ber of activated neutrophils may have altered local vessel permeability[6,7] although the possibility that chemotherapy may have contributed to this cannot be excluded. Overdose probably had no adverse affect on the outcome of the underlying disease as shown by a reduction of lymphnodes on CT scan. Meningitis, occurring three months after the overdose, was more likely due to steroid-induced immunosuppression with comorbidities (age, hypertension, emphysema) contributing to its lethal outcome. A role for pegfilgrastim cannot be fully excluded but, since it was undetectable in the serum on day +6 and recovery of ANC after subsequent courses was as expected, its role would seem to be negligible.

Recently the death of a patient with GSDIb was reported after 120 μg/kg of pegfilgrastim that were considered contributory to this event as aggravating pre-existing pulmonary arterial hypertension (PAH).[8] Different outcomes can be explained by the worse comorbidities (recurrent enteritis, total colectomy, interstitial nephropathy, PAH, liver adenomas, recent respiratory infection) of the GSDIb patient compared to our patient # 2.

WBC kinetics were similar in our patients, normalizing in both after d+20, whereas ANC peak was lower in patient # 1 as a consequence of the underlying disease. Hemoglobin and platelets were unaffected in patient # 1 whereas these dropped in patient # 2, more likely as an effect of chemotherapy rather than of pegfilgrastim. Serum drug levels peaked on d+1 in both patients and returned to baseline later in patient # 1 (d+16) *vs.* patient # 2 (d+6) in accordance with neutrophil-mediated kinetics of pegfilgrastim[9,10] by which patient # 2, whose neutrophil circulatory output was far greater than in patient # 1 (peak of 101×10^9/L *vs.* 20×10^9/L), cleared the drug more rapidly from the bloodstream.

Data on drug overdoses in humans are extremely rare to find. This report provides useful information to physicians indicating that overdose in children may have an uneventful outcome and in elderly patients may produce controllable side effects.

Carlo Dufour,[1] Barbara Cappelli,[2] Michaela Calvillo,[1] Francesca Fioredda,[1] Rossella Tonelli,[3] and Roberto Crocchiolo[4]

[1]Hematology Unit, G.Gaslini Children's Hospital, Genova, Italy;[2]Pediatric Immunohematology and Bone Marrow Transplantation Unit, San Raffaele Scientific Institute, Milano, Italy;[3]Mario Negri Institute for Pharmacology, Milano, Italy;[4]Unit of Lymphoid Malignancies, San Raffaele Scientific Institute, Milano, Italy.

Key words: neutropenia, pegfilgrastim, overdose, pharmacokinetic.

Acknowledgments: Fondazione Compagnia di San Paolo and ERG s.p.a, are acknowledged for supporting the activity of the Hematology Unit of G.Gaslini Children's Hospital.

Correspondence: Carlo Dufour, MD, Head Hematology Unit, G.Gaslini Children's Hospital, Largo G.Gaslini 5, 16147 Genova, Italy. Phone: 0039 01 56 36 694; Fax: 0039 010 56 36 714. E-mail: carlodufour@ospedale-gaslini.ge.it

Citation: Dufour C, Cappelli B, Calvillo M, Fioredda F, Tonelli R. Similar favorable outcome of pegfilgrastim overdose in patients with different age and underlying disease. Haematologica. 2010;95:684-685. doi:10.3324/haematol.2009.018333

