



[haematologica]  
2004;89:1161-1167

## Screening selected blood donors with biochemical iron overload for hemochromatosis: a regional experience

MARCO DE GOBBI  
SERGIO D'ANTICO  
FRANCO CASTAGNO  
DOMENICO TESTA  
ROBERTA MERLINI  
ALESSANDRO BONDI  
CLARA CAMASCHELLA

### A B S T R A C T

**Backgrounds and Objectives.** Hemochromatosis is a genetic disorder characterized by progressive iron overload which leads to early abnormalities of iron parameters (increased transferrin saturation =TS and serum ferritin=SF) and late clinical complications. The disease is prevalently due to C282Y and H63D mutations in the *HFE* gene, but additional molecular defects are present in a minority of patients.

**Design and Methods.** From January to December 2002 we screened first time blood donors of Piedmont, a region of North-western Italy, for TS>45%. Individuals with TS>45% underwent a second fasting check, SF assessment and molecular tests, investigating 12 hemochromatosis-associated molecular defects.

**Results.** A total of 13,998 subjects were screened; 868 (6.2%) had TS>45% and were recalled. Four hundred and eight-six underwent molecular testing. In this selected population allele frequencies of C282Y, H63D and S65C were 6.8%, 22.4% and 1.0%, respectively. No rare mutations were detected, except E168Q in *HFE*. When measured during fasting, TS was significantly higher in C282Y homozygotes and H63D/C282Y heterozygotes ( $p<0.05$ ) than in wild type subjects, but not in H63D homozygotes. Hyperferritinemia was documented in 32 cases, 9 with wild type genotype. Mean age, body mass index (BMI) and alcohol intake were higher in this group than in individuals with normal SF.

**Interpretation and Conclusions.** This study is an example of a large, two-step hemochromatosis screening with moderate effort and low cost, that enriches basal C282Y allele frequency by about three-fold. Screening based on genotyping only subjects found to have a TS>45% is feasible but, in order to be cost effective should be based on the identification of the two prevalent mutations even in an area where several forms of hemochromatosis have been reported.

**Key words:** screening, hemochromatosis, blood donors, HFE.

From the Department of Clinical and Biological Sciences, ASO San Luigi, University of Turin, Turin, Italy (MDG, RM, AB, CC); Blood Centre, Hospital San Giovanni Battista, Turin, Italy (SDA, FC); Transfusional Centre, Ospedale Civile Savigliano, Cuneo, Italy (DT) MDG and SDA contributed equally to the work.

Correspondence: Clara Camaschella, Dipartimento Scienze Cliniche e Biologiche Università di Torino, Azienda Ospedaliera S. Luigi, 10043 Orbassano, Turin, Italy. E-mail: clara.camaschella@unito.it

©2004, Ferrata Storti Foundation

**H**ereditary hemochromatosis (HH) is a recessive disorder of iron metabolism characterized by increased intestinal iron absorption, and progressive iron overload. HH can result in the development of liver cirrhosis, diabetes, cardiomyopathy, hypogonadism, other endocrine complications and susceptibility to liver cancer. Early diagnosis and treatment before the occurrence of organ damage leads to a normal life expectancy.<sup>1</sup> The majority of HH patients are homozygous for a mutation which results in a tyrosine for cysteine (C282Y) substitution at position 282 of the HFE protein.<sup>2</sup> In Italy about 64% of HH patients are C282Y homozygous<sup>4,5</sup> and the percentage is even lower in other Southern European countries.<sup>6-8</sup> Another mutation, which results in a histidine to aspartic acid substitution (H63D) at amino acid 63, is common in the general population and considered a polymorphic change. However,

H63D/C282Y compound heterozygotes,<sup>2</sup> or H63D homozygotes, are not infrequent among Southern European iron-loaded patients.<sup>4-8</sup> Other causal mutations in the *HFE* gene are rare and usually found in patients associated with C282Y heterozygosity.<sup>9-12</sup> The evidence is that HH is genetically heterogeneous, caused by mutations in at least 4 genes other than *HFE*, reflecting the complexity of iron regulation: transferrin receptor 2,<sup>13-14</sup> SCL40A1 encoding ferroportin 1,<sup>15-16</sup> hepcidin<sup>17-18</sup> and the recently identified hemojuvelin.<sup>19-21</sup> Most mutations in these genes are rare and restricted to family groups or small geographical areas<sup>22</sup> (and unpublished data). Only the deletion of valine at position 162 in ferroportin gene ( $\Delta 162\text{Val}$ ) recurs in different ethnic groups.<sup>23-26</sup> In Italy it is well known that there is a gradient of C282Y mutation frequency from northern to southern regions both in patients<sup>5</sup> and in nor-

mal controls<sup>27-28</sup> and where C282Y is less frequent, H63D and other genetic defects are present among patients.

