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VARIABILITY IN THE INTERACTION OF β-THALASSEMIA WITH THE α-CHAIN VARIANTS Hb G-PHILADELPHIA AND Hb RAMPS

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Variability in the interaction of β-thalassemia with the α-chain variants Hb G-Philadelphia and Hb Rampa

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Two unrelated families are reported in which a β-thalassemia trait occurred with a heterozygosity for Hb G-Philadelphia (α_2 68(E17)Asn→Lys β_2) in one family and with Hb Rampa (α_2 95(G2)Pro→ Serβ_2) in the other. The percentage of Hb G-Philadelphia was not influenced by the simultaneous presence of a β-thalassemia determinant, but that of Hb Rampa was decreased from 20% in the simple heterozygote to about 6% in persons with the Hb Rampa–β-thalassemia combination. Data from in vitro recombination experiments with isolated α^X, α^A, and β^A chains, with heme attached, indicated a preferential formation of Hb A over Hb Rampa but not over Hb G-Philadelphia in conditions of relative β-chain deficiency. This suggests that the rate of assembly of monomers to form dimers or tetramers can be an important mechanism of controlling the quantity of certain hemoglobin variants with critical substitutions in heterozygotes.

Abbreviations: ethylenediamine tetraacetic acid (EDTA), diethylaminoethyl (DEAE), carboxymethyl (CM), paramercuribenzoic acid (PMB)

Beta-thalassemia represents a group of genetic disorders which are characterized by a deficient synthesis of β chains. When such a condition occurs together with a heterozygosity for an α chain–abnormal hemoglobin, the formation of this variant may be either impaired or unaffected[1–8] (Table IV). In this report, we describe two families in which such a combination does occur. In the first family β-thalassemia trait was present together with the rather common α chain variant Hb G-Philadelphia, or α_2 68(E17)Asn→Lys β_2 (ref. 9); quantitative data show that the level of this Hb G is not affected by the simultaneous presence of a β-thalassemia determinant. Hb Rampa (α_2 95(G2)Pro→Serβ_2) was present in the second family. This variant was first characterized in 1971 in two East Indian families[10]; its amount in six heterozygotes was 23% to 48%, with an average of 38%.[10, 11] The carriers in a family of northern European ancestry living near Baltimore had a concentration of "about 15%."[12] The one heterozygote described in this paper had 20% Hb Rampa, but three other members of this family who had Hb Rampa had only 5% to 7% of the variant.

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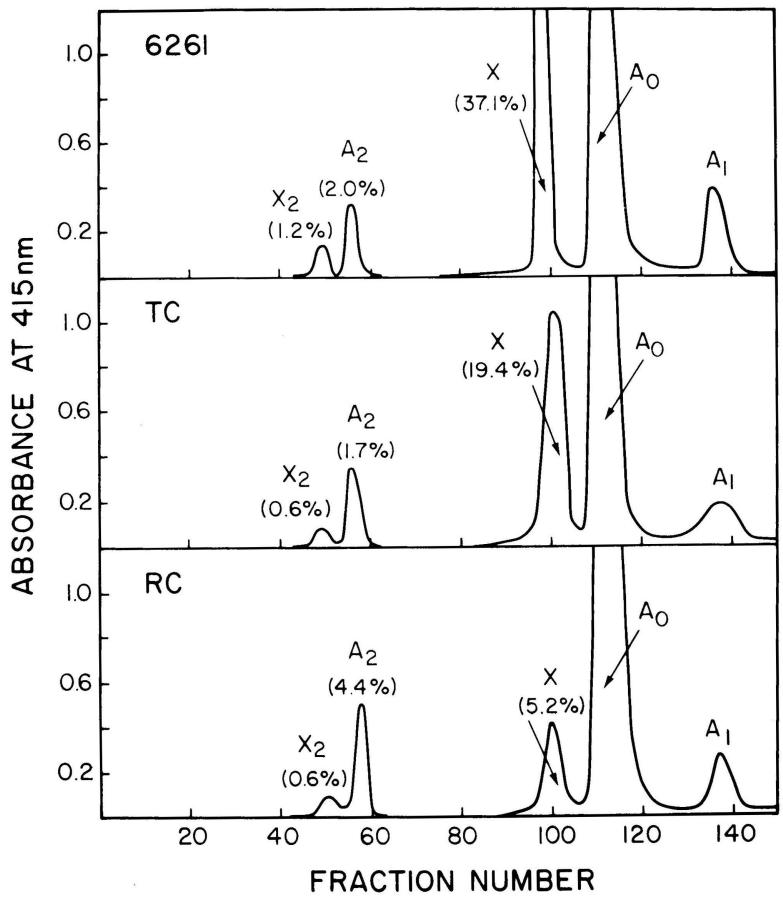

