Screening blood and bone marrow donors for haemochromatosis

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Hereditary haemochromatosis (HH) is one of the most common genetic disorders affecting populations of northern European origin. Isolation of the HFE gene, with its mutations C282Y and H63D, allows genetic diagnosis in a large number of cases. However, different mutation frequencies have been reported in populations of HH patients from various geographic regions. Screening studies for HFE mutations in countries with a high prevalence of HH should be implemented. In this study we present the results of two different programmes of genetic screening for HH, the first based on simple measurements of biochemical indices of iron metabolism in a population of 1,285 first time blood donors and the second based on HLA A3-B7 haplotype in 1,825 bone marrow donors in the Italian Bone Marrow Donor Registry enrolled by Blood Banks of the Transfusion Department of the Province of Cuneo. Both the programmes were successful in identifying subjects with HFE mutations, with a slight preference for the screening biochemical values, which could be used in all the Transfusion services.

Key words: haemochromatosis screening, HFE, population genetics

Introduction

Haemochromatosis is caused by abnormal iron metabolism. It tends to develop in patients over 45-50 years old as the abnormal iron metabolism leads to accumulation...
of excessive quantities of iron in the parenchyma of some target organs, whose structure and function become progressively compromised\(^1\). The incidence of hereditary haemochromatosis (HH) is significantly underestimated: populations in North Europe\(^2\) have a higher rate of HH, with 1 out of every 300 individuals being affected.

In 1996 a gene, called \(HFE\), was identified\(^3\). This gene is located on the short arm of chromosome 6, telomeric to the HLA system and about 4 megabases from the HLA-A locus. Mutations in the \(HFE\) gene can cause HH and have important implications for the diagnosis of haemochromatosis.

The first mutation identified\(^3\), called \(C282Y\), produces a cysteine to tyrosine substitution in position 282 of the \(HFE\) protein chain, thus preventing the formation of the disulphide bridge of the \(a\) 3 loop, which is indispensable for binding \(b2\)-microglobulin. Without this bond, the \(HFE\) molecule is unstable and cannot be expressed on the cell surface; instead, it is retained in the endoplasmic reticulum and in the first compartment of the Golgi apparatus, where it is broken down. The resulting absence of negative regulation of transferrin receptor 1 (TfR1) leads to an overload of iron in the liver and other organs and hence haemochromatosis\(^4,7\).

Subsequently, a second mutation of the \(HFE\) gene, called \(H63D\), was identified\(^4\). This mutation does not have any effect on \(b2\)-microglobulin binding or protein expression on the cell surface, but affects the \(a\) 1 domain in the loop between the third and fourth strand of the pocket corresponding to the antigen-presenting site of the HLA molecule\(^4\) and decreases the affinity of transferrin for TfR1\(^1,7\), although much less markedly than the \(C282Y\) mutation. The role of the \(H63D\) mutation in HH is not yet completely clear.

Other mutations of the \(HFE\) gene have recently been identified\(^8-12\); one of these, \(S65C\), located in exon 2 close to the \(H63D\) mutation, seems to be a genetic variant that increases the risk of developing mild forms of HH in association with \(C282Y\) or \(H63D\).

The \(C282Y\) mutation is prevalent in HH but with different frequencies in different populations: in fact it is found in 80–90% of HH patients in northern Europe\(^13,14\) while its frequency decreases to 64–76% in patients in southern Europe\(^15-17\), where there is an increased frequency of the other mutations\(^9\) of the \(HFE\) gene and of other genes involved in haemochromatosis, such as the \(TJRN2\) gene on chromosome 7 in the case of \(HFE3\)\(^18\), the gene for juvenile haemochromatosis on chromosome 1 in the case of \(HFE2\)\(^19\) or the \(SLC11A3\) gene of ferroportin in the cases of type 4 dominant haemochromatosis\(^20\).
A study of C282Y and H63D mutations and HLA typing in patients with haemochromatosis in the Province of Cuneo\textsuperscript{1,22} showed a correlation between the presence of the C282Y mutation and HLA A3-B7 haplotype, suggesting the possibility of using the HLA A3-B7 phenotype as an indicator for genetic screening of the population in order to identify carriers of the C282Y mutation early. The value of identifying such carriers early is that they are at higher risk of developing haemochromatosis and they could, perhaps, be encouraged to donate whole blood for their own benefit.