References

1. Green MD, Koelbl H, Baselga J, Galid A, Guillem V, Gascon P, et al. A randomized double-blind multicenter phase III study of fixed-dose single-administration pegfilgrastim versus daily filgrastim in patients receiving myelosuppressive chemotherapy. Ann Oncol. 2003;14(1):29-35.
2. Bruns I, Steidl U, Fischer JC, Czibere A, Kobbe G, Raschke S, et al. Pegylated granulocyte colony-stimulating factor mobilizes CD34+ cells with different stem and progenitor subsets and distinct functional properties in comparison with unconjugated granulocyte colony-stimulating factor. Haematologica. 2008; 93(3):347-55.
3. Beaupain B, Leblanc T, Reman O, Hermine O, Vannier JP, Suarez F, et al. French SCN Registry, Service d'Hémato Oncologie Pédiatrique. Is pegfilgrastim safe and effective in congenital neutropenia? An analysis of the French Severe Chronic Neutropenia registry. Pediatr Blood Cancer. 2009;53(6):1068-73.
4. Fioredda F, Calvillo M, Lanciotti M, Lanza T, Giunti L, Castagnola E, et al. Pegfilgrastim in children with Severe Congenital Neutropenia. Pediatr Blood Cancer. In press 2009.
5. Merli F, Bertini M, Luminari S, Mozzana R, Botto B, Liberati AM, et al. Long term results of a randomized study performed by Intergruppo Italiano Linfomi comparing Mini-CEOP vs P-VEBEC in elderly patients with diffuse large B-cell lymphoma. Leuk Lymphoma. 2007;48(2):367-73.
6. Spiekermann K, Roesler J, Emmendoerffer A, Elsner J, Welte K. Functional features of neutrophils induced by GCSF and GM-CSF treatment: differential effects and clinical implications. Leukemia. 1997;11(4):466-78.
7. Schilero GJ, Oropello J, Benjamin E. Impairment in gas exchange after granulocyte colony stimulating factor (GCSF) in a patient with the adult respiratory distress syndrome. Chest. 1995;107(1):276-8.
8. Donadieu J, Beaupain B, Rety-Jacob F, Nove-Josserand R. Respiratory distress and sudden death of a patient with GSDIb chronic neutropenia: possible role of pegfilgrastim. Haematologica. 2009;94(8):1175-7.
9 Yang BB, Kido A, Shibata A. Serum pegfilgrastim concentrations during recovery of absolute neutrophil count in patients with cancer receiving pegfilgrastim after chemotherapy. Pharmacotherapy. 2007;27(10):1387-93.
10. Holmes FA, Jones SE, O'Shaughnessy J, Vukelja S, George T, Savin M, et al. Comparable efficacy and safety profiles of once-per-cycle pegfilgrastim and daily injection filgrastim in chemotherapy-induced neutropenia: a multicenter dose-finding study in women with breast cancer. Ann Oncol. 2002;13(6):903-9.

Homozygous deletion of *HFE* is the common cause of hemochromatosis in Sardinia

We recently characterized an *Alu*-mediated recombination causing the loss of the complete *HFE* gene sequence. Here, we describe the case of a novel homozygous patient. We further show that *HFE* deletion results from a founder effect and that it represents the common cause of hemochromatosis in Sardinia.

We recently reported the case of a 47-year old woman with a moderate iron overload due to an *Alu*-mediated recombination causing the loss of the complete *HFE* gene sequence.[1] The same chromosomal alteration was identified by Pelucchi and co-workers in another woman. Despite a younger age at diagnosis (29 years), their patient showed a more impressive iron overload.[2]

A third case, a man, came to our attention. At the age of 44 he showed a transferrin saturation level of 80% and a serum ferritin level of 2080 μg/L. He was not genotyped for the p.C282Y and p.H63D variations because of an inability to amplify the *HFE* exons 2 and 4. We confirmed absence of the *HFE* gene in this patient but we were also interested in its origins. Indeed, the patient and both previously reported women were of Sardinian descent.

The Sardinian population is genetically differentiated from the other Caucasian populations.[3] It represents a genetic isolate where the p.C282Y mutation is considered as rare or even absent.[4] This led us to assume that the *HFE* deleted allele was present at the population level and, related to a founder effect, was the common cause of hemochromatosis in this Mediterranean island.

