Hemoglobin S/hemoglobin City of Hope compound heterozygote with a SubSaharan genetic background and severe bone marrow hypoplasia.

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Key words: hemoglobin S, hemoglobin City of Hope, immune deficiency, sickling anemic phenotype, African haplotypes, parvovirus B19.

Abstract. Hemoglobin City of Hope (Hb CH) (*HBB*: c.208G>A, beta 69 (E13)Gly>Ser) is a rare, anomalous change. Seven independent carriers reported so far, had not displayed any hematological manifestations. The ethnic origin of the known instances is presumably heterogeneous, although they are mainly Mediterraneans or equatorial West Africans. We describe the case of a compound heterozygote in trans for Hb S (Glu6Val) and Hb City of Hope (Gly69Ser) in an anemic two year-old boy with a severe immune-deficient phenotype and fatal chronic parvovirus B19 infection. Haplotype with the Hb S was Bantu; while it was a mixed atypical Benin/Cameroon for Hb CH. Remote ancestral origin of the City of Hope mutation in this family seems to be SubSaharan African. The compound heterozygosis in trans for hemoglobins S and City of Hope, jointly with an unfavorable *HBB* control region background and a viral chronic infection, seemed the cause of the fatal outcome in the patient. When accompanied by other Hb deleterious mutations in trans, Hb CH should not be considered any longer as an innocuous or functionally silent variant.

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Heterocigoto compuesto para hemoglobina S/hemoglobina City of Hope con trasfondo genético subsahariano e hipoplasia severa de médula ósea.

   Resumen. La hemoglobina City of Hope (Hb CH) (*HBB*: c.208G>A, beta 69 (E13)Gly>Ser) es una variante infrecuente, considerada como anómala. Ninguno de los siete heterocigotos simples, genéticamente no relacionados, reportados hasta ahora, ha mostrado hemopatología. El origen étnico de esos casos es presuntamente heterogéneo, pero la mayoría parece mediterráneo o africano-ecuatorial occidental. Se describe el caso de un niño de dos años de edad con fenotipo hipoplásico mieloeritroideo severo e infección crónica por parvovirus B19, heterocigoto compuesto en trans para las hemoglobinas S (Glu6Val) y City of Hope (Gly69Ser). El haplotipo en fase con la Hb S fue Bantú, mientras que el de la Hb CH fue un combinado atípico Benin/Camerún. El origen ancestral remoto de la mutación City of Hope (y de la Hb S) en esta familia es africano subsahariano. La heterocigosis compuesta en trans para las hemoglobinas S y City of Hope y una secuencia génica predisponente en la región de control de *HBB,* conjuntamente con la infección por parvovirus B19 pueden ser la causa del curso fatal del paciente. En presencia de otras mutaciones de hemoglobina deletéreas, la Hb City of Hope no debiera ser considerada una variante inocua o funcionalmente silenciosa.

INTRODUCTION

   Hemoglobin City of Hope (Hb CH) (HBB: c.208G>A, beta 69 (E13)Gly>Ser) seems to be a rare β globin variant, which was found initially in Caucasoid families (1, 2); later in a Turkish one (3); in an Italian family from Naples (4); in a Nigerian newborn and his mother in Madrid, Spain (5, 6) and in 2 out of 2105 hemoglobin samples collected along 5 years in Switzerland (7). This functionally silent mutation has been discovered in single heterozygous healthy individuals who had not shown any hematological or clinical manifestations (1-6). The exception is a Turkish patient with β⁰-thalassemia intermedia and moderate microcytic hypochromic anemia (3), whose normal heterozygous mother carried the Gly69Ser substitution on a β globin-like cluster haplotype I background. In the Italian family the haplotype was IX.

