophoresed in 0.8% agarose and transferred to nitrocellulose filters.\(^12\) It was hybridized with a specific 32P-labeled α-globin gene probe prepared by nick-translation, as
hybridized with a -globin-specific probe, two fragments are produced, i.e., an 11.0-Kb fragment, containing the 1; gene, and a 12.0-Kb fragment, containing the 1; , 7; , and 1; genes (Fig. 1). DNA from the rightward heterozygous -thalassemia-2 genotype yields the two normal fragments from the normal chromosome and an additional 16.0-Kb fragment from the rearranged chromosome, while in the homozygous state or heterozygous state, the unaffected subject. This results in an almost balanced globin chain synthesis, which could, in turn, explain the quasi-normal volume and hemoglobinization of the red blood cells. However, the same amelio-

described by Maniatis et al., from -globin gene cloned in the plasmid JW 101. The filters were also hybridized with a -specific probe prepared by nick-translation of a Hind I fragment of the pBR322 plasmid.

RESULTS

In normal DNA digested with Bam HI and hybridized with an -globin-specific probe, the duplicated -globin loci reside in a DNA fragment 14.5 kilobase (Kb) in length. Restriction maps of 8 of the 10 -thalassemia carriers showed that they had only the shortened 10.5-Kb fragment, characteristic of homozygous -thalassemia-2 (-/-). The remaining 2 showed both the 14.5- and 10.5-Kb fragments, which indicates the silent carrier state or heterozygous -thalassemia-2 (-/-). We further characterized the nature of -thalassemia-2 by Bgl II digestion. When digested with this enzyme and hybridized with an -globin-specific probe, normal DNA yields 12.5- and 7.0-Kb fragments that contain the 1; and 1; globin structural loci, respectively. The -thalassemia-2 haplotype (-/) can be produced by two different unequal crossover mechanisms, indicated as the rightward or leftward deletion. Digestion of the DNA from the leftward deletion -thalassemia-2 haplotype produces only the 7.0-Kb fragment, while a 15.8-Kb fragment replaces the two normal fragments in DNA from the rightward deletion. We found the rightward deletion in all the carriers analyzed, both in the homozygous and heterozygous state.

We also analyzed our samples for -thalassemia-1 haplotype (-) by digesting DNA with Bgl II and hybridizing with a nick-translated -globin-specific probe. When normal DNA is cleaved with Bgl II and hybridized with a -globin-specific probe, two fragments are produced, i.e., an 11.0-Kb fragment, containing the 1; gene, and a 12.0-Kb fragment, containing the 1; , 7; , and 1; genes (Fig. 1). DNA from the rightward heterozygous -thalassemia-2 genotype yields the two normal fragments from the normal chromosome and an additional 16.0-Kb fragment from the rearranged chromosome, while in the homozygous state or heterozygous state, the unaffected subject. This results in an almost balanced globin chain synthesis, which could, in turn, explain the quasi-normal volume and hemoglobinization of the red blood cells. However, the same amelio-

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\begin{array}{|c|c|c|c|c|c|c|c|c|c|}
\hline
\text{Subjects} & \text{Sex} & \text{RBC} \times 10^{12} / \text{L} & \text{Hb} (g/dl) & \text{MCV} (fL) & \text{MCH} (pg) & \text{HbA}_{2} (%) & \text{HbF} (%) & \text{G6PD} (U/gHb) & \text{a-Globin Genes Arrangement} \\
\hline
\text{LG} & M & 5.67 & 17.1 & 30.3 & 88 & 5.24 & 0.80 & 0.88 & 0 & -/- \\
\text{S.D.} & M & 4.92 & 14.4 & 29.4 & 70 & 5.00 & — & 0.86 & 0 & -/- \\
\text{P.T.} & F & 5.25 & 13.6 & 26.0 & 79 & 5.51 & 0.73 & — & 0 & -/- \\
\text{C.L.} & M & 5.70 & 14.3 & 26.2 & 79 & 5.15 & 0.62 & 0.73 & 8.4 & -/- \\
\text{P.S.} & M & 5.50 & 15.0 & 27.2 & 80 & 5.21 & 0.50 & 0.83 & 7.2 & -/- \\
\text{R.G.} & F & 4.90 & 13.2 & 27.2 & 83 & 5.13 & 0.73 & 0.98 & 9.0 & -/- \\
\text{S.M.B.} & F & 4.63 & 12.1 & 26.9 & 77 & 5.17 & 0.51 & — & 6.1 & -/- \\
\text{G.S.} & M & 5.86 & 15.7 & 27.5 & 80 & 4.44 & 0.55 & 0.89 & 5.8 & -/- \\
\text{V.A.} & F & 5.05 & 13.7 & 27.1 & 84 & 5.66 & — & — & 0 & -/- \\
\text{A.F.} & M & 5.31 & 14.9 & 28.2 & 83 & 4.76 & 2.7 & 1.30 & 7.2 & -/- \\
\hline
\text{Normal} & & 15.9 ± 2.1 & 30.9 ± 3.9 & 89.1 ± 10.2 & 2.55 ± 0.44 & 0.37 ± 0.56 & (3.66-6.69) & (12.5-19.1) & (26.9-35.0) & (79-102) & (1.86-2.98) & (0.11-1.13) & 1.02 ± 0.12 & (3.88-5.81) & (10.5-16.3) & (25.4-34.6) & (77-100) & (1.53-3.09) & (0.14-0.98)
\hline
\text{Hetero.} & & 13.3 ± 1.6 & 21.9 ± 4.6 & 66.5 ± 11.5 & 4.99 ± 1.02 & 0.98 ± 1.12 & (4.99-7.38) & (11.5-15.3) & (19.5-25.1) & (59-85) & (4.09-6.51) & (0.16-2.5) & 1.83 ± 0.52 & (4.02-7.61) & (9.1-14) & (18.5-25.8) & (55-78) & (4.19-6.29) & (0.12-3.35)
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*Means ± 2 SD and ranges in parentheses.
rating effect was also found in two β^0-thalassemia heterozygotes with only one α-structural gene deleted. A possible explanation for one of these cases might be the association of the G6PD defect of the Mediterranean type. This defect, when associated with heterozygous β-thalassemia, has already been found to lead to greater volume and increased Hb content/cell, but was never seen to produce red blood cells with normal indices. This effect has been attributed to a younger mean red blood cell population, resulting from a chronic slight hemolysis. Another possibility, however, may be the undetected presence of an associated nondeletion α-thalassemia resulting in an effective (αα^+ /−α) genotype. In our population, such nondeletion defects have been observed with a frequency of 25% of all the α-thalassemia-2 haplotypes.

We conclude, therefore, that the interaction of heterozygous β^0-thalassemia with the deletion of either one or two α-globin genes may produce normal red blood cell indices. Moreover, from our data it seems that the association of the G6PD defect may also contribute to this effect.

Based on the evidence produced in this and a previous study, the finding of normal red blood cell indices in several β-thalassemia heterozygotes seems to represent the maximum expression of the ameliorating tendency due to the coinheritance of α-thalassemia. This extreme effect is relatively frequent, since in the Southern Sardinian population, 3.5% of β^0-thalassemia heterozygotes were found to have normal hematologic characteristics, probably due to the coinheritance of α-thalassemia gene(s). Therefore, in populations where α-thalassemia is also prevalent, β-thalassemia carrier screening based on MCV/MCH values may miss a significant proportion of carriers. In the light of these results, it would seem advisable to incorporate testing for HbA2 in the first set of tests in genetic screening programs for heterozygous β-thalassemia in populations where α-thalassemia is also prevalent.

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