Fig. 1. DEAE-cellulose chromatograms of red cell hemolysates from three subjects with variable amounts of Hb Rampa (=X), Hb A_2, and Hb A_2-Rampa (=X_2). Subjects T. C. and R. C. are identified in Table I and in the text. Subject 6261 is a Hb Rampa heterozygote described in one of the original publications[11]; a sample from this person was provided through the courtesy of Dr. G. Brittingham, Cleveland, Ohio.

Data from in vitro recombination experiments with isolated chains obtained from Hb A and the above-mentioned hemoglobin variants suggest a preferential formation of Hb A over that of certain α chain variants with rather critical substitutions. It is suggested that this type of posttranslational control is at least in part responsible for the variable amounts of abnormal hemoglobins in heterozygotes.

Materials and methods

Hematologic methods and hemoglobin analyses. Blood samples were collected in EDTA and analyzed immediately. The samples collected from members of the Canadian family were transported in ice, by air, special delivery, to Augusta, Ga. Blood counts and red cell indices were obtained with a Coulter Model S cell counter (Coulter Electronics, Inc., Hialeah, Fla.), and other routine hematologic studies were made with standard methods.[13] Hemoglobin electrophoresis was done on starch gel by a previously described method.[14] The hemoglobins A, X(= either Hb G-Philadelphia or Hb Rampa), X_2 (= Hb G_2 or Hb A_2-Rampa), and A_2 were quantified by DEAE-cellulose chromatography.[15] The four hemoglobins were separated completely from each other and from the minor Hb A_1 fraction; some chromatographic separations are shown in Fig. 1. The percent of alkali-resistant hemoglobin (F_{AD}) was determined by the method of Betke et al.[16]

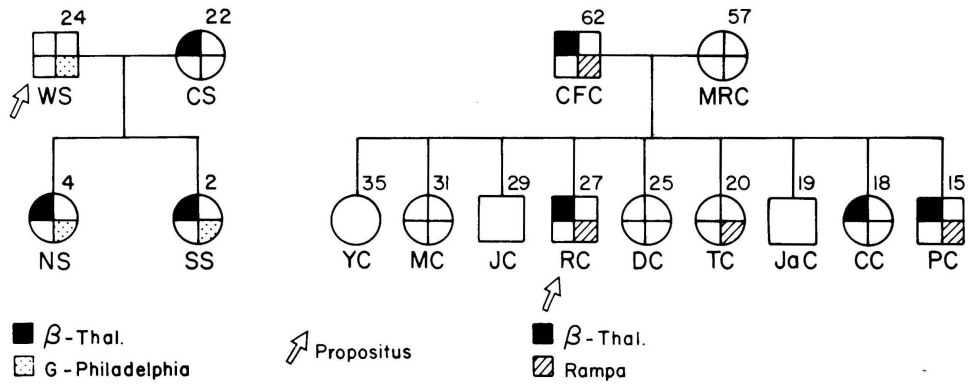

Fig. 2. Pedigrees of Family S from Georgia (left) and Family C from Canada (right). Ages are indicated for each member.

