

## Different geographic origins of Hb Constant Spring [ $\alpha_2$ codon 142 TAA→CAA]

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**Background and Objectives.** The occurrence of Hb CS is usually limited to the geographic area which includes Southern China and South East Asia. In 1968 Hb CS was also found to occur in the Mediterranean area where it was originally described as Hb Athens. We investigated the independent origin of these termination codon mutations of the  $\alpha_2$ -globin gene by determining the  $\alpha$ -cluster haplotype and comparing the hematologic data from Hb CS-Hb H patients and their family members.

**Design and Methods.** We studied one Hb CS-Hb H patient of Greek origin and a Sicilian family in which one individual was affected by Hb CS-Hb H. The haplotype of the Hb CS allele was determined and compared to the haplotype of an Hb CS-Hb H individual of Chinese origin.

**Results.** The haplotype found for the Greek and Sicilian Hb CS was the same but differed significantly from the Asiatic Hb CS mutation.

**Interpretation and Conclusions.** The Hb CS mutation found in both Mediterranean patients arose independently in the Mediterranean area. The difference in clinical manifestation of the Hb CS-Hb H disease in both patients is less common but consistent with similar variation in the clinical expression of analogous Hb Icaria-Hb H disease patients.

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Key words: hemoglobin, thalassemia, Hb CS,  $\alpha$ -globin gene mutation, Hb H disease

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**H**emoglobin Constant Spring (CS) is characterized by an elongated  $\alpha$  chain due to a T→C transition of codon 142 of the  $\alpha_2$ -globin gene. Heterozygosity for this mutation is usually associated with mild anemia, microcytosis and thalassemic red cell morphology. Compound heterozygosity of an  $\alpha^0$ -thalassemia determinant with Hb CS results in a form of Hb H disease more severe than that caused by the interaction with a deletional  $\alpha^+$  allele. The severity of the clinical picture in patients with the Hb CS/ $\alpha^0$  combination is correlated with the higher degree of  $\alpha$ -chain deficiency observed in patients with this genotype in comparison with the deletional Hb H disease. The occurrence of Hb CS is usually limited to the geographic area which includes Southern China and South East Asia. In Thailand particularly, the frequency of this  $\alpha$ -globin mutant attains polymorphic levels; about 50% of the patients with symptomatic Hb H disease in this area have compound heterozygosity of Hb CS with the common S.E. Asian  $\alpha^0$  deletion ( $-^{\text{SEA}}$ ). Hb CS was reported in a Greek patient in 1968 by Sofroniadou *et al.*<sup>1</sup> under the name of Hb Athens. It was subsequently confirmed at the protein level to be identical to Hb CS.<sup>2</sup> We report here two cases of Hb H disease, resulting from the interaction of Hb CS with a deletional  $\alpha^0$  determinant, one in a Greek family and one in a Sicilian family.

### Design and Methods

Blood samples were collected into vacutainers with Li Heparin or Na<sub>2</sub> EDTA as anticoagulants and hematology was assessed in Greece and Sicily according to standard methods.<sup>3</sup> Genomic DNA was isolated from white blood cells by high salt and send to a laboratory in The Netherlands.<sup>4</sup> The *in vitro* synthetic ratio between the  $\alpha$ - and non- $\alpha$ -globin chains was measured according to standard methods.<sup>5</sup> Southern blotting was performed using the restriction enzymes EcoRI, BglII, RsaI, HindIII, XbaI, BamHI and SacI. The following probes

**Table 1. Hematology of families A and B.**

Indiv.	Sex	Hb g/dL	RBC ( $10^{12}/L$ )	Ht (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	HbA2 (%)	HbF (%)	HbH (%)	Hb CS (%)	$\alpha/\beta$ ratio	Inclusion bodies (%)	Genotype
A II.1	M	9.5	5.6	38	68	16.9	25	2.7	2.5	17.8	0.9	n.d.	many	$\alpha^{CS}\alpha/-_{Med1}$
B I.1	M	14.6	5.09	44	86	28.7	33	2.2	1.04	absent	0.7	1.01	n.d.	$\alpha^{CS}\alpha/\alpha\alpha$
I.2	F	10.2	5.11	29	57	20.0	35	1.03	1.15	9.2	absent	0.2	2.5	$-\alpha^{3.7}/-_{Cal}$
II.1	M	10.3	4.8	30	62	21.5	34	2.3	n.d.	absent	absent	n.d.	n.d.	$\alpha\alpha/-_{Cal}$
II.2	F	5.6	3.43	20	58	16.3	28	0.8	2.0	10.0	0.8	0.48	4.8	$\alpha^{CS}\alpha/-_{Cal}$

n.d. = not done.