Population screening for HH has been advocated to avoid complications caused by excess iron deposition.<sup>29</sup> However, the issue is controversial because of the low penetrance of *HFE* mutations and of the uncertain genotype-phenotype correlation.<sup>30-32</sup> Pilot genetic screenings in Italy<sup>27,33-34</sup> indicate that population-based genetic screening is not justified because of the low frequency of C282Y mutation (between 0.016-0.022) in our country. Screening subjects for increased transferrin saturation seems more appropriate. Among the different screenings carried out in Italy only one was based on biochemical parameters of iron overload.<sup>35</sup> This was carried out on samples from different geographical areas, but, at present, systematic regional screenings are lacking.

We carried out a regional biochemical/genetic screening of HH on almost 14,000 voluntary first time blood donors in Piedmont, a region of North-Western Italy, in order to determine the prevalence of subjects with a biochemical phenotype of iron overload, to verify the correlation between *HFE* mutations and iron parameters in young healthy subjects and to check iron parameters in a large cohort of potential blood donors, an issue important for planning blood donations.

## Design and Methods

### Samples

The screening was organized by the local National Health Service and a representative of transfusion centers of Piedmont, a region of North-Western Italy, in collaboration with the referral center for molecular diagnosis. Piedmont has approximately 3,500,000 inhabitants. Subjects presenting for evaluation of eligibility for blood donation in the 12 months from January to December 2002 in all transfusion centers had their transferrin saturation (TS) measured: in a proportion of subjects this determination was carried out in a non-fasting state. Individuals (both females and males) with a TS >45% were recalled by a letter explaining the problem, offering the possibility of an interview with one of the authors. Since about 75% of individuals were phlebotomized to collect blood when first evaluated, a second check was offered after an interval of at least two months.

Consenting donors had a repeat, fasting TS assay together with serum ferritin (SF) determination and a genetic test. At that time a medical history was collected on the basis of a structured questionnaire aimed at collecting information on family history, daily alcohol intake, body mass index and other parameters of potential relevance to iron overload. Serum iron (SI), ST and SF were measured by each transfusion center

using standards methods. TS was indirectly calculated using the formula:  $SI \mu\text{g/dL} \div (ST \text{ mg/dL} \times 1.42)$  on the basis of molecular weight of transferrin. SF was considered to be abnormal when > 300  $\mu\text{g/L}$  in males and > 200  $\mu\text{g/L}$  in females.<sup>36</sup>

All individuals undergoing genetic tests gave informed consent, according to the guidelines of our institution, and were informed of the results. Furthermore they had the opportunity to have a genetic counseling in order to organize family screening and phlebotomy, if necessary.

### Molecular analysis

Genetic analyses were carried out in a single reference laboratory to which all the samples and data were sent. DNA was prepared by standard protocols and samples were genotyped using a reverse hybridization assay (Haemochromatosis StripAssay, Nuclear Laser, Settala, Milan, Italy) for the simultaneous detection of *HFE* and *TFR2* mutations.

Polymerase chain reaction (PCR) was carried out in a Thermal Cycler Perkin Elmer GeneAmp® PCR System 2400 (Perkin Elmer Biosystem, Foster City, CA, USA). Thirty-five cycles were performed (94°C for 15 seconds, 58°C for 30 seconds and 72°C for 30 seconds) with a final extension at 72°C for 3 minutes. PCR products were hybridized to the teststrips and detected by enzymatic color reaction.<sup>11</sup> Eleven *HFE* (V53M, V59M, H63D, H63H, S65C, Q127H, E168Q, E168X, W169X, C282Y, Q283P) and 1 *TFR2* (Y250X) mutations were investigated. Selected cases underwent direct sequencing screening for ferroportin 1 mutations. Primers were obtained from public databases at the following address: <http://www.ncbi.nlm.nih.gov/>.

Direct sequencing was performed according to standard methods described elsewhere.<sup>25</sup>