Structural characterization of the abnormal hemoglobins. A large quantity of Hb X was isolated from the blood of the propositus of the Canadian family and from that of his sister T. C. (see pedigree of Fig. 2) by DEAE-cellulose chromatography. Separation of the variant α chain, separation and identification of the tryptic peptides by cation exchange chromatography, and amino acid analysis followed procedures that are described in detail elsewhere.[17] The variant in the Georgia family was characterized by a radioimmunologic method.[18]

Other procedures. The α and β chains (with heme attached) of the hemoglobins A, Rampa, G-Georgia ($\alpha_2$95(G2)Pro→Leu β_2; ref. 19), and G-Philadelphia were isolated as PMB derivatives by CM and DEAE-cellulose chromatography. The relative affinities of the α^A, α^{Rampa}, $\alpha^{G-Georgia}$, and $\alpha^{G-Philadelphia}$ chains for normal β^A chains were determined through quantitation by chromatography of the Hb A and Hb Rampa, Hb A and Hb G-Georgia, and Hb A and Hb G-Philadelphia that were formed when variable amounts of β chains were added to a mixture of equal amounts of the appropriate α chains. Details of this procedure have been given elsewhere.[20] Hb G-Georgia ($\alpha_2$95(G2)Pro→Leu β_2) was selected as a third hemoglobin type to be studied because of its similarity to Hb Rampa ($\alpha_2$95(G2)Pro→Ser β_2), the availability of this variant, and the limited quantity of pure Hb Rampa that was at hand for these in vitro recombination experiments. The α-PMB subunits of Hb Rampa, Hb G-Georgia, Hb G-Philadelphia, and Hb A had a similar stability; no noticeable precipitation occurred during the recombination experiments with the β-PMB subunits nor during the dialysis of these mixtures in developers to which excess β-mercaptoethanol was added. Recovery of the hemoglobins from the DEAE-cellulose chromatograms exceeded 95% in all cases.

Blood samples obtained for globin synthesis studies from the four members of the Georgia family were collected in EDTA. Washed cells (2 ml) were incubated in the home of the propositus for 120 min in a medium containing *l*-^{14}C-leucine. This incubation procedure and the chromatography of the globin on columns of CM-cellulose were done as previously described.[17, 23] The α/β chain synthesis ratios and the α^G/total α chain synthesis ratios were calculated from the recovered radioactivity of the entire fraction corresponding to each of the different chains.

Results

Family from Georgia. The propositus W. S., a 24-year-old healthy black man, participated in a routine testing program for hemoglobin abnormalities at the local Health Department and was found to have a slowly moving variant, which was identified as Hb G-Philadelphia by radioimmunoassay. Subsequent testing of members of his immediate family (for pedigree see Fig. 2) showed that the same variant was present in his two daughters. His wife (C. S.) did not have an abnormal hemoglobin but had hematologic stigmata of a β-thalassemia trait. Stained peripheral blood smears from this woman revealed microcytosis with poikilocytosis, ovalocytosis, and some target cells. These findings, together with an elevated level of Hb A_2, supported the diagnosis of β-thalassemia trait. Careful evaluation of the blood from the two children suggested a similar degree of mi-

Table I. Hematologic and hemoglobin composition data in the families with Hb G-Philadelphia and Hb Rampa[B, C]

Subject[A]	*Age (yr)*	*Condition*	*Hemoglobin (gm/dl)*	*PCV L/L*	*RBC 10^{12}/L*	*MCV (fl)*
W. S.	24	A-G-Phila.	15.1	0.465	5.64	84
C. S.	22	A-β-thal.	11.4	0.370	4.87	77
N. S.	4	G-Phila.-β-thal.	12.6	0.377	5.31	72
S. S.	2	G-Phila.-β-thal.	12.7	0.372	5.28	72
C. F. C.	62	Rampa-β-thal.	11.8	0.372	5.95	62
M. R. C.	57	AA	13.1	0.413	4.48	90
M. C.	31	AA	14.1	0.485	4.57	104
R. C.	27	Rampa-β-thal.	11.4	0.368	5.73	63
D. C.	25	AA	13.9	0.431	4.62	91
T. C.	20	A-Rampa	13.2	0.419	4.26	96
C. C.	18	A-β-thal.	12.3	0.378	5.68	65
P. C.	15	Rampa-β-thal.	12.3	0.396	5.75	68

