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The S65C mutation in Spain. Implications for iron overload screening

Hereditary hemochromatosis is related to mutations of the HFE gene. The role of the S65C mutation of this gene was evaluated in a Spanish population, consisting of 100 controls and 41 patients who had resulted positive to screening for iron overload. Only one patient was heterozygous for the S65C mutation, so the S65C mutation is infrequent in our area. Nevertheless, it is advisable to search for this mutation in cases with iron overload and heterozygosity for the C282Y or H63D mutations of the *HFE* gene.

Sir,

Hemochromatosis is a hereditary disorder that is common in people of European descent. Most cases are attributable to homozygosity for the mutation C282Y in the *HFE* gene, or to compound heterozygosity for the C282Y and the H63D mutations.[1,2] However, a few individuals with clinical hemochromatosis lack these phenotypes.[1,2] Other mutations of the *HFE* gene could account for some of these cases.[3]

In this regard, a third mutation in the *HFE* coding region (exon 2, nt 193A→T, S65C) has been described in hemochromatosis patients (compound heterozygotes) and controls in Brittany and the USA.[4,5] The proximity of the S65C and H63D substitutions suggests that the former plays a role in hemochromatosis.[6] The S65C mutation was evaluated in 141 individuals in whom C282Y and H63D homozygosities and compound heterozygosity had been ruled out. The S65C mutation was studied with a *Hinf*1 digest using the conditions for the H63D analysis.[2,4]

These subjects included 100 control subjects (selected from a large group so that all *HFE* mutations would be represented) and 41 patients who were positive to screening tests for iron overload (transferrin saturation exceeding 50% and serum ferritin exceeding 450 µg/L) (Table 1). In the latter group, 2 patients had iron overload (measured in a liver biopsy) and 33 patients had hepatitis C virus infection: their characteristics have been reported elsewhere.[7]

None of the cases in the control group had the S65C mutation and only one patient out of 41 who screened positive for iron overload was heterozygous for the S65C mutation (genotype CC/HH/SC). When both groups were combined, the estimated allelic frequency was 0.36± 0.03%.

The frequency of the S65C substitution was lower in our population than in others from different areas (5.5% in Utah, 1.95% in Brittany and 0.6% in Alabama),[4,5] in contrast to the frequency of the H63D mutation (15-20%), which was higher.[8,9] It should be pointed out that the frequency observed in our study is probably an overestimation given that we included a number of patients with positive iron overload screening tests.[7] The role of this mutation in iron abnormalities of hepatitis C virus infected patients is negligible.[7]

Table 1. Genotype results of the population in which the S65C substitution was studied.

Genotype C282Y	Genotype H63D	CONTROLS (N#100) +/total	HCV AND OTHER (N#41) +/total
+/-	-/-	0/40	0/4
-/-	+/-	0/30	0/17
-/-	-/-	0/30	1/20*

**This patient was heterozygous (SC) and had hepatitis.*

However, a number of arguments justify the evaluation of this substitution in patients in whom the C282Y and H63D mutations do not account for the iron overload. First, the laboratory analysis of this mutation is fairly simple.[4] Second, some compound heterozygotes for S65C have iron overload.[4,5] Finally, using the crystal structure of the HFE molecule, the position of the S65C mutation within the α1 domain suggests that it has a role in the function of the *HFE* protein.[10]

In conclusion, the S65C mutation of the *HFE* gene is very rare in our area of Spain (Northeast). Nevertheless, its evaluation is important when iron overload is demonstrated and heterozygosity for the other mutations (C282Y and H63D) is present.

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References

1. Pietrangelo A, Camaschella C. Molecular genetics and control of iron metabolism in hemochromatosis. Haematologica 1998; 83;456-61.

2. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary hemochromatosis. Nat Genet 1996; 13:399-408.
3. de Villiers JN, Hillermann R, Loubser L, Kotze MJ. Spectrum of mutations in the HFE gene implicated in haemochromatosis and porphyria. Hum Mol Genet 1999; 8:1517-22.
4. Mura C, Raguenes O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 1999; 93:2502-5.
5. Barton JC, Sawada-Hirai R, Rothenberg BE, Acton RT. Two novel missense mutations of the HFE gene (I105T and G93R) and identification of the S65C mutation in Alabama hemochromatosis probands. Blood Cells Mol Dis 1999; 25:146-54.
6. Aguilar Martínez P, Biron C, Blanc F, et al. Compound heterozygotes for hemochormatosis gene mutations: may they help to understand the pathophysiology of the disease? Blood Cells Mol Dis 1997; 23:269-76.
7. Remacha AF, Carrasco M, Sardá MP, Barceló MJ, Baiget M. HFE mutation analysis in patients with hepatitis C virus with positive screening for iron overload. Haematologica 1999; 84:284-5.
8. Baiget M, Barceló MJ, Gimferrer E. Frequency of the HFE C282Y and H63D mutations in distinct ethnic groups living in Spain. J Med Genet 1998; 35:701.
9. Sánchez M, Bruguera M, Bosch J, Rodés J, Ballesta F, Oliva R. Prevalence of the Cys282Tyr and His63Asp HFE gene mutations in Spanish patients with hereditary hemochromatosis and in controls. J Hepatol 1998; 29:725-28.
10. Bennett MJ, Lebron JA, Bjorkman PJ. Crystal structure of the hereditary haemochromatosis protein HFE complexed with transferrin receptor. Nature 2000; 403:46-53.