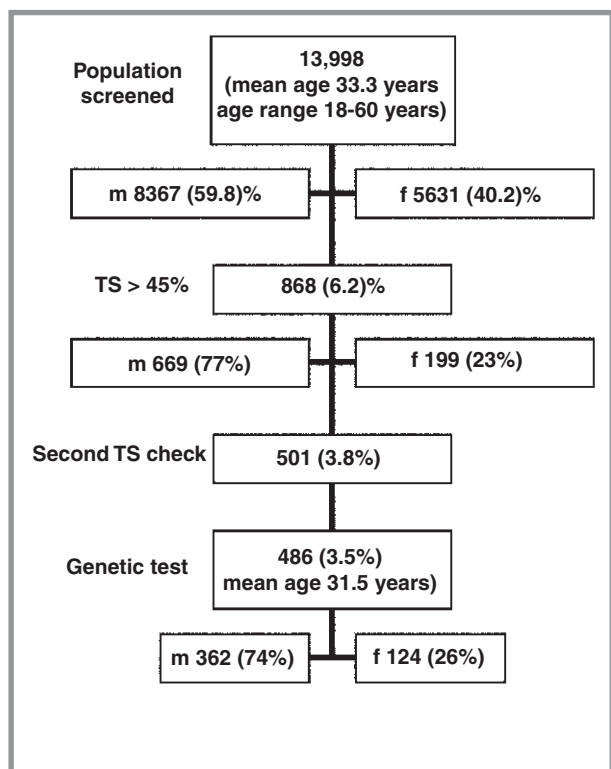
### Statistical analysis

We calculated allele and genotype frequencies for *HFE* mutations in the series. Enrichment factors for C282Y and H63D alleles were also calculated comparing two different groups of blood donors divided according to TS level at the second fasting check (group A with TS <45% and group B with TS >45%). Differences among TS and SF according to *HFE* genotypes were evaluated by the non-parametric Mann-Whitney (GraphPad Prism 3.0, GraphPad Software, Inc) test.

## Results

### First step: biochemical studies

The screening protocol is shown in Figure 1. Briefly, from January to December 2002, 13,998 voluntary first time blood donors underwent biochemical screening for



**Figure 1.** Flow chart of examinations for the detection of iron overload-related genotypes. Percentage values given in the central lane are percentages of the original numbers of donors. m: males; f: females.

HH, based on TS. The screening essentially covered first time blood donors of the whole Piedmont region, since 17 out of 19 transfusion center participated in the screening; only two small centers, usually accounting for 8% of the total number of donors, did not participate.

TS was >45% at first determination in 868 donors (6.2%), of whom 501 presented for re-testing. However, 15 individuals refused the genetic test for different reasons. Thus 486 DNA samples from individuals with an abnormal TS at a single determination underwent genetic testing.

### Second step: genetic studies

The results obtained, reported as number and percentages of the genotypes observed are summarized in Table 1. We found 4 (3 males and 1 female) C282Y homozygous subjects, 18 (2 females and 16 males) C282Y/H63D compound heterozygotes and 30 (13 females and 17 males) H63D homozygotes. Forty subjects (8%) were C282Y heterozygotes; H63D heterozygotes accounted for 28.6% of the cases. Rare genotypes found were: heterozygous S65C in 9 subjects, S65C/H63D compound heterozygosity in one individual and E168Q heterozygosity in two individuals, one of

**Table 1.** HFE genotypes identified in the 486 potential blood donors with TS >45%.

Genotypes	n.	%	95% C.I.
C282Y homozygote	4	0.8	0.01-1.59
H63D/C282Y	18	3.7	2.0-5.4
H63D homozygote	30*	6.2	3.8-8.0
C282Y heterozygote	40	8.2	5.6-10.4
H63D heterozygote	139*	28.6	24.6-32.6
H63D/S65C	1	0.2	
S65C heterozygote	9	1.9	0.9-3.5
Wild type	245	50.4	46.0-54.8

\*E168Q associated with H63D in 2 subjects.

**Table 2.** Allele frequency in the 486 individuals with TS > 45% at first determination and according to TS at the second determination (group A: <45%; group B: > 45%).

	All series		Group A		Group B	
	n	%	n	%	n	%
C282Y	66	6.8	29	3.9	37	16.7 <sup>^</sup>
H63D	218	22.4**	170	22.8	50	21.6
S65C	10	1.0**	10	1.2	0	0
Wild type	678	69.8	541	72.1	135	61.7
Total	972		750		222	

<sup>^</sup>p<0.001 vs group A; TS: transferrin saturation; \*\*H63D: 24.3%; S65C: 1.3% after correction for disequilibrium.

whom was an H63D homozygote and the other an H63D heterozygote. The other mutations investigated (V53M, V59M, H63H, Q127H, E168X, W169X and Q283P in HFE, Y250X in TFR2) were not found in this study. Approximately 50% of the cases (63/124 females and 182/362 males) had a wild type genotype.

The allele frequency of the different mutations is reported in Table 2. The overall C282Y frequency was 6.8%, that of H63D 22.4% and that of S65C 1.0%. The total allele frequency corrected for the observed *disequilibrium* (H63D, S65C and C282Y variants are mutually exclusive and never found *in cis*) overlaps the basal one (Table 2).

At the second fasting test the TS was <45% in 375 cases (group A) and >45% in 111 samples (group B) (96 males and 15 females, mean age 34 years), representing 23% of the sample. The C282Y allele frequency was enriched from 3.9% to 16.7% in group B, compared to in group A (p<0.001), whereas that of H63D was not (Table 2). In terms of genotypes, the enrichment concerned only C282Y homozygotes (from 0 to 3.6%) and C282Y/H63D compound heterozygotes (from 1.6 to 10.8%) but not H63D homozygotes (6.4 vs 4.5%) (*data not shown*).

