S65C frequency in Italian patients with hemochromatosis, porphyria cutanea tarda and chronic viral hepatitis with iron overload

We studied the frequency of S65C variant in patients with hereditary hemochromatosis, porphyria cutanea tarda and chronic viral hepatitis with hepatic iron overload. Results suggest that S65C has a role in mild to moderate forms of iron overload in the compound heterozygote state with C282Y and possibly with H63D.

Two missense mutations, C282Y and H63D, were originally described in the HFE gene.[1] Another variant, S65C (193 A→T), was found to be enriched in French hereditary hemochromatosis (HH) probands with at least one non-C282Y and non-H63D chromosome, but the role of this mutation in HH is controversial.[2-4] HFE mutations confer susceptibility to porphyria cutanea tarda (PCT)[5] and to the development of iron accumulation in patients with chronic viral hepatitis (CH).[6] There are no data on S65C frequency in these groups of patients in Italy.

Since S65C variant is found only in non-C282Y and non-H63D chromosomes[2] we excluded C282Y and H63D homozygotes and compound heterozygotes. We selected 36 unrelated probands with HH, 28 patients with PCT, 76 with CH and iron overload, and 258 healthy controls.

The diagnoses of HH, PCT and CH were based on classical, previously reported criteria.[5-7] Serum iron indices and hepatic iron stores were measured as described elsewhere.[7] Genomic DNA was extracted from peripheral blood leukocytes. C282Y, H63D and S65C mutations were analyzed using standard polymerase chain reaction (PCR) and restriction enzyme digestion with Rsa I, Bcl I and Hinf I, respectively.[1,2] The frequency of S65C mutation in HH probands, PCT, CH patients and healthy controls was compared by Fisher's exact test.

The number of non-C282Y non-H63D chromosomes analyzed was 54 from patients with HH, 47 from those with PCT, 123 with CH and 444 in controls. The prevalence of S65C allele in controls (0.009) was lower than that reported in northern European populations.[8] and not significantly different to that found in the other groups studied: HH (0.0185), PCT (0.0213), and CH (0.024). In C282Y heterozygous HH patients the prevalence of S65C in *at risk* chromosomes was 0.2 (1/5), 20 times higher than in controls; in H63D heterozygous PCT patients, the prevalence of S65C was 14 times higher than in controls (Table 1). No additional mutation in the HFE sequence was found in our patients, as in the S65C carriers with iron overload described by Mura *et al.*[2] Our results are in agreement with those reported by Mura *et al.*[2] in the C282Y heterozygotes in whom the frequency of S65C was 0.16 in HH compared to 0.02 in controls (p = 0.028, Fisher's exact test). Although it is difficult to determine the biological effect of the S65C mutation because of its low frequency in the population and its mild phenotype, together these results suggest that S65C variant may have a role in the development of iron overload in the compound heterozygous state with C282Y. S65C variant is located close to H63D and may affect the affinity of transferrin receptor for transferrin similarly.[9] Accordingly, Beutler *et al.*[4] showed that the S65C mutation affects transferrin saturation, although to a lesser extent than the C282Y and H63D mutations. Thus, S65C/C282Y compound heterozygosity should have a very mild phenotype expression and a low penetrance. The clinical and histologic data of the patients carrying the S65C mutation are reported in Table 2. Interestingly the C282Y/S65C woman with HH has two other factors that could have favored the development of iron overload in the range of HH: early amenorrhea and the β-thalassemia trait.[10] It is possible that a compound heterozygote of S65C mutation with H63D could also develop a mild form of iron overload and that the presence of other factors, either acquired or inherited may influence the expression of iron overload. In the PCT patient the absence of other additional factors suggests that the mutated genotype itself is implicated in the clinical manifestations of the disease.[5] Due to the complex mechanisms involved in the development of iron overload in PCT[5] and CH patients,[6] larger studies are needed to clarify the role of S65C in these patients.

Table 1. Allele frequency of the S65C variant in non-C282Y, non-H63D chromosomes of patients with hereditary hemochromatosis (HH), porphyria cutanea tarda (PCT), chronic viral hepatitis (CH) with iron overload and in healthy controls.