   We describe the case of a compound heterozygote in trans for Hb S/Hb City of Hope seen in a two-year old boy referred for diagnosis, who died from a septic shock a year later. His mother, maternal grandmother, aunt and one sister, carried the Hb CH mutation, without any hematological manifestation. His normal father and paternal aunt carried a Hb S chromosome. The maternal City of Hope syntenic associations

were $-; -; +; -; -; -; -; -; +; +$ plus the beta gene promoter 5' untranslated region motif $(AC)_2(AT)_8(T)_5$; and the paternal ones for the Hb S haplotype were respectively $-; -; -; -; -; +; +; -; +; +$ and motif $(AC)_2(AT)_6(T)_9$.

This report of a β globin compound heterozygote in trans for the Gly69Ser and Hb S (Glu6Val) mutations, displaying a severe anemic phenotype complicated with a fatal parvovirus B19 chronic infection, challenges the 'quasi-normality' hypothesis of the Hb CH mutation of a possibly remote SubSaharan origin.

CASE REPORT

The last child of 3-sibship was born through a Cesarean delivery after a normal 39-week gestation, to a 33 year-old mother and as a healthy 3.4 kg, 50 cm boy. After the first 4 uneventful months he was presented with diarrhea and dehydration, that was successfully treated. One month later, he acquired varicella from his sister and at 6 months, he suffered a pneumococcal meningitis, complicated with a subdural collection and convulsions. At 7 months of age he was found to have anemia, leucopenia, thrombocytopenia and red cell morphologic alterations, but the low MCV and reticulocyte values were atypical for any mixed Hb S /β thalassemia combination (Table I) an enlarged liver and spleen were discovered. The bone marrow was found "normal" in two consecutive studies. Erythrocytic G6PD and pyruvate kinase were normal. Viral infections (hepatitis B, C, parvovirus, HIV and EBV) were apparently absent, but a high IgM anti-CMV was found; immune studies (complement, immunoglobulins, lymphocyte populations, NBT test) and clotting factors, were all found normal. At 2 years of age he was briefly hospitalized due both, to a severe respiratory infection with bronchoconstriction and to generalized convulsive cri-

ses. Brain MRI revealed an improvement of the subdural lesion, which however was producing a cortical irritation not previously curtailed with anticonvulsants. The bone marrow was still normal. Transaminases were increased, and liver and spleen biopsies revealed only reactive hepatitis and congestion, respectively. Lymphocyte populations showed an inverse CD4/ CD8 index, which continued to be so all along his second year. Cytomegalovirus (CMV) antibodies found were of the IgG type, without any IgM and supressor CD8 lymphocytes were the predominant ones. He received several blood transfusions and at 3 years of age he was admitted again with another upper respiratory infection, high fever, leuco-, neutro-, thrombocytopenia and anemia. Enlarged liver and spleen were found and a splenectomy was performed because of a large (694 g) and polysplenic organ. He never suffered from vasoocclusive episodes. A month later the child died at 3.1 years of age with 9.2 kg of weight and 93 cm stature.The autopsy revealed as positive findings a left pleural (250 cc), pericardial (150 cc) and peritoneal (30 cc) effusions, severe myocarditis, subendocardial hemorrhage and ventricular hypertrophy. These were attributed by the pathologist to a non-cytomegalovirus viral infection, later proved to be of parvovirus B19 as the precipitating cause of death, in an immune deficient child.

Hemoglobin phenotype

Initial phenotype ascertainment for any hemoglobin with anomalous electrophoretic migration besides Hb S, was done by isoelectrofocusing in 5.05% polyacrylamide gel with ampholyte pH 7-9, according to standard procedures (8).

Stability tests

Isopropanol and heat stability tests were performed according to standard procedures (8).