**Table 3. Serum iron parameters at the first and second determinations according to HFE genotypes.**

Genotypes (number of individuals)	1 <sup>st</sup> TS % (mean±Sd)	2 <sup>nd</sup> TS % (mean±Sd)	SF (µg/L) (mean±Sd)	SF/ALT (mean±Sd)
C282Y homozygotes (n=4)	67±13.8	60±16.4*	372±65*	19.16±12.23*
H63D/C282Y (n=18)	59±11.1	47±14.4*	248±187	9.65±6.94*
H63D homozygotes (n=30)	57±8.6	37±18.15	76±59	5.37±9.01
C282Y heterozygotes (n=40)	56±8.8	46±11.1	152±172	5.02±5.03
H63D heterozygotes (n=139)	54±8.9	35±16.7	139±237	6.2±13.64
Wild type (n=245)	53±7.4	34±13.8	106±138	4.5±5.7

TS: transferrin saturation; SF: serum ferritin; Sd: standard deviation; \* $p < 0.05$  vs wild type.

### Genotype/phenotype correlation

Table 3 reports the correlation between genotypes and iron parameters. Three genotypes maintained a mean TS >45% at the fasting check: C282Y homozygotes, C282Y/H63D compound heterozygotes, and also C282Y heterozygotes, although the mean value in these cases was reduced from 56 to 46%. Only in C282Y homozygotes and in C282Y/H63D compound heterozygotes was the mean TS at the second check significantly higher than that of wild type individuals. The mean SF was also found to be slightly elevated only in the two series of patients with the at risk genotypes; in C282Y homozygotes it was significantly higher than in wild type subjects ( $p < 0.05$ ). The SF/ALT ratio, which corrects SF concentration for hepatocellular damage,<sup>37</sup> was also significantly higher in C282Y homozygotes and compound heterozygotes than in wild type individuals (Table 3).

In the whole series with TS>45% at the first determination, 32 subjects (6.6%), all but one of whom were males, had an increased SF. As for genotype relationship, all 4 C282Y homozygotes but only 4/18 compound heterozygotes had an increased SF. Of these 32 hyperferritinemic individuals, 15 had HFE mutations in the heterozygous state (4 C282Y, 10 H63D and 1 S65C heterozygotes), but 9 cases had a wild type genotype. Five individuals with severe hyperferritinemia (>1000 µg/L) had genotypes not at risk (3 H63D heterozygotes and 2 wild type). Sequence analysis of ferroportin 1 in 4 subjects gave normal results. Hyperferritinemic subjects were statistically older (mean age 43.8 years) than subjects with a normal SF (mean age 33.1,  $p < 0.01$ ). This was even more evident in hyperferritinemic subjects without *at risk* genotypes (mean age 45.7 years) (*data not shown*). The results of the questionnaire allowed us to analyze acquired factors potentially involved in an increased SF. An alcohol

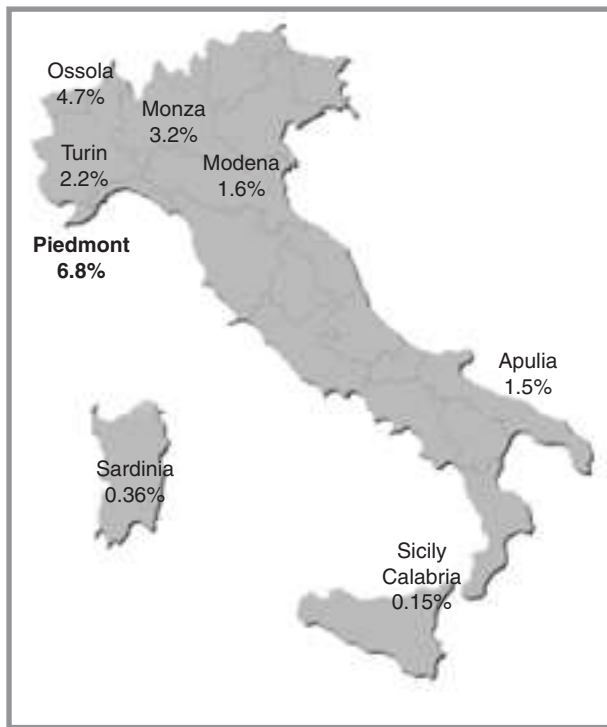
intake >80 g/daily for males and >40 g/daily for females and a body mass index >25 were statistically more frequent among hyperferritinemic subjects (10% vs 1%,  $p < 0.001$  and 59.4 vs 22.5%,  $p < 0.001$ , respectively) than in the whole series.