[A]See pedigrees in Fig. 2.
[B]Determined by DEAE-cellulose chromatography.[15]
[C]Hb A is the sum of the Hb A_0 and Hb A_1 (Fig. 1); the values given for the members of Family C include the small amount of Hb F.
[D]Determined by DEAE-cellulose chromatography (Family S) and by alkali denaturation (Family C).
PCV = packed cell volume; RBC = red blood cells; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin.

Table II. Globin synthesis studies in the four members of Family S from Georgia

	cpm recovered [A]			*Ratios*[B]	
Subject	*β*	α^A	α^G	*Total α/β*	α^G/*total α*
W. S.	5124	2548	1084	0.71	0.30
C. S.	6248	7904	—	1.27	—
N. S.	5832	4624	1612	1.07	0.26
S. S.	13,196	5312	4492	0.74	0.46

[A]Total cpm/zone isolated from whole cell globin after 120 min incubation.
[B]The average total α/β ratio observed in our laboratory (Augusta, Ga.) for normal black adults is 0.91 ± 0.1 (1 S.D.; n = 17) ref. 24; the same ratio is 1.33 (with a range of 1.19-2.38, n = 13) for β-thalassemia heterozygotes.[17]

crocytosis, but the poikilocytosis and anisocytosis were more marked in the 7-year-old N. S. than in her 2-year-old sister S. S. (Fig. 3). The smear from the father did not show any morphologic abnormalities. Table I lists the hematologic data and the proportions of the hemoglobins G and G_2 in the heterozygotes; serum iron and total iron-binding capacity determinations were not made due to lack of appropriate samples. The father had a Hb G (i.e., Hb G + Hb G_2) level of 32.7%, for his daughter N. S. the level was 33.5%, and for his youngest daughter S. S. it was 44.5%. Both children had elevated levels of Hb A_2 + G_2; for N. S. this was 5.3%, and for S. S. 5.7%.

The synthesis ratios of total α chain/β chain and of α^G/total α are listed in Table II. These data suggest an α-thalassemia–like effect in W. S. and S. S., a β-thalassemia–like effect in C. S., and a nearly balanced synthesis in N. S. The α^G/total α ratios corresponded fairly well with the level of Hb G (=Hb G + Hb G_2) in W. S. (0.30% vs. 32.7%), in N. S. (0.26% vs. 33.5%), and in S. S. (0.46% vs. 44.5%).

Family from Canada. R. C., a 27-year-old French Canadian, was hospitalized be-

MCH (pg)	Hb X_2[B] (%)	Hb A_2[B] (%)	Hb X[B] (%)	Hb A[B, C] (%)	Hb F[D] (%)
26.9	0.8	2.3	31.9	63.0	2.0
23.6	0	7.6	0	89.0	3.4
23.9	1.7	3.6	31.8	56.8	6.1
24.3	2.3	3.4	42.2	48.7	3.8
18.8	0.5	4.6	6.1	88.8	<1.0
27.6	0	2.8	0	97.2	<1.0
29.2	0	2.6	0	97.4	<1.0
18.8	0.6	4.4	5.2	89.8	<1.0
28.6	0	2.4	0	97.6	<1.0
29.2	0.6	1.7	19.4	78.3	<1.0
20.5	0	5.6	0	94.4	1.2
20.1	0.6	5.7	4.2	89.5	<1.0

cause of shortness of breath and anemia with a Hb level of 9 gm/dl. Peripheral blood showed numerous target cells, elliptocytes, and anisocytosis. The erythrocyte osmotic resistance was markedly increased. The serum iron concentration and the iron-binding capacity were within normal limits. On electrophoresis a small amount of a Hb fraction migrating in a position between that of Hb A and Hb S was detected on several occasions. Negative results were obtained in the solubility test used for the detection of Hb S and with a heat stability test. The Hb F concentration (as percent F_{AD}) was usually less than 1%.