TABLE I
BLOOD CELL COUNTS AND HEMOGLOBIN ANALYSES IN THE PROPOSITUS DURING HIS FIRST
TWO YEARS OF LIFE (n= NUMBER OF INDEPENDENT TESTS)

Parameter	Age 7-12 months		Age 13-24 months		Control values
	Range[b]	n	Range[b]	n	
WBC ($\times 10^3/mm^3$)	5.0-5.5	6	3.8-6.0	5	5.0-9.5
RBC ($\times 10^6/mm^3$)	3.3-4.9	6	3.7-4.15	5	4.7-6.1
Hb (g/dL)	7.2-10.9	6	8.6-9.4	5	11-14
Hematocrit (%)	20.3-34.5	6	26.7-30.4	5	33-41
MCV (fL)	56.5-70.2	6	70.0-72.0	3	80-94
MCH (pg)	16.7-25.1	6	21.4-24.1	3	27-31
MCHC (g/dL)	29.5-34.7	6	30.5-33.2	3	33-37
Platelets ($\times 10^3/mm^3$)	184-264	6	96.0-212	5	130-400
Ferritin (ng/mL)	-		332.8	1	6.9-294
Anisocytosis	+ to +++	5	++	4	0
Microcytosis	+	3	++	3	0
Hypochromia	+	3	++	3	0
Reticulocytes (%)	0.1-1.6	7	-		0.5-2.3
Hb CH[a] (%)	55.6	1	-		0
Hb A$_2$ (%)	< 4.3	1	-		2.1-3.0
Hb F (%)	1.7	1	-		< 4.1
Hb S (%)	38.4	1	-		0

[a] initially mistaken as Hb A.   [b] Values were selected to exclude results within four months of any blood transfusions.   (-) : Not measured.

DNA analysis

DNA was extracted from 5 ml of a venous EDTA anticoagulated blood sample, by a saline method (9), after informed consent of each adult and the granted permission from the parents in the children's case, according to the bioethical institutional guides. PCR amplification for 81 bases in the 5´untranslated promoter region, exon 1 and part of exon 2 of the beta globin gene (bases 218 to 746, GenBank accession number EF450778) was performed using primers KM29 and RS42 as previously reported (10). Mutant Hb S (Glu6Val) was confirmed with the restriction enzyme *Dde I*. The β globin codon 2 (C>T) polymorphism (SNP rs713040) was ascertained by restriction digestion of the PCR product with the enzyme *Hpy188 I* and by sequencing, respectively in both cases.

The single strand conformational polymorphism (SSCP) analysis of the PCR product was done in all the studied family members (10 individuals), in 8% non-denaturing polyacrylamide-bis acrylamide gels (29:1). Bands were visualized by a silver nitrate stain (11). The complete sequence of the PCR product of those individuals showing anomalous SSCP migration was performed under request in all cases by Macrogen Service, Seoul, Korea.

α-Thalassemia-2 determinants

The ascertainment of the two most common α chain deletions $-\alpha^{-3.7}$ and $-\alpha^{-4.2}$ was performed according to Baysal and Huisman (12).

Haplotype analysis

The following polymorphic sites of the hemoglobin ("beta globin-like") gene cluster on chromosome 11 were analyzed: site 1: 5'ε (*Hinc II*); site 2: 5' $^{G}\gamma$ (*Xmn I*); site 3: $^{G}\gamma$ (*Hind III*) site 4: $^{A}\gamma$(*Hind III*); site 5: ψβ(*Hinc II*); site 6: 3' ψβ (*Hinc II*); site 7: 5' β (*Hinf I*); site 8: β (*Ava II*); site 9: 3' β (*Hpa I*) and site 10: 3' β (*BamH I*). PCR amplification for sites 2 to 7 was performed using primers previously reported (13), also for the 5' ε site (14). Primers for sites 8 to 10 were designed using Primer 3 Program (http://frodo.wi.mit.edu/) as follows:

– *Ava II* site: 5'-TTTCTGGGTTAAGGCAATAG and 5'-CAAGAAAGCGAGCTTAGTGA
– *Hpa I* site: 5'-TTAATTATCAACCCAAAGTA and 5'-AGCAGGCTGTTCAGTTTCCA (designed on purpose outside the *Kpn I* long repetitive sequence insertion) (15).
– *BamH I* site: 5'-CCTGAGAGAAACAAGCAATG and 5'-ATTAGATCCCGTTTGTCCAT.