### Discussion

The controversy on the need for hemochromatosis screening is mainly related to the penetrance issue.<sup>30-32</sup> Depending on its evaluation, penetrance is reported to vary between 1% and 79% even in males. There is a general agreement that the clinical penetrance is low,<sup>30,38-39</sup> whereas the biochemical penetrance (increased TS, increased SF) is common.<sup>30,38-40</sup> Increased TS (> 50%) was extensively used for HH screening before the discovery of the *HFE* gene, since it is relatively inexpensive. Nowadays screening TS in order to select candidates for genotyping (two-step screening) limits costs and restricts the genetic analysis to subjects with the biochemical phenotype.<sup>41-42</sup>

We have applied a two-step screening strategy to Italian subjects presenting for evaluation of blood donor eligibility in a region where previous pilot genetic screenings showed a low frequency of the C282Y mutation.<sup>27</sup> Our study is the largest two-step screening in Europe, based on the search for multiple defects, and is the first screening in Italy on a regional scale. The number of individuals screened is a representative sample (around 0.4%) of the Piedmont population.

First time blood donors represent an ideal target for HH pilot screening. They are prevalently males (60% in our series) and young (mean age around 30 years); if carriers of *at risk* genotypes, they are in the presymptomatic stage of the disease, but express the biochemical phenotype fully. In addition, they are healthy,



**Figure 2. Allelic frequencies of C282Y mutations in Italy. Results of the following studies in the general population are shown: Ossola,<sup>48</sup> Monza,<sup>56</sup> Turin,<sup>27</sup> Asiago,<sup>49</sup> Pavia,<sup>34</sup> Modena,<sup>33</sup> Apulia,<sup>57</sup> Sardinia,<sup>58</sup> Sicily.<sup>59</sup> The result of the present study on donors in Piedmont selected for TS > 45%, to strengthen the enrichment of the HH genotypes, is in bold.**

so that influences of other diseases on iron parameters are excluded. Examining blood donors has the advantage that the first phase of the screening requires a moderate effort at low cost, since our current national guidelines recommend checking serum iron levels in this setting. Problems are encountered in the second phase, because of partial adhesion. However, we consider the 57% participation of the recalled subjects to the second step a success. Usually only a proportion of first time donors become regular donors, a consistent percentage (55–65%) remaining occasional donors. Reasons for the low compliance, other than the physiologic loss of donors, are screening or disease refusal and non-acceptance of genetic tests. None of the 4 young C282Y homozygotes we identified had clinical signs of iron overload, but all 4 had full biochemical expression. A mean TS >45% at the second check was recorded in C282Y homozygotes, C282Y heterozygotes and in H63D compound heterozygotes, consistent with the effect of the C282Y mutation in increasing TS. In agreement with other studies<sup>28,33-34</sup> most subjects with increased TS had a wild type genotype. The existence of other genetic defects in these patients has been

hypothesized<sup>34</sup> but we did not carry out family studies to investigate this interpretation.

Hyperferritinemia was present in C282Y homozygotes and in some compound heterozygotes, but was independent of *at risk* genotypes in several cases. The differential diagnosis of hyperferritinemia is complex, because of the possible presence of other genetic disorders (e.g. hyperferritinemia-cataract syndrome)<sup>44,45</sup> or acquired factors (e.g. alcohol intake or metabolic alterations).<sup>46,47</sup> Some subjects with a high serum ferritin had an elevated body mass index or high alcohol intake. The significance of these factors in increasing SF levels was further strengthened by the findings of an association between alcohol intake, body mass index and SF levels in the whole series. We conclude that in our population both genetic and acquired factors co-operate in altering iron parameters.

From our results we predict 7 C282Y homozygotes in the series of 864 subjects with increased TS; this figure corresponds to approximately 1:2000 individuals of the originally screened population. Therefore we estimate that approximately 1750 C282Y homozygous subjects express the biochemical phenotype in Piedmont. Due to the study design, our prediction obviously underestimates genotypes potentially at risk that are not expressed. However, the regional distribution of the C282Y mutation is not uniform and it is particularly prevalent in populations of Celtic ancestry, such as those in Ossola valley,<sup>48</sup> or in other areas of Northern Italy.<sup>49</sup> Mass screening could, therefore, be planned.<sup>48</sup>

By comparing genotype and allele frequencies of mutations with the results of other population screening studies carried out in Northern Italy<sup>27,33-34</sup> (Figure 2) we observed, in our selected population, an enrichment of the C282Y allele frequency of at least 3-fold, which was further increased to 7.5–10-fold in the group with TS >45% at the second test. We observed a much greater reduction of subjects with TS >45% between the first and second determinations than that reported in other studies performed in primary care patients.<sup>50</sup> One possible explanation is that 75% of our subjects had blood collected between the first and second TS measurements. TS values are highly variable, and are influenced by recent dietary iron uptake and diurnal variation, thus repeated TS measurements are recommended for phenotypic screening.<sup>51</sup> This approach will be more cost-effective, because it restricts the number of genetic tests. Our results show that there was no C282Y loss at the second check. However, there was a limited loss of C282Y/H63D compound heterozygotes, probably related to blood collection between the first and the second test in subjects with mild iron overload. Therefore, the ideal approach would be to determine TS in a fasting state at the first check. It is well known that several forms of hemochromatosis have been charac-