To characterize the contribution of the *HFE* deletion in the Sardinian population, we first established the frequency of this mutation in a sample set of 198 controls

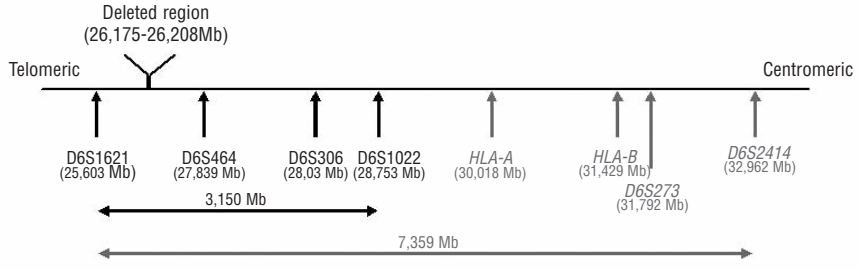

Figure 1. Physical map of the chromosome 6 region flanking *HFE*. Distance between the D6S1621 and D6S2414 marker is indicated in grey; the arrow marks a possible ancestral haplotype. Distance between the D6S1621 and D6S1022 markers is indicated in black; the arrow encompasses only alleles shared by all the mutation carriers.

Table 1. Allelic distribution of markers surrounding *HFE*. *The parentheses mark differences between the 6 individuals; these differences are probably related to a recombination event on chromosome 6 of the mutation carrier number 3.

Patient (P) or Mutation Carrier (MC)	Alleles								
	D6S1621	*D6S464*	*D6S306*	*D6S1022*	*HLA-A*	*HLA-B*	*D6S273*	*D6S2414*	*D6S1611*
P1	302	215	259	148	02	58	148	193	242
P2	302	215	259	148	02	58	148	193	238
MC1	302, 308	215, 231	259	148, 160	02, 32	18, 58	146, 148	193	246, 248
MC2	302, 304	215, 219	253, 259	148, 152	02, 30	18, 58	148, 152	193	238, 242
MC3	302	215, 229	259, 261	148, 152	01, 11	35, 49	136, 150	193	240, 242
MC4	302	215, 219	253, 259	145, 148	02, 32	18, 58	148, 150	193, 197	238, 246
Deduced haplotype*									
	302	215	259	148	(02)	(58)	(148)	(193)	

who originated from different districts of the island (90% of them were from South Sardinia, while the others originated from central and Northern parts of the island). Genotype analysis was performed using a rearrangement specific PCR, as previously described.[1] The *HFE* deletion was detected at the heterozygous state in 4 subjects, giving a carrier frequency of 2.02% and an estimated homozygous frequency of 0.01% (one person in 10,000). We also looked for the p.C282Y mutation. It was identified at the heterozygous state in one of 193 subjects (0.52%).

We next proceeded to analyze polymorphic microsatellite repeat markers flanking *HFE*. Based on results from the consanguineous family reported in our initial study,[1] we selected a set of informative markers and defined a first chromosomal region of 12.049 megabases (Mb). This chromosomal region encompassed two HLA-class I loci which were added to the haplotypes analysis. Final results are summarized in Table 1 and Figure 1. A conserved haplotype of 3.150 Mb was deduced from the consideration of alleles shared by the 2 homozygous cases identified by us (P1, P2), and the 4 mutation carriers (MC 1-4). However, a recombination event could account for differences in HLA-A, HLA-B and D6S273 alleles of one of the study subjects (MC 3 in Table 1). This allowed us to consider a common haplotype of 7,359 Mb.

By their position on the same maritime routes, the histories of the West Mediterranean islands are very similar. Studies based on HLA polymorphisms have confirmed singularity of these populations, and have further revealed a close relationship between the Sardinian and Corsican populations. However, it must be pointed out that the HLA-A*2-B*58 haplotype is frequent in Sardinia (4.9%) and almost absent in the other West Mediterranean islands (Corsica and Balearic Islands). In fact, this haplotype could be of African origin.[5] If so, comparing frequency of the *HFE* deleted allele (2%) to that of the HLA-A*2-B*58 haplotype (4.9%), one may assume that deletion of the *HFE* gene arose in the Sardinian population after contacts with African populations. But an African ancestry for the *HFE* deletion cannot be completely excluded.