PCR products were digested with the appropriate restriction endonuclease and the fragments were visualized on 8% polyacrylamide gels.

Beta promoter polymorphism motifs

Primers 5'-AGAGCACATTGATTTGTATTTA and 5'AGCTTTGTCTACCATAATTCAG were designed (Primer 3 Program) to study the polymorphic promoter motifs $(AC)_n (AT)_x(T)_y$ (16), including the -551 and -521 SNPs. The structure of each individual motif was assessed by sequencing.

Parvovirus B19 infection

Parvovirus B19 infection was detected by the polymerase chain reaction (PCR) in DNA samples extracted from paraffin-embedded post-mortem tissues (liver, kidney and heart) using primers previously reported (17) and by examining 4μ tissue sections stained with hematoxylin-eosin (17).

RESULTS

Initial hemoglobin phenotyping of the patient at 14 months of age, both by electrophoresis (cellulose acetate, polyacrylamide, acid agar) and isoelectrofocusing, showed him to be an apparent heterozygote Hb A/S, but he had recently received a blood transfusion. A second analysis of a new sample performed several months later showed a less intense band for "Hb A" and a typical band for Hb S; his father was a heterozygote HbA/S and his mother was an apparent homozygote Hb A/A.

The molecular study of codon 6 of the HBB gene showed the change Glu6Val, corresponding to the Hb S mutation both in one paternal chromosome and in one child chromosome, which his mother and two sisters did not carry.

SSCP analysis of the PCR product including exon 1 and part of exon 2 of the *HBB* locus showed an anomalous migration pattern in the patient, his mother, one of his sisters (individual code 605, Fig. 1), maternal grandmother and in one of the latter three additional daughters (individual code 507), being different from that of the children's father. DNA sequencing revealed in all of them, the heterozygous Gly69Ser substitution (GGC→AGC), which corresponded to hemoglobin City of Hope.

Hemoglobin stability tests and hemoglobin F concentration were normal in the grandmother (code 405), mother (code 503) and the patient's carriers maternal
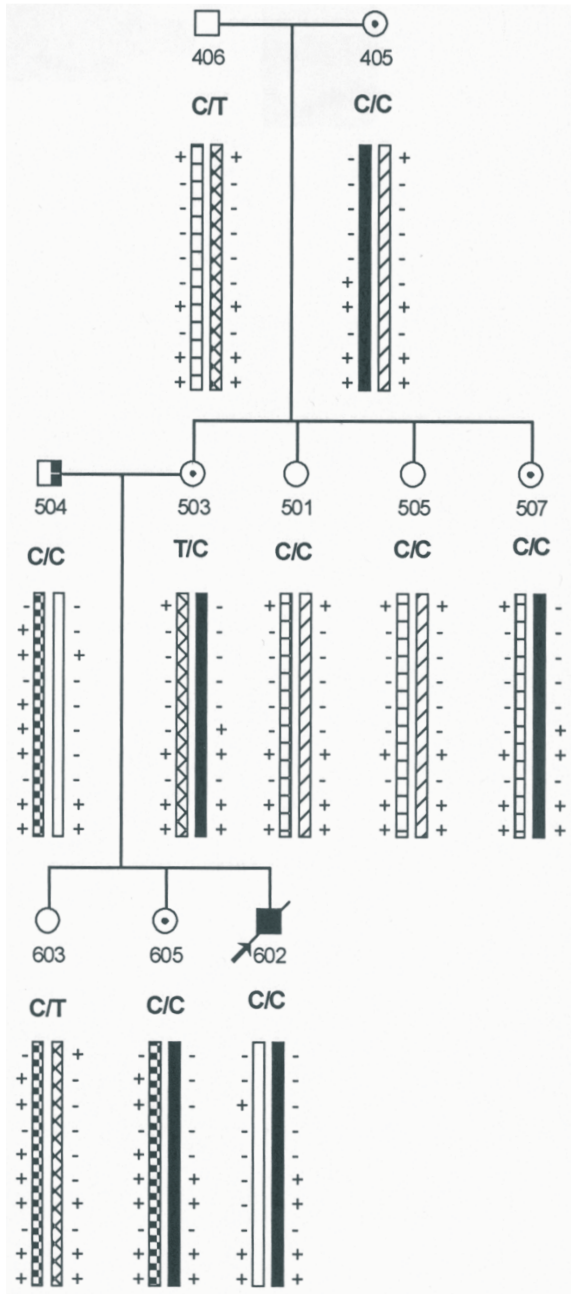
Order of sites from top to bottom: beta-globin codon 2 (C or T), 5'ε (Hinc II); 5'Gγ (Xmn I); Gγ (Hind III); Aγ (Hind III); ψβ (Hinc II); 3' ψβ (Hinc II); 5'β (Hinf I); β (Ava II); 3'β (Hpa I) and 3'β (BamH I).