terized in Italy.<sup>5,10,13,16,17,20</sup> All previous Italian screenings identified C282Y-H63D<sup>27,33-35</sup> and rarely E168X mutations.<sup>48</sup> Using an assay for multiple mutations we found no other HFE mutations but S65C<sup>52</sup> with a very low prevalence (1%) and E168Q which, in our region, has been already described to be associated with H63D.<sup>53</sup> From our experience we conclude that the two-step screening is feasible on a large scale and suitable for blood donors. However, TS should be determined on samples from fasting subjects and, to be cost effective, the screening should be based on the identification of the two common HFE mutations.

This type of screening can detect young HH adults who would be ideal blood donors. A revision of the restriction on the use of *healthy hemochromatosis* blood has been advocated.<sup>48</sup>

Two recent studies have shown that HH donors do not present a greater risk to blood safety than other donors<sup>54</sup>

and may significantly augment the allogeneic blood supply.<sup>55</sup> Finally, in to measure iron parameters in first time donors it could be useful to plan the donation frequency prospectively.

*All the authors contributed to the design of the study and to the conception of the experimental work. All reviewed the manuscript and approved the final version. The authors reported no potential conflicts of interest.*

*We thank G. Rivetti and A. Orlando of the Assessorato Sanità Regione Piemonte. We are indebted to the following colleagues of the Piedmont transfusion centers: Catapano, Valderrama (Avis Torino), Patroncini (Ospedale Maria Vittoria, Torino), Tucciarone, Girotto (Ivrea) A. Ferrari (Pinerolo), Dallavalle (Alessandria), Pollis, Vaniglia (Casale Monferrato), Gipponi (Novi Ligure), Balduzzi (Tortona), Balbo (Cuneo), Gianarda (Mondovì), Di Marco (Savigliano), L. Ferrari (Novara), Cirinna', Tarditi (Borgomanero), Zucchinetti, Anatra (Verbania), Cerutti (Domodossola), Bosetti (Vercelli).*

*This work was partially supported by grants from Regione Piemonte, MIUR (PRIN) and Telethon (n. GPO0255Y01) to CC.*