To conclude, we show that *HFE* deletion results from a founder effect rather than that of a mutational hotspot. We also demonstrate that, with an estimated homozygous frequency of one person in 10,000, *HFE* deletion is the common cause of hemochromatosis in Sardinia.

Gérald Le Gac,[1] Rita Congiu,[2] Isabelle Gourlaouen,[1] Milena Cau,[3] Claude Férec,[1] and Maria Antonietta Melis[3]

[1]Inserm U613, Etablissement Français du Sang, Bretagne, Université de Bretagne Occidentale, Brest, F-29200 France; [2]Ospedale Microcitemico ASL8, Cagliari, Italy; [3]Dipartimento Scienze biomediche e biotecnologica, Università di Cagliari, Italy

Key words: hemochromatosis, gross deletion, Sardinia, founder effect.

Correspondence: Dr. Gerald Le Gac, Inserm U613, EFS – Bretagne, 46, rue Félix Le Dantec, 29200 Brest, France. Phone: international +33.0298445064. Fax: international +33 (0)298430555. E-mail: gerald.legac@univ-brest.fr

Citation: Le Gac G, Congiu R, Gourlaouen I, Cau M, Férec C, and Melis MA. Homozygous deletion of HFE is the common cause of hemochromatosis in Sardinia. Haematologica. 2010; 95:685-687. doi:10.3324/haematol.2009.016816

References

1. Le Gac G, Gourlaouen I, Ronsin C, Géromel V, Bourgarit A, Parquet N, et al. Homozygous deletion of HFE produces a phenotype similar to the HFE p.C282Y/p.C282Y genotype. Blood.

2008;112(13):5238-40.
2. Pelucchi S, Mariani R, Bertola F, Arosio C, Piperno A. Homozygous deletion of HFE: the Sardinian hemochromatosis? Blood. 2009;113(16):3886.
3. Lampis R, Morelli L, De Virgiliis S, Congia M, Cucca F. The distribution of HLA class II haplotypes reveals that the Sardinian population is genetically differentiated from the other Caucasian populations. Tissue Antigens. 2000;56(6):515-21.
4. Candore G, Mantovani V, Balistreri CR, Lio D, Colonna-Romano G, Cerreta V, et al. Frequency of the HFE gene mutations in five Italian populations. Blood Cells Mol Dis. 2002;29(3):267-73.
5. Grimaldi MC, Crouau-Roy B, Amoros JP, Cambon-Thomsen A, Carcassi C, Orru S, et al. West Mediterranean islands (Corsica, Balearic islands, Sardinia) and the Basque population: contribution of HLA class I molecular markers to their evolutionary history.

HAMP promoter mutation nc.-153C>T in non p.C282Y homozygous patients with iron overload

In a recent paper, Island and colleagues[1] described a heterozygous hepcidin (*HAMP*) promoter mutation, nc.-153C>T, which in association with *HFE* p.C282Y homozygosity appeared to lead to very severe iron overload (IO). They demonstrated *in vitro* that this *HAMP* mutation decreases transcriptional activity of the hepcidin promoter, alters IL6 total responsiveness and impairs binding of the SMAD protein complex to the BPM-RE.

HFE genotypes other than the homozygous p.C282Y (YY) or the compound heterozygous state for the p.C282Y and p.H63D mutations are not considered responsible for causing symptomatic forms of IO. This led us to re-evaluate 30 unrelated patients with non-YY *HFE* genotype associated with the diagnostic criteria of hemochromatosis who were referred to our laboratory. Among them, 12 were previously reported H63D homozygotes.[2] The diagnosis of IO was based on either liver biopsy and/or therapeutic criteria (phlebotomies). Informed written consent was obtained from all patients according to the French regulation.

Table 1. Characteristics of 3 iron loaded male patients with the *HAMP* nc.-153 C>T.