Haplotype origin: left: paternal; right: maternal. Filled bar: Hb CH; open bar: Hb S.

Symbols: ● Hb CH carrier; ◨ Hb S carrier; ■ Hb S/ Hb CH carrier.

Fig. 1.   Beta globin-like gene cluster haplotypes segregation in the family.

aunt (code 507) and sister (605). The patient sample was unavailable for those studies, since he had died prematurely, but previous studies had shown no increase in Hb F (Table I).

Haplotypes of the β globin-like gene cluster were assessed; these have been reported as prognostic factors in sickle cell disease (18) and are also useful markers to trace the ethnic origin of the HbS mutation (19, 20). Figure 1 shows haplotype segregation in the family. Haplotype –; – ; +; – ; – ; – ; – ; – ; + ; + in phase with the HbS chromosome, has a Bantu origin (19), except for the (–) *Ava II* site, whereas haplotype – ; – ; – ; – ; – ; +; +; – ; +; + , in phase with the Hb CH mutation, seems to be an atypical Benin/Cameroon haplotype (21), with the same exception of the (–) *Ava II* site.

Since previous reports of Hb City of Hope carriers have not mentioned it as being a potentially abnormal variant, three additional possible influencing factors were studied which could explain the patient's evolution and fatal outcome: common α-thalassemia-2 and β-thalassemia determinants, β-chain promoter silencer motifs and a parvovirus B19 infection.

Neither common α-thalassemia-2 determinants ($-\alpha^{3.7}$ and $-\alpha^{4.2}$ deletions) nor β-thalassemia common mutations (changes in codon 39, codon 8 and -27 to -31 upstream bases) were present in the index case, his parents or grandparents and thus, they could not be invoked as concomitant causes for the disease.

The β locus promoter motifs are silencing regulatory sequences to which beta protein 1 (BP1) binds to regulate β globin gene expression (22). Motif $(AC)_2(AT)_7(T)_7$ is the most common worldwide, being considered the reference sequence (16). Promoter motifs are in allelic disequilibrium with haplotypes of the β globin-like gene cluster (23).

Motif $(AC)_2(AT)_8(T)_5$ was in cis with Hb CH, whereas $(AC)_2(AT)_6(T)_9$ was in phase with Hb S (Table II). The $(AC)_2(AT)_8(T)_5$ configuration is almost absent from the Mediterranean basin populations and has a frequency of 54.9% in China (22), being also observed in Cameroon Hb S chromosomes, with a T base at -551 position (16). The $(AT)_6(T)_9$ motif with a C base at -551 position is in allelic disequilibrium with the Bantu haplotype (22, 23). All the found promoter motif configurations in the Hb CH and the Hb S chromosomes (Table II) support the beta globin-like gene cluster African SubSaharan ethnic origin in the present family. The T base at codon 2 polymorphism (histidine in position 3) present in the grandfather was a useful marker to establish haplotype segregation in this family (Fig. 1).