In the light of paragraph 17 of the DMS 26.1.2001 normative, in which Regional authorities in collaboration with Transfusion Centres, are urged to promote preventive health care initiatives on the basis of evaluations of data from donors and donations, since January 2000 those donors at the Transfusion Department of the Province of Cuneo who were found to have an HLA A3-B7 haplotype were invited to undergo testing to determine the presence of mutations of the HFE gene, if they wished. At the same time, the Departmental Transfusion Service of the Santa Croce e Carle (Cuneo), Mondovì and Savigliano health facilities adhered to a programme, approved and funded by the Region of Piedmont, of screening new blood donors for hereditary haemochromatosis. The initial screening of subjects, who were then to undergo HFE genotyping, was based on a biochemical strategy, involving calculation of the transferrin saturation index. This report presents the results of the two different types of screening programme and discusses which would be the better approach for population screening for mutations of the HFE gene in real-life situations.

**Materials and methods**

**Group 1: Screening of bone marrow donors based on HLA typing**

The screening based on HLA typing was carried out between January 2000 and December 2002 in a group of 1,825 bone marrow donors, (870 males, 955 females, mean age 35 years, range 19-51), of whom 146 were found to be HLA A3-B7 positive (77 males and 69 females). These 146 individuals were sent a letter inviting them to undergo a test to identify mutations of the HFE gene. Ninety-six donors (49 males, 47 females, mean age 33 years, range 20-51) accepted the invitation and were typed for HFE gene mutations after DNA extraction, carried out using QIAamp\textsuperscript{®} Mini kit columns (Qiagen, Milan). The search of population delle mutazioni del gene HFE in base alle diverse realtà.

**Materiali e metodi**

**Gruppo 1: Screening in base alla tipizzazione HLA sui donatori di midollo osseo**

Lo screening in base alla tipizzazione HLA è stato eseguito dal gennaio 2000 al dicembre 2002, su un gruppo di 1,825 donatori di midollo osseo, (870 maschi, 955 femmine, età media 33 anni, range 19-51), di cui 146 risultarono HLA A3-B7 positivi (77 maschi e 69 femmine). Ad essi è stata inviata la lettera di invito ad eseguire il test per la ricerca delle mutazioni del gene HFE.

Hanno aderito 96 donatori (49 maschi, 47 femmine, età media 33 anni, range 20-51) che sono stati tipizzati per le mutazioni del gene HFE dopo estrazione del DNA, eseguita mediante le colonne QIAamp\textsuperscript{®} Mini kit (Qiagen, Milano).

La ricerca delle mutazioni del gene HFE e del gene TFR2 è stata effettuata con il test di mutazione genica dell’emocromatosi, secondo le istruzioni della ditta, (Haemochromatosis Strip Assay, Vienna Lab, Wien, Austria, distribuito dalla ditta Nuclear Laser Medicine, Settala, Milano). Il test permette di eseguire la ricerca di 11 mutazioni del gene HFE: C282Y, H63D, S65C, V53M, V59M, H63H, Q127H, E168Q, E168X, W169X, Q283P e la mutazione Y250X del gene TFR2. È stata eseguita un’amplificazione multiplex delle sequenze dei geni HFE e TFR2 utilizzando primers biotinilati; i prodotti di PCR sono stati ibridizzati ad oligonucleotidi aminomodificati ed immobilizzati su una membrana di nylon. Le strisce contenenti le sonde sono state ibridizzate con 10 mL di DNA amplificato e denaturato in un ugual volume di NaOH 1N per 5', poi incubate in 1 mL di soluzione di ibridizzazione (SSC 6X 0,1% SDS) in bagnomaria a 45 °C per 30'. Dopo 3 lavaggi di stringenza a 45 °C, le membrane sono state incubate con fosfatasi alcalina coniugata a streptavidina per 15' a temperatura ambiente. Dopo altri 3 lavaggi, gli ibridi biotinilati sono stati rivelati utilizzando un appropriato substrato colorato (NBT/BCIP).