Differences in phenotype, growth factor requirements, pattern of expression of adhesion molecules and rate of apoptosis displayed by three new myeloid sister leukemic cell lines

We established three new human myeloid cell lines from one patient, in the presence of granulocyte-macrophage colony-stimulating factor (UPM1-GM), interleukin-3 (UMP1-IL-3) or without exogenous growth factors (UPM1). The 3 lines were characterized by phenotypic, genotypic and functional studies. These cell lines may provide useful tools to study different aspects of leukemic cell biology.[1]

Three sister cell lines were established from a bone marrow sample (80% myeloblasts), collected in a resistant phase after 2 courses of induction chemotherapy (mitoxantrone/cytarabine) of an acute myeloid leukemia, FAB type M4Eo. The cells were initially cultured in RPMI 1640 with 20% fetal bovine serum.[2] The 3 cell lines share cytogenetic abnormalities with the patient's karyotype (43-45,XX,del(5)(q13q33), -7,-16,-18, add(21)(q22),+1-2mar,dmin), but have some differences between them (Table 1). The 3 lines express myeloid antigens and are strongly positive for LFA-1 (CD18/CD11a) (Table 1).

Table 1. Cytogenetic and immunologic characterization of UPM-1, UPM1-GM and UPM1-IL3 cell lines.

	UPM1	*UPM1-GM*	*UPM1-IL3*
Chrom. abnormalities	*del(8),hsr*	*+11,del(22)(q13)*	*add(1)(p36),add(11)(p15),del(22)(q13)*
Phenotype			
CD34	**88%**	**73%**	**63%**
CD38	**10%**	**86%**	**97%**
CD117	**72%**	**43%**	**72%**
HLA-DR	82%	72%	74%
CD13	99%	89%	92%
CD33	96%	86%	89%
CD15	**25%**	**43%**	**45%**
CD71	88%	85%	82%
MPO	**27%**	**36%**	**34%**
CD7	**40%**	**28%**	**31%**
CD69	42%	69%	36%
CD54	3%	10%	16%
CD11a	98%	95%	95%
CD11b	**7%**	**22%**	**14%**
CD11c	**8%**	**40%**	**20%**
CD18	98%	95%	98%
others	neg	neg	neg
CD34/CD33	89%	65%	66%
CD33/CD15	**35%**	**58%**	**50%**

(Others) B-lineage (CD19, CD10 and cytoplasmic CD22) and T-lineage (CD3, CD2 and cytoplasmic CD3) markers, CD14, CD16, CD21, CD23, CD56, CD61, LF, TdT; (neg) < 20% of positive cells; antigens with different expression in the three cell lines are in bold.

UPM1 cells are small and morphologically less differentiated, grow in suspension (doubling time-196 hours) and have little CD38 expression. UPM1 cells grow slower than the 2 other lines, as measured by thymidine incorporation (see ref. #3 for details) and clonogenic assays, and have a higher percentage of spontaneous apoptotic cells, as measured by sub-G1 peak and AO/EB staining.[4] Interleukin-3 (IL-3) (10 U/mL) and granulocyte-macrophage colony-stimulating factor (GM-CSF) (10 ng/mL) increased UPM1 proliferation (3.3 times, p=0.032, and 2.6 times, p=0.049, respectively) and decreased apoptosis[5] by 40%. UPM1 growth was also stimulated by granulocyte colony-stimulating factor (G-CSF, 10 ng/mL) and stem cell factor, SCF (10 ng/mL), flt3 ligand, FL (100 ng/mL) and the GM-CSF+IL-3, PIXY321(10 ng/mL)+SCF, or PIXY321+FL combinations (more than 2.0 times the proliferation of control cells).

The UPM1-GM cell line was established in the presence of GM-CSF. This is the fastest growing line (doubling time-89 hours), with larger, more