*Manuscript received July 16, 2004. Accepted July 27, 2004*

## References

- Camaschella C, De Gobbi M, Roetto A. Hereditary hemochromatosis: progress and perspective. *Rev Clin Exp Hematol* 2000;4:302-21.
- Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996;13:399-408.
- Merryweather-Clarke AT, Pointon JJ, Shearman JD, Robson KJ. Global prevalence of putative hemochromatosis mutations. *J Med Genet* 1997;34:275-8.
- Carella M, D'Ambrosio L, Totaro A, Grifa A, Valentino MA, Piperno A, et al. Mutation analysis of HLA-H gene in Italian hemochromatosis patients. *Am J Hum Genet* 1997; 60:828-32.
- Piperno A, Sampietro M, Pietrangelo A, Arosio C, Ludica L, Montosi G, et al. Heterogeneity of hemochromatosis in Italy. *Gastroenterology* 1998;81:996-1002.
- Borot N, Roth M, Malfroy L, Demangel C, Vinel JP, Pascal JP, et al. Mutations in the MHC class I-like candidate gene for hemochromatosis in French patients. *Immunogenetics* 1997;84:320-4.
- Papanikolaou G, Politou M, Terpos E, Fourlemadis S, Sakellaropoulos N, Loukopoulos D. Hereditary hemochromatosis: HFE mutation analysis in Greeks reveals genetic heterogeneity. *Blood Cells Mol Dis* 2000; 26:163-8.
- Cardoso CS, Oliveira P, Porto G, Oberkanins C, Mascarenhas M, Rodrigues P, et al. Comparative study of the two more frequent HFE mutations (C282Y and H63D): significant different allelic frequencies between the North and South of Portugal. *Eur J Hum Genet* 2001;9: 843-8.
- Wallace DF, Dooley JSE, Walker AP. A novel mutation of HFE explains the classical phenotype of genetic hemochromatosis in a C282Y heterozygote. *Gastroenterology* 1999;116:1409-12.
- Piperno A, Arosio C, Fossati L, Vigano M, Trombini P, Vergani A, et al. Two novel nonsense mutations of HFE gene in five unrelated Italian patients with hemochromatosis. *Gastroenterology* 2000;119:441-5.
- Oberkanins C, Moritz A, de Villiers JN, Kotze MJ, Kury F. A reverse-hybridization assay for the rapid and simultaneous detection of nine HFE gene mutations. *Genet Test* 2000;4:121-4.
- Pointon JJ, Wallace D, Merryweather-Clarke AT, Robson KJ. Uncommon mutations and polymorphisms in the hemochromatosis gene. *Genet Test* 2000; 4: 151-61.
- Camaschella C, Roetto A, Cali' A, De Gobbi M, Garozzo G, Carella M, et al. The gene encoding transferrin receptor 2 is mutated in a new type of hemochromatosis mapping to 7q22. *Nat Genet* 2000;25:14-5.
- Roetto A, Totano A, Piperno A, Piga A, Longo F, Garozzo G, et al. New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. *Blood* 2001; 97: 2555-60.
- Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, et al. A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. *Nat Genet* 2001;28:213-4.
- Montosi G, Donovan A, Totaro A, Garuti C, Pignatti E, Cassanelli S, et al. Autosomal-dominant hemochromatosis is associated with a mutation in the ferroportin (SLC11A3) gene. *J Clin Invest* 2001;108: 619-23.
- Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nat Genet* 2003; 33:21-2.
- Roetto A, Daraio F, Porporato P, Caruso R, Cox TM, Cazzola M, et al. Screening hepcidin for mutations in juvenile hemochromatosis: identification of a new mutation (C70R). *Blood* 2004;103:2407-9.
- Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. *Nat Genet* 2004;36:77-82.
- Lanzara C, Roetto A, Daraio F, Rivard S, Ficarella R, Simard H, et al. The spectrum of hemochromatosis gene mutations in 1q-linked juvenile hemochromatosis. *Blood* 2004;103:4317-21.
- Lee PL, Beutler E, Rao SV, Barton JC. Genetic abnormalities and juvenile hemochromatosis: mutations of the HJV gene encoding hemojuvelin. *Blood* 2004; 103: 4669-71.
- De Gobbi M, Barilaro MR, Garozzo G, Sbaiz L, Alberti F, Camaschella C. TFR2 Y250X mutation in Italy. *Br J Haematol* 2001;114:243-4.
- Devalia V, Carter K, Walker AP, Perkins SJ, Worwood M, May A, et al. Autosomal dominant reticuloendothelial iron overload associated with a 3-base pair deletion in the ferroportin 1 gene (SLC11A3). *Blood* 2002;100:695-7.
- Wallace DF, Pedersen P, Dixon JL, Stephenson P, Searle JW, Powell LW, et al. Novel mutation in ferroportin 1 is associated with autosomal dominant hemochromatosis. *Blood* 2002;100:692-4.
- Roetto A, Merryweather-Clarke AT, Daraio F, Livesey K, Pointon JJ, Barbabietola G, et al. A valine deletion of ferroportin 1: a common mutation in hemochromatosis type 4? *Blood* 2002;100: 733-4.
- Cazzola M, Cremonesi L, Papaioannou M, Soriani N, Kioumi A, Charalambidou A, et al. Genetic hyperferritinemia and reticuloendothelial iron overload associated with a three base pair deletion in the coding region of the ferroportin gene (SLC11A3). *Br J Haematol* 2002;119:539-46.
- Restagno G, Gomez AM, Sbaiz L, De Gobbi M, Roetto A, Bertino E, et al. A pilot C282Y hemochromatosis screening in Italian newborns by TaqMan technology. *Genet Test* 2000;4:177-81.
- De Gobbi M, Daraio F, Oberkanins C, Moritz A, Kury F, Fiorelli G, et al. Analysis of HFE and TFR2 mutations in selected blood donors with biochemical parame-