Genotype		S65C/neither C282Y nor H63D allele			
C282Y	H63D	HH	PCT	CH	Controls
+/-	-/-	0.2 (1/5)	0 (0/2)	0 (0/3)	0 (0/10)
-/-	+/-	0 (0/13)	0.14 (1/7)	0.038 (1/26)	0 (0/58)
-/-	-/-	0 (0/36)	0 (0/38)	0.021 (2/94)	0.011 (4/376)

Paola Trombini, Viviana Mauri,* Alessandra Salvioni,* Chiara Corengia,* Cristina Arosio,° Alberto Piperno**

**Clinica Medica, Università Milano - Bicocca, Ospedale S.Gerardo, Monza, Italy; °Istituto Auxologico Italiano, Ospedale S.Luca, Milan, Italy*

Funding: VM is supported by a grant from the Association for the Study of Hemochromatosis and Iron Overload Disorders, Monza.

Key words: hemochromatosis, HFE, S65C, iron overload.

Correspondence: Alberto Piperno, M.D., Clinica Medica Ospedale S.Gerardo, via Donizetti 106, 20052 Monza, Italy. Phone: international +39-039-2333361/57 - Fax: international +39-039-322274 - E-mail: alberto.piperno@unimi.it

Table 2. Main data of the patients carrying the S65C allele.

Subject	Sex	Age (yrs)	Disease	HFE genotype	TS (%)	SF (mg/L)	Scheuer's grade	Liver histology	HIC (mmol/g)	IR (g)
D.F.*	F	45	HH	C828Y/S65C	68	800	4	Normal	257	7
V.V.	M	64	PCT	H63D/S65C	41	520	2	Mild fibrosis	59.4	1.8
F.E.	M	52	CH	H63D/S65C	68	501	3	CAH	-	-
A.L.	M	33	CH	wt/S65C	35	571	3	CAH	-	-
L.B.	M	70	CH	wt/S65C	49	413	2	CAH	-	1.2

*D.F.*carries β-thalassemia trait and developed amenorrhea before the age of 40. TS = transferrin saturation; SF = serum ferritin; HIC = hepatic iron concentration; IR = iron removed by phlebotomy; CH = chronic hepatitis C; CAH = chronic active hepatitis.*

References

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
2. Mura C, Raguenes O, Férec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. Blood 1999; 93:2502-5.
3. Arya N, Chakrabrati S, Hegele RA, Adams P. HFE S65C variant is not associated with increased transferrin saturation in voluntary blood donors. Blood Cells Mol Dis 1999; 25: 354-7.
4. Beutler E, Felitti VJ, Ho NJ, Gelbart T. Commentary on HFE S65C variant is not associated with increased transferrin saturation in voluntary blood donors by Naveen Arya, Subrata Chakrabrati, Robert A. Hegele, Paul Adams. Blood Cells Mol Dis 1999; 25:358-60.
5. Sampietro M, Piperno A, Lupica L, et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. Hepatology 1998; 27:181-4.
6. Vergani A, Bovo G, Trombini P, et al. Semiquantitative and qualitative assessment of hepatic iron in patients with chronic viral hepatitis: relation with grading, staging and haemochromatosis mutations. Ital J Gastroenterol Hepatol 1999; 31:395-400.
7. Piperno A, Sampietro M, Pietrangelo A, et al. Heterogeneity of hemochromatosis in Italy. Gastroenterology 1998; 114:996-1002.
8. Beutler E, Felitti V, Gelbart T, Ho NJ. The effect of HFE genotypes on measurement of iron overload in patients attending a health appraisal clinic. Ann Intern Med 2000; 133: 329-37.
9. Pietrangelo A, Camaschella C. Molecular genetics and control of iron metabolism in hemochromatosis. Haematologica 1998; 83:456-61.
10. Piperno A, Mariani R, Arosio C, et al. Haemochromatosis in patients with β-thalassemia trait. Br J Haematol 2000; 111:908-14.