Parvovirus B19 is, predominantly, an erythrophilic virus with a potent inhibitor effect on erythropoiesis, due to its tropism for the dividing erythrocyte precursors. Viral infection destroys immature erythrocytes (reticulocytes) with the resulting decrease in hemoglobin levels. Parvovirus B19 can infect other organs (kidney, heart, liver) causing myocarditis, hepatitis, etc. and several other eventually fatal complications (24). In at least two of the patient post-mortem tissues the virus was present, as demonstrated by the PCR results and the histopathological lesions consisting of nucleolar hypertrophy and brick-red peri-nuclear staining, which are characteristic findings of parvovirus B19 infection (17, 25).

DISCUSSION

More than 700 structural β globin locus point mutations have been described (26), many of them apparently "silent". Little is known about their pathogenic effects in compound heterozygotes; in them, the combination of two substitutions could enhance abnormal consequences of one mutation upon the other, either in the cis or trans configurations in terms of susceptibility to erythrophilic (and other) viral infections. Very scarce information is still available on the regulator influence of the beta gene promoter enhancer or supressor sequences on most of those rare variants. They display quite stratified, but still badly estimated frequencies worldwide.

Ethnic origin of the City of Hope mutation

Hemoglogin City of Hope frequency could have been underestimated so far, since traditional electrophoresis and cation exchange high performance liquid chromatography (HPLC) cannot detect it, and only combined techniques including mass spectrometry can be expected to be totally successful (7). In two independent, more reliable gene frequency estimations done in

TABLE II
β GLOBIN PROMOTER POLYMORPHISMS SEGREGATION IN PARENTS AND CHILDREN: MOTIF (AC)n(AT)x(T)y AND -551T>C. CHROMOSOMAL HAPLOTYPE IN EACH INDIVIDUAL IS SEPARATED BY A SLASH; LEFT: PATERNAL; RIGHT: MATERNAL

Individual code	Motif $(AC)_n(AT)_x(T)_y$	-551T>C
Father (504)	$(AC)_2(AT)_6(T)_9$ / $(AC)_2(AT)_{11}(T)_7$	C/T
Mother (503)	$(AC)_3(AT)_7(T)_5$ / $(AC)_2(AT)_8(T)_5$	T/T
Daughter (603)	$(AC)_2(AT)_{11}(T)_7$ / $(AC)_3(AT)_7(T)_5$	T/T
Daughter (605)	$(AC)_2(AT)_{11}(T)_7$ / $(AC)_2(AT)_8(T)_5$	T/T
Index case (602)	$(AC)_2(AT)_6(T)_9$ / $(AC)_2(AT)_8(T)_5$	C/T

present-day western Caucasoid European samples, it was found to be around 1/2200 (6, 7). This variant has been reported in different genetic backgrounds from several populations: in two Caucasoids (at least one of them Mediterranean) (1, 2); two other Mediterranean ones (from Turkey and Naples) (3,4) and one SubSaharan West African (from Nigeria) (5, 6). There was no published information about the ethnic origin of the two carriers in Switzerland recently reported (7).

β globin-like gene cluster haplotypes in phase with the City of Hope carrier chromosomes are known only for the Italian and Turkish cases, and now in the family herein reported as well, being them different in each instance. One possible explanation for this difference might be recombinant events between the 5' and 3' subhaplotypes within a 9 Kb interval, an interpretation already suggested for the Italian and Turkish families (4). In our family the 3' subhaplotype is different from those of the other families. Another possibility is a rare de novo mutation in each case, which seems less likely than a recombination event, according to Zago et al. (27). These authors, studying several polymorphic markers (including 7 restriction sites) along the beta-like gene cluster in chromosomes with atypical haplotypes, suggest that those are generated by any of three different mechanisms: isolated nucleotide changes in one of the polymorphic restriction sites, as the negative one we found in all examined family members for the *Ava II* site; simple or double crossovers between typical βS or βA haplotypes; or gene conversion (27).