The patient's parents and five of his eight siblings were available for study (Fig. 2). His father (C. F. C.), a brother (P. C.), and a sister (C. C.) also had the abnormal hemoglobin. Hematologic data and the hemoglobin composition data of the eight members of this family are listed in Table I. These show that (1) the propositus, his father, and siblings T. C. and C. C. are β-thalassemia heterozygotes as evidenced by elevated levels of Hb A_2 and low MCV and MCH values; (2) in R. C., C. F. C., and P. C. the abnormal hemoglobins formed 4% to 6%, whereas in the one sibling (T. C.) with normal hematologic values it formed nearly 20%; and (3) the three remaining members (including the mother of the propositus) had normal hematologic values and no hemoglobin variant. The level of Hb F in all subjects fell within the normal range.

STRUCTURAL ANALYSES. All expected tryptic peptides (except T-12) were recovered from a digest of the α^X chain, and the amino acid composition of the peptides T-1 through T-10, T-13, and T-14 was the same as that of corresponding fragments of the α chain of Hb A. The composition of T-11 was Lys 1.00 (1); Asp 2.20 (2); Ser 0.93 (0); Pro 0.05 (1); Val 2.22 (2); Phe 0.96 (1); the values between parentheses are those of the T-11 peptide of the α chain of Hb A. These results indicate a substitution of the prolyl residue by a seryl residue in the third position of the T-11 peptide, corresponding to position 95 (G2) of the α chain, and identify the variant as Hb Rampa.[10, 12]

QUANTITATIVE ANALYSES. Table I lists the hemoglobin composition data for the members of Family C who had Hb Rampa. Two distinct modes were observed for the total amount of α^{Rampa} chain containing hemoglobins (=X + X_2), namely 20.0% in T. C. and 5.8% average in the three members of Family C who had a β-thalassemia (see also Fig. 1).

In vitro recombination experiments. Mixtures of equal quantities of α^A and the α

