Origin and spread of HbG-San Josè in Southern Italy

We found that the HbG-San Josè mutation was associated with haplotype VII [- - - - / - +] in four families from Campania and in one family from Calabria, and with the haplotype IV [+ - + + / - +] in two families from Eastern Sicily. These results support the hypothesis that the mutation had a unique origin in Southern Italy and a subsequent recombination event gave rise to the background heterogeneity.

HbG-San José or β7(A4)Glu→Gly, first described in a family of Calabrian origin,[1] has been reported in a few families from Southern Italy, Calabria[2] and Eastern Sicily,[3,4] and in Mexico.[5] The mutant allele has been found associated with haplotype IV in a cluster of four families and three unrelated heterozygotes identified in Grammichele, a small town near Catania.[6] These findings have led to the hypothesis that the mutation had a unique origin in Eastern Sicily and that its prevalence in Grammichele was due to a founder effect.[6]

Here we report the restriction fragment length polymorphism (RFLP) characterization of haplotypes associated with the HbG-San José allele in seven families originating from Southern Italy. The observed heterogeneity of the chromosomal background of the haplotypes gave new insights into the origin and spread of the mutation in Italy.

Blood samples were collected into vacutainers with Na_2EDTA as anticoagulant. Hematologic parameters were measured by standard methods. Ion exchange high performance liquid chromatography (HPLC) of hemoglobin (Hb) was carried out using the Diamat System (Bio-Rad Laboratories, Richmond, CA, USA). DNA was prepared from white blood cells by the high salt method. A DNA 609 bp fragment was amplified using the primers: 5'-TAAGCCAGTGCCAGAAGAGCC-3' (-138//-158) and 5'-CACTCAGTGTGGCAAAGGTG-3' (+432//+541). DNA sequencing was performed by polymerase chain reaction (PCR) amplification and direct sequencing.[7] RFLP characterization of the β-globin gene cluster was carried out as previously reported[7] or by digestion of the specific PCR amplified fragments.[8]

Seven unrelated families originating from three different regions of Southern Italy, four from Naples, one from Calabria and two from Eastern Sicily were studied. The heterozygous subjects did not have any clinical symptoms. They had normal hematologic parameters and 28-38 % of a Hb variant eluted after the HbA2. The GAG→GGG mutation at codon 7, leading to the Glu→Gly amino acid substitution in the β-globin chain, was characterized by DNA sequencing. Hind III/Gγ and Aγ, Hinc II/φβ and 3' φβ, Ava II/β and Bam HI/3' β RFLPs were characterized in all the patients and their relatives. Family linkage analysis indicated that the HbG-San Josè mutation was associated with two different haplotypes: VII and IV (Figure 1). Both haplotypes showed the same 3' subhaplotypes [- +] due to the absence of the Ava II/β and to the presence of the Bam HI/3' β restriction site . In contrast, the four families from Campania and the patient from Calabria had the 5' subhaplotype [- - - -], whereas the two families from Eastern Sicily had the 5' subhaplotype [+ - + +].

This heterogeneity may be explained by two mechanisms: 1) a unique origin of the mutation followed by a recombination event; 2) recurrent origin. Two considerations render the first hypothesis more likely. First, the Hb variant allele is associated in all cases with the same 3' subhaplotype [- +]. Second, this 3' subhaplotype is rare, being associated only with haplotypes IV, VI and VII, which have a frequency of 3.3%, 0% and 9.8%, respectively, in the Mediterranean population.[9] It appears unlikely that recurrent mutational events have occurred on a rare 3' subhaplotype. In this unicentric hypothesis the heterogeneity of the 5' subhaplotype can be explained by a subsequent recombination event occurring in a 12 kb region located between the two subhaplotypes (Figure 1), which is reported to have high recombination rate.[10]

The results reported here indicate that HbG-San Josè-haplotype VII spread in large areas of Southern Italy, such as Campania and Calabria and that HbG-San Josè-haplotype IV spread in a restricted region around Catania, including Grammichele.[6] The clustering of HbG-San Josè-haplotype IV in Eastern Sicily might be due to a founder effect, as already proposed for Gram-