Patient #	HG224	HG707	HG118
Age at diagnosis (years)	54	75	69
HFE genotype			
• p.C282Y	absent	absent	absent
• p.H63D	homozygote	homozygote	absent
• p.S65C	absent	absent	heterozygote
• other	none	none	none
Serum ferritin (µg/L)	1270	2220	2330
Transferrin Saturation (%)	51	95	90
Liver biopsy	yes	yes	yes
• Cirrhosis	no	no	yes
• Fibrosis	yes	no	
• LIC (µmole/g)	145	193	565
• LIC/age	2.7	2.6	8.3
Clinical manifestations possibly related to IO	dark skin joint pain hepatomegaly	fatigue joint pain hepatomegaly	dark skin hypertrophic cardiomyopathy
Other			
• Alcohol	yes	stopped 2 years ago	NA
• Viral hepatitis	no	no	NA
• Overweight	BMI: 26	BMI: 30.5	NA
Family history of IO or hemochromatosis	yes	no	yes
Phlebotomies	>5 g iron removed	>5 g iron removed	regularly over 4 years

LIC: liver iron concentration; BMI: body mass index; NA: not available.

DNA sequencing of the *HAMP* gene found no mutation in the coding sequence or in the exon-intron junctions of the 30 patients. However, two gene substitutions, a C to T replacement at position -153 upstream of the ATG and a C to T substitution in intron 1 at position -66, 5' to exon 2 (c.91-66C>T, rs#2293689) were identified in 3 patients. Both sequence alterations were found in all 3 patients but we could not confirm whether they were in linkage disequilibrium or whether they were inherited on different chromosomes, as family segregation could not be performed. The patients, 3 white men living in different cities of the Southern part of France, were referred separately by three different physicians. To the best of our knowledge the men are not related. They all had ferritin levels over 1000 µg/L and transferrin saturation over 50%. They had been regularly phlebotomized for more than one year. Two of them, a 75-year old and a 54-year old male respectively, were p.H63D homozygotes (Table 1). The third patient, diagnosed at 69 years of age, was simply heterozygote for the p.S65C substitution on the *HFE* gene. His medical record indicated that he had myocardiopathy, cirrhosis and a liver biopsy compatible with the diagnosis of hemochromatosis (Table 1). Two patients had a family history of iron overload. Both substitutions, nc.-153C>T and c.91-66T>C, were also found in one out of 224 unrelated control chromosomes from white individuals of the same region. The iron status of this anonymous control was not available. The *HAMP* nc.-153C>T mutation was thus present at a high allele frequency (0.05) in this series of non-YY iron loaded patients. It was also found in one control subject, with a low allele frequency of 0.004 in this group (P=0.008).[3]

Barton and colleagues[4] did not detect *HAMP* nc.-153C>T in any of 191 *HFE* p.C282Y homozygotes from the HEIRS (Hemochromatosis and Iron Overload Screening) Study. They also screened a control group with various ethnic backgrounds, including non-Hispanic whites, Hispanics, blacks and Asian subjects, and found this mutation in only one Hispanic woman with apparently no IO at a control sample. They concluded that routine testing to detect *HAMP* nc.-153C>T is not indicated in population-based hemochromatosis and IO screening programs in North America. However, the data presented here indicate that in a selected sample of iron loaded individuals, especially those with *HFE* non-YY genotypes, the search for this mutation could be of interest as it can possibly explain IO in certain patients. Indeed, we found that 2 out of 12 iron loaded H63D homozygotes[2] had the *HAMP* nc.-153 C>T as a potential genetic factor of IO. Screening for this mutant may be, therefore, indicated in this particular population, or for individuals with high transferrin saturation and unexplained IO, as suggested by Loréal.[4]

Patricia Aguilar-Martinez, Muriel Giansily-Blaizot, Michael Bismuth, Séverine Cunat, Hélène Igual and Jean François Schved

Laboratory of Hematology, CHU de Montpellier, Montpellier, France.

Acknowledgments: the authors are grateful to Dr Béatrice Bonafoux and Dr Nathalie Funakoshi for their helpful contribution.

Funding: supported by a grant from la Recherche Clinique, CHU de Montpellier, AOI 2004.