The reported Nigerian Hb CH carrier (5) and the present family both have an African background, since the β globin-like gene cluster haplotype in cis in the Venezuelan Hb CH is a mixed Benin/Cameroon one, except again for a (–) *Ava II* site. Furthermore, the promoter polymorphic motif $(AC)_2(AT)_8 (T)_5$ in cis with the City of Hope mutation in this family has been also reported in Cameroon chromosomes (22). This might indicate a very old SubSaharan predominant origin for the mutation, which is apparently unknown so far in non-Mediterranean Caucasoids or in Mongoloids.

Functional analysis of hemoglobin in the compound heterozygote Hb S/Hb CH

Reported single heterozygotes for Hb CH (at least in 8 non-independent individuals from seven independent families) (1-6) lack hematological manifestations; the only compound heterozygote excepted is a Turkish woman with a $β^o$-thalassemia intermedia phenotype (3). Thus presumably the Gly69Ser substitution has no important effects on the β chain functional properties in single heterozygotes. The glycine residue at position 69 is external to the active center and is not involved in the α1β1 or α1β2 interactions in normal tetramers, and has no direct contact with the heme group (1). Neither Hb Rambam (also known as Hb J-Cambridge) a Gly69Asp substitution (28), nor Hb Kenitra, a beta 69 Gly>Arg change associated with an alpha thalassemia show abnormal hematological parameters attributed to the β69 substitution (29).

The three point mutations in the beta chain at codon 69 (City of Hope, Rambam and Kenitra) are the only ones reported to date in the HbVar database (26).

Only one out of seven hemoglobinopathies quoted in that database (26), due to the compound heterozygosity Hb S /Hb X (beta 6 Glu>Val plus other beta mutation) is in trans, as occurred in the Hb S/Hb CH case. Reported mutations flanking codon 69 (3 beta 68 and 4 beta 70) all show either mild clinical manifestations or a severe hemolytic anemia (Hb Mizuho, a beta 68 Leu>Pro mutation) (26). Another un-

stable low oxygen affinity hemoglobin (Hb Nishinomiya) due to a Leu-Gly insertion between codons 69 and 70 causes spherocytic anemia (30).

Hemoglobin Jamaica Plain (Hb JP, $\beta^{Glu6Val, Leu68Phe}$ in cis) is a sickling-hemoglobin with markedly reduced oxygen affinity (31); molecular modeling of the compound mutant protein $\beta^{Glu6Val, Leu68Phe}$ suggested a destabilization of the oxy conformation in Hb JP, due probably to unfavorable steric interactions of the phenylalanine residue within the folded beta globin chain (31). Hb Loves Park ($\beta^{Leu68Phe}$, also known as Hb Rockford) has been reported to be a low affinity stable hemoglobin (32).

Thus, it seems that changes in sequences adjacent to codon 69 may have some functional consequences, which could possibly be further enhanced by a Hb S mutation in trans.

Despite the beta 69Gly>Ser substitution being located in the heme pocket region, hemoglobin City of Hope is reputed to be a functionally "silent" change, and it is electrophoretically indistinguishable from Hb A; however the serine residue has a side chain slightly longer than glycine, and this could cause some steric disturbance in the conformation of the folded molecule. Such a situation might be functionally important and thus symptom-prone, when the beta chain conformation in trans is besides, an abnormal one as is the case with Hb S. It has been shown that the area between residues $\beta66$ and the close $\beta73$, is an important area of contact between tetramers of the polymer Hb S/Hb X in trans (X being a β-chain variant), affecting polymerization of the hybrid hemoglobin ($\alpha_2 \beta^S \beta^X$) (33).