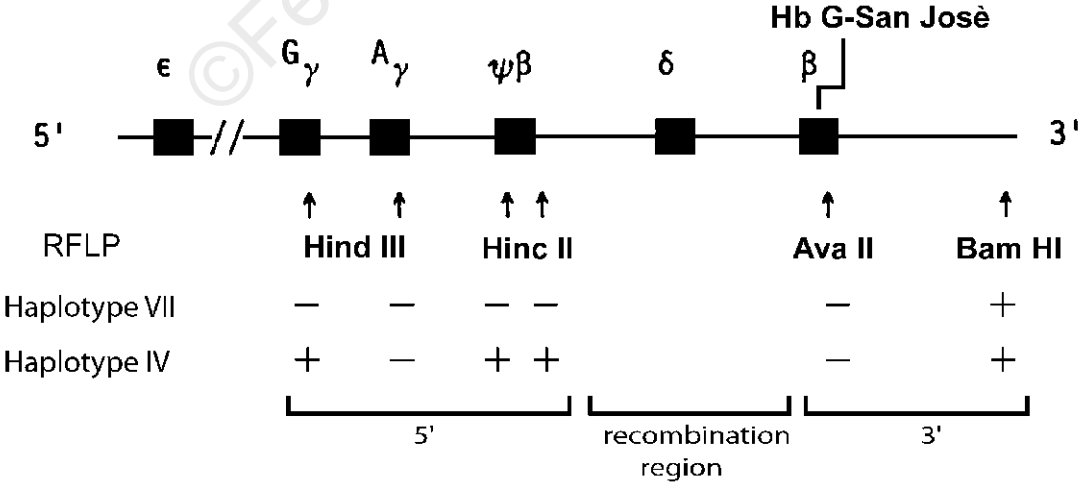

Figure 1. Top: β-like globin gene cluster with the HbG-San Josè allele and with the position of the principle RFLPs. Bottom: Haplotypes associated with the HbG-San Josè allele. Left, the 5' subhaplotype; right the 3' sub-haplotype; in the middle the region of recombination of the two subhaplotypes.

michele.[6] Knowledge of the haplotype associated with the HbG-San Josè in other families, particularly from Western Sicily, would be of great interest in order to trace the spread of this mutation.

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References

1. Hill RL, Swenson TR, Schwartz HC. Characterization of a chemical abnormality in Hb G. J Biol Chem 1960; 235: 3182-7.
2. Brancati C, Marinucci M, Borghesi V, Tentori L. HbG-San José in a Calabrian family. Atti XXVI Congresso Nazionale Italiano Ematologia, 1977; Torino. p. 777.
3. Musumeci S, Schilirò G, Pizzarelli G, et al. Hemoglobin G San Josè [β2 7 (A4) Glu to Gly α2], β thalassemia, and α thalassemia in a Sicilian family. Hum Genet 1979; 52:239-47.
4. Schilirò G, Li Volti S. Did the HbG San Josè gene arise in Sicily? J Med Genet 1986; 23:281.
5. Espinosa Jurcott J, Landero de Ruiz N, Armenta Olvera T, Murillo M, Ruiz Reyes G. Hemoglobin G-San Josè associated with hereditary spherocytosis in a Mexican family. Rev Invest Clin 1981; 33:383-6.
6. Cremonesi L, Travi M, Li Volti S, Testa R, Schilirò G, Ferrari M. Evidence for the single origin of HB G-San Josè in Sicily. Hemoglobin 1989; 13:579-84.
7. Lacerra G, Fioretti G, Hani A, et al. Hb O-Arab [β121 (GH4)Glu→Lys]: association with DNA polymorphisms of African ancestry in two Mediterranean families. Hemoglobin 1993; 17:523-35.
8. Kulozik AE, Lyons J, Kohne E, Bartram CR, Kleihauer E. Rapid and non-radioactive prenatal diagnosis of β thalassaemia and sickle cell disease: application of the polymerase chain reaction (PCR). Br J Haematol 1988; 70:455-8.
9. Antonarakis SE, Boehm CD, Serjeant GR, Theisen CE, Dover GJ, Kazazian HH Jr. Origin of the β S-globin gene in blacks: the contribution of recurrent mutation or gene conversion or both. Proc Natl Acad Sci USA 1984; 81:853-6.
10. Chakravarti A, Buetow KH, Antonarakis SE, Waber PG, Boehm CD, Kazazian HH. Nonuniform recombination within the human β-globin gene cluster. Am J Hum Genet 1984; 36:1239-58.