- ters of iron overload. *Haematologica* 2003;88:396-401.
29. Godard B, ten Kate L, Evers-Kiebooms G, Ayme S. Population genetic screening programmes: principles, techniques, practices, and policies. *Eur J Hum Genet* 2003;11 Suppl 2:S49-87.
  30. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G>A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet* 2002;359:211-8.
  31. Adams PC. Population screening for hemochromatosis - are we finding people with a disease or a biochemical curiosity? *Semin Gastrointest Dis* 2002; 13:89-94.
  32. Ajioka RS, Kushner JP. Clinical consequences of iron overload in hemochromatosis homozygotes. *Blood* 2003; 101: 3351-3.
  33. Cassanelli S, Pignatti E, Montosi G, Garuti C, Mariano M, Campioli D, et al. Frequency and biochemical expression of C282Y/H63D hemochromatosis (HFE) gene mutations in the healthy adult population in Italy. *J Hepatol* 2001;34:523-8.
  34. Barosi G, Salvaneschi L, Grasso M, Martinetti M, Marchetti M, Bodini U, et al. High prevalence of a screening-detected, HFE-unrelated, mild idiopathic iron overload in Northern Italy. *Haematologica* 2002;87:472-8.
  35. Velati C, Marliani E, Rigamonti D, Barillari G, Chiavilli F, Fugiani P, et al. Mutations of the hemochromatosis gene in Italian candidate blood donors with increased transferrin saturation. *Hematol J* 2003;4:436-40.
  36. EASL International Consensus Conference on Haemochromatosis. *J Hepatol* 2000;33:485-504.
  37. Triadou P, Regnat-Lusinchi A, Girot R. Use of ferritin/alanine aspartate transaminase ratio as an iron overload marker independent of liver cell damage. *Eur J Haematol* 1989;43:413-27.
  38. Deugnier Y, Jouanolle AM, Chaperon J, Moirand R, Pithois C, Meyer JF, et al. Gender-specific phenotypic expression and screening strategies in C282Y-linked haemochromatosis: a study of 9396 French people. *Br J Haematol* 2002; 118: 1170-8.
  39. Jackson HA, Carter K, Darke C, Guttridge MG, Ravine D, Hutton RD, et al. HFE mutations, iron deficiency and overload in 10,500 blood donors. *Br J Haematol* 2001;114:474-84.
  40. Olynyk JK, Cullen DJ, Aquilia S, Rossi E, Summerville L, Powell LW. A population-based study of the clinical expression of the hemochromatosis gene. *N Engl J Med* 1999;341:718-24.
  41. Adams PC. Population screening for haemochromatosis. *Gut* 2000;46:301-3.
  42. McGrath JS, Deugnier Y, Moirand R, Jouanolle AM, Chakrabarti S, Adams PC. A normogram to predict C282Y hemochromatosis. *J Lab Clin Med* 2002;140:6-8.
  43. Njajou OT, Alizadeh BZ, van Duijn CM. Is genetic screening for hemochromatosis worthwhile? *Eur J Epidemiol* 2004; 19: 101-8.
  44. Cazzola M. Hereditary hyperferritinemia/cataract syndrome. *Best Pract Res Clin Haematol* 2002;15:385-98.
  45. Hetet G, Devaux I, Soufir N, Grandchamp B, Beaumont C. Molecular analyses of patients with hyperferritinemia and normal serum iron values reveal both L ferritin IRE and 3 new ferroportin (slc11a3) mutations. *Blood* 2003;102:1904-10.
  46. Moirand R, Mortaji AM, Loreal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 1997;349:95-7.
  47. Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology* 1999; 117: 1155-63.
  48. Salvioni A, Mariani R, Oberkanins C, Moritz A, Mauri V, Pelucchi S, et al. Prevalence of C282Y and E168X HFE mutations in an Italian population of Northern European ancestry. *Haematologica* 2003;88:250-5.
  49. Pozzato G, Zorat F, Nascimben F, Gregorutti M, Comar C, Baracetti S, et al. Haemochromatosis gene mutations in a clustered Italian population: evidence of high prevalence in people of Celtic ancestry. *Eur J Hum Genet* 2003;9:445-51.
  50. Phatak PD, Sham RL, Raubertas RF, Dunigan K, O'Leary MT, Braggins C, et al. Prevalence of hereditary hemochromatosis in 16031 primary care patients. *Ann Intern Med* 1998;129:954-61.
  51. Edwards CQ, Kushner JP. Screening for hemochromatosis. *N Engl J Med* 1993; 328:1616-20.
  52. Mura C, Raguene O, Ferec C. HFE mutations analysis in 711 hemochromatosis probands: evidence for S65C implication in mild form of hemochromatosis. *Blood* 1999;93:2502-5.
  53. Menardi G, Perotti L, Prucca M, Martini S, Prandi G, Peano G. A very rare association of three mutations of the HFE gene for hemochromatosis. *Genet Test* 2002; 6: 331-4.
  54. Sanchez AM, Schreiber GB, Bethel J, McCurdy PR, Glynn SA, Williams AE, et al. Prevalence, donation practices, and risk assessment of blood donors with hemochromatosis. *Retrovirus Epidemiology Donor Study (REDS)*. *JAMA* 2001; 286: 1475-81.
  55. Leitman SF, Browning JN, Yau YY, Mason G, Klein HG, Conry-Cantilena C, et al. Hemochromatosis subjects as allogeneic blood donors: a prospective study. *Transfusion* 2003;43:1538-44.
  56. Mariani R, Salvioni A, Coreggia C, Erba N, Lanzafame C, De Micheli V, et al. Prevalence of HFE mutations in upper Northern Italy: study of 1132 unrelated blood donors. *Drug Liver Dis* 2003; 35: 479-81.
  57. Pietrapertosa A, Vitucci A, Campanale D, Palma A, Renni R, Delios G, et al. HFE gene mutations in an Apulian population: allele frequencies. *Eur J Epidemiol* 2003; 18:685-9.
  58. Melis MA, Cau M, Congiu R, Ruvoletto L, Cao A, Galanello R. Frequency of hemochromatosis C282Y and H63D mutations in Sardinia. *Genet Test* 2002;6:327-9.
  59. 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;29:267-73.