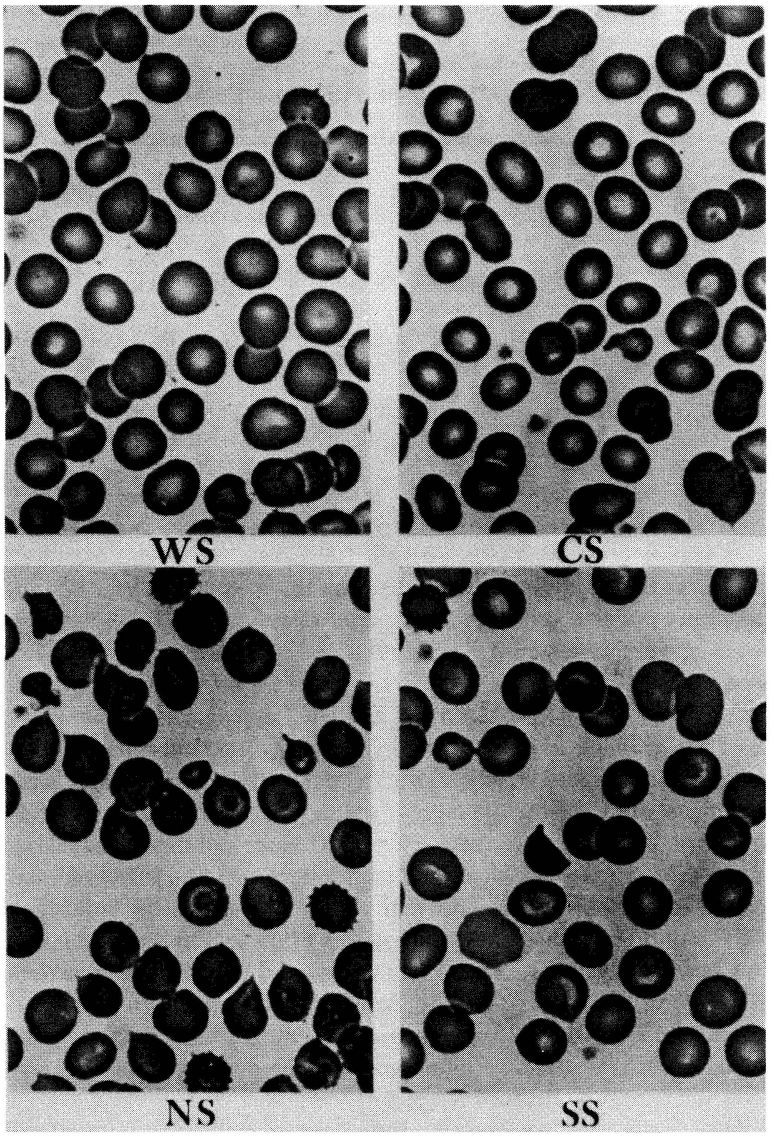

Fig. 3. Peripheral blood smears of the four members of Family S from Georgia. *WS*, Father with Hb G-Philadelphia trait; *CS*, mother with β-thalassemia trait; *NS*, daughter with Hb G-Philadelphia $-\beta$-thalassemia; *SS*, daughter with Hb G-Philadelphia $-\beta$-thalassemia. C. S. and N. S. also have a heterozygous α-thalassemia-2 condition, and S. S. is homozygous for the same α-thalassemia-2 determinant.

chain of either G-Philadelphia, G-Georgia, or Rampa were combined with normal β^A chains in ratios of β/total α chain varying between 0.25 and 2.0. The available amount of pure α^{Rampa} chain permitted only two experiments with this variant. Three experiments involving the α chain of Hb G-Georgia and four involving the $\alpha^{G-Philadelphia}$ chain were performed. Starch gel electrophoretic examination of the hemoglobins in the mixtures showed the presence of two major hemoglobin components (Hb A and the expected variant) and, in addition, some free β^A or α^X and α^A chains in the experiments with high or

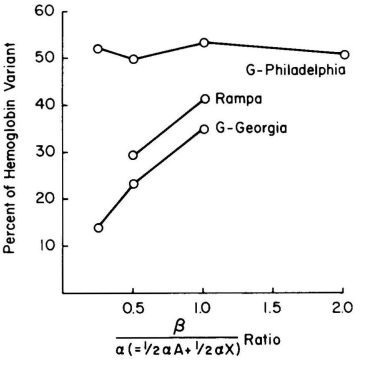

Fig. 4. Relative amounts of the α chain variants Hb G-Philadelphia, Hb G-Georgia, and Hb Rampa in mixtures prepared by combining free β^A chains with free $\alpha^A + \alpha^X$ chains in ratios as indicated.

low β/α ratios, respectively. The hemoglobins were quantified by DEAE-cellulose chromatography, and the calculations of the relative amounts of the two hemoglobins were based on the total amount of hemoglobin recovered from the column but excluding the free chains.

Fig. 4 represents the results of these recombination experiments, which indicate that considerable differences exist in the quantities of the hemoglobin variants recovered from the mixtures. Thus Hb G-Philadelphia averaged about the expected 50%, and its formation was independent of the β/α ratio in the mixtures. However, in mixtures of $\alpha^A + \alpha^{Rampa}$ with β^A chains or of $\alpha^A + \alpha^{G-Georgia}$ with β^A chains, the formation of either Hb Rampa or Hb G-Georgia was greatly dependent upon the β/α ratio, and only some 25% to 30% of the variant was present in mixtures in which the $\alpha^A:\alpha^X:\beta^A$ ratio was 1:1:1. These data suggest that in in vitro conditions of relative β-chain deficiency, the formation of Hb A is favored over that of either Hb Rampa or Hb G-Georgia.

Discussion

The two families are of interest because they contain the first instances to be reported of a combination of a β-thalassemia heterozygosity and a heterozygosity for either Hb G-Philadelphia or Hb Rampa.