In addition to the eventual intrinsic effect of the concurrence of these mutations in trans herein reported, haplotype backgrounds in the beta-like gene cluster (19, 21) and in the beta gene promoter region (16, 22, 23) probably produce important consequences on the severity and course of the phenotype. The Bantu haplotype of the beta globin-like gene cluster in cis with the Hb S mutation in the Venezuelan family may be associated with serious immunodeficiency and sickle cell anemia. This has been formerly attributed (23) to the promoter $(AC)_2(AT)_6(T)_9$ Bantu configuration, which has the weakest affinity for the repressor BP1 protein, producing a greater beta gene transcription in cis of the abnormal hemoglobin. Since erythrocytic concentration of Hb S is a prime determinant in its intracellular polymerization, the low BP1 repressor effect associated with the Bantu motif could result in an increased beta S chain production. It is a condition that favors polymerization, sickling and red cell destruction, with its ensuing effects (23).

Additionally our index case was homozygous -/- for the *Xmn I* (-158C>T substitution 5' to $^G\gamma$) site. Base T at -158 position creates a recognition sequence for *Xmn I*, which has been associated with a three to eleven fold increase in $^G\gamma$ gene expression (34) and a significant raise in Hb F levels (35), which can ameliorate sickle cell disease manifestations; as expected, Hb F was not raised in our patient.

On the other hand, the $(AC)_2(AT)_8(T)_5$ Cameroon configuration in cis with the City of Hope mutation has shown high mRNA expression levels in plasmid constructs expressed in transfected murine erythroleukemia cells (16). However, relative synthesis levels of β^S and β^A mRNAs in heterozygotes are balanced (around 50% of each one) but it is not so for the relative percentage of blood hemoglobin proteins (36), as was observed in the present case which had 55.6% Hb CH and 38.5% Hb S (Table I) at 11 months of age.

Whether this potentially high level of the City of Hope hemoglobin in the patient influenced its interaction with the Hb S in trans, is presently unknown.

Parvovirus B19 infection and compound heterozygosity

This compound heterozygous in trans Hb S /Hb CH patient was apparently healthy during his first 4 months of life. Since then, he suffered repeatedly from different infections; an enlarged liver and spleen were detected already at seven months of age.

Although his hematological parameters were early almost "normal"; at the age of three years he was suffering from aplastic anemia, dying finally due to an acute cardiac failure and a septic shock. Since he was born in apparent good health and remained so for several months, an external cause seemed to be the precipitating factor for his ailments from then on. The parvovirus B19 infection demonstrated 5 years post-mortem, is an established noxious variable, being most probably in direct relation to the serious cardiac lesions as was proven by both histopathological and viral DNA findings.

It is known that clinical manifestations associated with parvovirus B19 infection depends on the patient's immunological and hematological status. In immunocompetent individuals, B19 infection is usually benign. Immunodeficient subjects may become chronically infected, and patients with hematological disorders like sickle cell disease are at high risk of suffering a severe aplastic crisis (37) because B19 infection can suppress erythropoiesis. Furthermore a sickle cell anemic patient has been reported to develop a fulminant myocarditis following a B19-related aplastic crisis (38). This also occurred in our patient who was not however a Hb S/Hb S homozygote, but instead an apparent Hb S/Hb A heterozygote who later was proven to be a compound heterozygote Hb S/unrevealed Hb CH.

This patient is the first reported compound heterozygote in trans for hemoglo-

bins S and City of Hope, jointly with an unfavorable genetic background of a likely African origin from both parental lines in whom a deleterious viral chronic infection precipitated a fatal outcome. The β globin Gly69Ser mutation therefore should not be considered any longer as a functionally silent one, at least in its trans combination with any other known abnormal β globin hemoglobin. This notion would be of great help in the genetic counseling of carrier couples under a similar risk. They are to be considered within the same recurrence risk group as any other pair of single heterozygous carriers at least of hemoglobin S. That should be also the same conclusion in the face of any eventual cross between a single Hb S heterozygous and a "homozygous" Hb A/Hb A partner not adequately tested for the presence of Hb CH.

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