**Table 2. Haplotype analysis of the Hb CS alleles.**

	5'HVR (kb)	XbaI	SacI	Inter- $\zeta$ HVR	Intra- $\zeta$ HVR	RsaI	3'HVR (kb)
Mediterranean Hb CS (n=4)	5.5	-	+	L	PZ	-	4.0
Chinese Hb CS (n=1)	3	+	+	M	PZ	+	2.1

were used for hybridization; the 1.7 kb AluI fragment from pSG21 containing the IZHVR, the 1.7 kb fragment from the p $\zeta$ BR, the 1.5 kb PstI fragment containing the  $\alpha_1$  globin gene and the 4.0kb HinfI fragment from p $\alpha$ 3'HVR.64 containing the 3'HVR.6 DNA of the  $\alpha_1$  and  $\alpha_2$ -globin genes was selectively amplified using a forced water circulation thermocycler. To detect point mutations in the  $\alpha$ -globin genes the  $\alpha$ -gene specific amplified fragments were subjected to single strand conformation analysis (SSCA) followed by direct solid phase sequencing as previously described.<sup>7</sup>

## Results

The SSCA analysis of the 3' non-translated fragment of the  $\alpha_2$ -globin gene revealed the reproducible pattern associated with the TAA $\rightarrow$ CAA transition at the termination codon; the identification was confirmed by DNA sequencing.

The characterization of the  $\alpha^0$  mutations disclosed a  $-\text{MED}1$  deletion in the Greek proband and a  $-\text{CAL}$  deletion in the Sicilian family. Hematologic findings (Table 1) indicate the diagnosis of Hb H disease in the three individuals who were compound heterozygotes for an  $\alpha^0$  and an  $\alpha^+$  determinant. The Hb CS mutation was found to be on an identical haplotype in both the Greek and Sicilian patients. This haplotype is completely different from that harboring the Asiatic Hb CS mutation (Table 2).

## Discussion

We observed a different clinical expression of Hb H disease in the two patients of Sicilian pedigree with this disease. The proband ( $\alpha^{CS}\alpha/-_{CAL}$ ) is rather severely anemic, with pronounced splenomegaly and requires occa-

sional blood transfusions, whereas her mother ( $-\alpha^{3.7}/-_{CAL}$ ), is almost asymptomatic, has never had hemolytic crises and received transfusions only during pregnancy. This is consistent with the observation that the clinical manifestations of Hb H patients with non-deletion  $\alpha$ -thalassemia defects in the  $\alpha_2$ -globin gene tend to be more severe than those in patients with single  $\alpha$ -gene deletion determinants. In contrast the Greek (AII.1) proband, carrying a similar genotype as the Sicilian (BII.2) proband, shows milder hematologic signs and preserves adequate hemoglobin levels (9.5 g/dL). This milder clinical expression of Hb CS-Hb H disease is less common, but is consistent with a similar variation in the clinical expression of analogous Hb Icaria-Hb H disease patients.<sup>8-10</sup>

On the basis of the difference in haplotype between the Asiatic Hb CS mutation on the one hand and the Greek and Sicilian Hb CS mutation on the other, we suggest that the Hb CS mutation found in both Mediterranean patients arose independently in the Mediterranean area. Its observation in two geographically close populations indicates that it was probably transferred by gene flow between Greece and Sicily, as has been observed for other disease-causing mutations, for example a common low density lipoprotein receptor gene mutation (G528D) underlying familial hypercholesterolemia.<sup>11</sup>

Hemoglobin CS has also been detected in the United Arab Emirates in a few individuals with Hb H disease.<sup>12</sup> The  $\alpha$ -haplotype associated with Hb CS in this country has not yet been characterized. It is likely that the Greek Hb CS haplotype spread out, like Hb Icaria, farther than Sicily and now contributes to the morbidity from Hb H disease in the Mediterranean area and in the near East.

## Contributions and Acknowledgments

CLH, PCG, LFi were involved in DNA sequencing and haplotype analysis, writing the paper and scientific input. JT, CK, and EK, medical and scientific staff in Greece, played a role in recognition and hematologic analysis of the Greek family. AR, MF, GS, medical and scientific staff in Sicily, played a role in recognition and hematologic analysis of the Sicilian family.

### Disclosures

*Conflict of interest: none.*

*Redundant publications; no substantial overlapping with previous papers.*

### Manuscript processing

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### Potential implications for clinical practice

In Mediterranean countries Hb H disease may result from the interaction of Hb C with a deletional  $\alpha^0$  mutant.

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