The observation of Hb G-Philadelphia in a black family is a rather common one; heterozygotes for this variant are reported to have either 20% to 25%, 30% to 35%, or 40% to 45% of the variant (for a review of the literature see refs. 21 and 22). These differences in proportion can be explained by assuming variable numbers of active α-chain structural loci; for instance, the $\alpha\alpha^G/\alpha\alpha$, the $-\alpha^G/\alpha\alpha$, and the $-\alpha^G/-\alpha$ genic arrangements are considered to explain adequately the variation in the percentages of Hb G-Philadelphia in heterozygotes.[21, 23] The $-\alpha^G/\alpha\alpha;\beta^A/\beta^A$ genic arrangement results in 30% to 35% of the Hb G-Philadelphia variant and is the most common one.[21–23] The finding of 32.7% Hb G + Hb G_2 in subject W. S. in our first family suggests that he has this condition. This propositus married a person with a β-thalassemia trait, and their two daughters inherited both abnormal conditions. One of these (N.S.) had 5.3% Hb A_2 + Hb G_2 and 33.5% Hb G + Hb G_2, suggesting the presence of a β-thalassemia trait which did not influence the relative production of the α chain variant because the levels of Hb G + Hb G_2 in her and in her father were the same. The other daughter (S. S.) also had a β-thalassemia trait (Hb A_2 + Hb G_2 equaled 5.7%), but in her, the level of Hb G + Hb G_2 was considerably higher

Table III. Genotypes of the members of the Georgian family with Hb G-Philadelphia, β-thalassemia, and α-thalassemia-2

Subject	*Relationship*	*Hb G[A] (%)*	*Total α/β*	*Genotype[B]*
W. S.	Father	32.7	0.71	$-\alpha^{G}/\alpha\alpha;\beta^{A}/\beta^{A}$
C. S.	Mother	—	1.27	$-\alpha/\alpha\alpha;\beta^{A}/\beta^{Th}$
N. S.	Daughter	33.5	1.07	$-\alpha^{G}/\alpha\alpha;\beta^{A}/\beta^{Th}$
S. S.	Daughter	44.5	0.74	$-\alpha^{G}/-\alpha;\beta^{A}/\beta^{Th}$

[A]Includes G and G_2.
[B]The minus sign (−) indicates the presence of an α-thalassemia-2 determinant without commitment as to whether this is due to deletion or suppression of an α-chain structural gene. The evidence for considering the Hb G-Philadelphia gene to be linked to an α-thalassemia-2 determinant (thus, $-\alpha^{G}/$) is reviewed elsewhere.[21, 23]

Table IV. Quantities of some α chain variants in heterozygotes and in persons with the Hb X-β-thalassemia condition

Hb variant	*Substitution*	*Method of quantitation[A]*	*%X in AX[B]*	*Number of cases*	*%X in X-β-thal.[B]*	*Number of cases*	*References*
J-Paris	12 Ala → Asp	CA-elution	26	1	16	1	1
J-Oxford	15 Gly → Asp	CA-elution	23-26	4	25	1	2
Hasharon	47 Asp → His	CA-elution	30-40	Many	17-19	14	3
J-Sardegna	50 His → Asp	SG-elution, densitometry	16-20 23-35	3 4	21-24 33	2 1	4,5
Q-India	64 Asp → His	DEAE-Seph., CA-elution	— 20	 1	9 8-19	1 6	6,7
G-Philadelphia	68 Asn → Lys	DEAE-cell.	33[C]	1	33.5;44.5[D]	2	This paper
Inkster	85 Asp → Val	SB-elution, CA-elution	21-23	12	19-21	2	8
Rampa	95 Pro → Ser	DEAE-cell.	20	1	5-7	3	This paper

[A]CA = cellulose acetate electrophoresis; SG = starch gel electrophoresis; SB = starch block electrophoresis; DEAE-Seph. = DEAE-Sephadex chromatography; DEAE-cell. = DEAE-cellulose chromatography.
[B]Decimals of the reported percentages are omitted.
[C]This value agrees with values published in refs. 21 and 22.
[D]Difference between these two values is discussed in detail in the text.

(44.5%), which is most easily explained by assuming an $-\alpha^{G}/-\alpha$ genic arrangement in this child.

In retrospect, the mother of this family probably had the $-\alpha/\alpha\alpha;\beta^{A}/\beta^{Th}$ genic arrangement (i.e., a β-thalassemia trait with an α-thalassemia-2 trait); her husband had the $-\alpha^{G}/\alpha\alpha;\beta^{A}/\beta^{A}$ genic arrangement (Hb G trait and α-thalassemia-2 trait), and their two daughters had the $-\alpha^{G}/\alpha\alpha;\beta^{A}/\beta^{Th}$ (Hb G trait, α-thalassemia-2 trait, and β-thalassemia trait) and the $-\alpha^{G}/-\alpha;\beta^{A}/\beta^{Th}$ (Hb G trait, homozygous α-thalassemia-2, and β-thalassemia trait) genic arrangements, respectively. The results of the biosynthetic studies support these conclusions. The decreased α/β ratio (i.e., 0.71) in the father is comparable to the average values obtained for persons with a similar condition.[21] The α/β of 1.27 in the mother is somewhat lower than is usually observed in persons with a β-thalassemia trait[17] (Table II) and is consistent with the simultaneous presence of an α-thalassemia-2 trait (the $-\alpha/\alpha\alpha$ genic arrangement) and a β-thalassemia trait. The condition in N. S. is comparable to that seen in her mother except for an additional Hb G heterozygosity, and the nearly balanced α/β ratio of 1.07 is somewhat surprising. The effect of the α-thalassemia-2

homozygosity in the other daughter (S. S.) with 44.5% Hb G + Hb G_2 and a β-thalassemia trait is considerable, and this α-thalassemia-2 homozygosity is probably responsible for relatively low α/β ratio of 0.74 found in her; as expected, this ratio is in between the average values of 0.86 and 0.63 reported for persons with the $-\alpha^G/\alpha\alpha;\beta^A/\beta^A$ and $-\alpha^G/-\alpha;\beta^A/\beta^A$ genic arrangements, respectively.[21] The genotypes of the individual members of this family are summarized in Table III.

The second family is of added interest because it indicates the presence of the gene for Hb Rampa in persons of French ancestry in Canada, and it contains three members with the interaction of Hb Rampa and β-thalassemia determinants. Of considerable interest is the difference in amount of Hb Rampa in the Canadian heterozygote (who has 20% Hb Rampa and therefore the $\alpha\alpha^X/\alpha\alpha;\beta^A/\beta^A$ genic arrangement[23]) and in the three subjects with Hb Rampa–β-thalassemia combination (5% to 7%). The effect of the simultaneous occurrence of β-thalassemia on the percentage of Hb Rampa is much more marked than for any other variant studied thus far (Table IV); a decrease of less than 50% was observed for Hb J-Paris and Hb Hasharon, whereas the presence of a β-thalassemia did (almost) not influence the percentage of the other five variants listed in this table.

The data from in vitro recombination experiments involving β^A, α^A, and α^X chains indicate that in the case of Hb Rampa and Hb G-Georgia the quantities of these hemoglobin variants recovered from these mixtures depend on the ratio between the β chains and the $\alpha^A + \alpha^X$ chains that are present. A similar dependence was observed for Hb St. Luke's (α95Pro→Arg) but not for Hb G-Philadelphia (α68Asn→Lys) and Hb G-Montgomery (α48Leu→Arg) (Family S, and ref. 23). These results suggest that the formation of dimers and/or tetramers of certain α chain variants with rather critical substitutions (such as those involving the prolyl residue in position α95) is impaired in mixtures with a low β/α ratio and that under these in vitro conditions the formation of Hb A is favored over that of the variant. These data may offer an explanation for the low level of Hb Rampa in persons with a relative β-chain deficiency, i.e., the Hb Rampa–β-thalassemia condition.

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