I donatori risultati positivi per una o più mutazioni (39) sono stati indagati per i seguenti parametri: sideremia, ferritina, transferrina ed indice di saturazione della transferrina calcolato secondo la formula: sideremia/ (transferrina x 1,42).

La tipizzazione HLA di classe I è stata eseguita mediante metodica di microlinfocitotossicità, utilizzando piastre del commercio (Biostest, Trezzano sul Naviglio, Milano).
for mutations of the HFE and TFR2 genes was performed using a haemochromatosis gene mutation test, according to the manufacturer's instructions (Haemochromatosis Strip Assay, Vienna Lab, Vienna, Austria, distributed in Italy by Nuclear Laser Medicine, Settala, Milan). This test can identify 11 mutations of the HFE gene: C282Y, H63D, S65C, V53M, V59M, H63H, Q127H, E168Q, E168X, W169X, Q283P as well as the Y250X mutation of the TFR2 gene. Multiplex amplification of the HFE and TFR2 gene sequences was performed using biotinylated primers; the PCR products were hybridised to amino-modified oligonucleotides and fixed on a nylon membrane. The strips containing the probes were hybridised with 10 mL of DNA, amplified and denatured in an equal volume of NaOH 1N for 5', and then incubated in 1 mL of hybridisation solution (SSC 6X 0.1% SDS) in a bain marie at 45 °C for 30'. After 3 stringent washes at 45 °C, the membranes were incubated with streptavidin-conjugated alkaline phosphatase for 15' at room temperature. After another 3 washes, the biotinylated hybrids were read using an appropriately stained substrate (NBT/BCIP). The subjects who were found to have one or more mutations (n=39) then had the following parameters measured: serum iron, ferritin, transferrin and transferrin saturation index, calculated using the formula: serum iron/(transferrin x 1.42). Class I HLA typing was carried out by the microlymphocytotoxicity method, using commercially available plates (Biotest, Trezzano sul Naviglio, Milan).

**Group 2: Biochemical screening of new blood donors**

The biochemical screening carried out on new blood donors in the period January-December 2002 was based on measuring serum iron, ferritin and transferrin, and calculating the transferrin saturation index using the formula: serum iron/(transferrin x 1.42). During this period 1,285 new blood donors (705 males and 580 females, mean age 32 years, range 18-61) were screened, of whom 140 (100 males and 40 females) were found to have a saturation index greater than 45%. All these subjects were sent a letter inviting them to undergo a second, control test at least one month later in a fasting state. Of these 140 donors, 128 presented for the repeat measurements of serum iron, transferrin, ferritin, ESR and transferrin saturation index, and subsequent search for HFE gene mutations.

The mutational analysis in this group was carried out in the Molecular Biology Laboratory of the Department of Clinical and Biological Sciences of San Luigi Hospital, Orbassano (Turin), led by Professor Camaschella, co-

**Gruppo 2: screening biochimico sui nuovi donatori di sangue**

Lo screening biochimico, eseguito sui nuovi donatori di sangue nel periodo gennaio-dicembre 2002 in base ai seguenti parametri: sideremia, transferrina, calcolo dell’indice di saturazione della transferrina secondo la formula: sideremia/(transferrina x 1,42), è stato effettuato su un gruppo di 1.285 nuovi donatori di sangue, (705 maschi e 580 femmine, età media 32 anni, range 18-61) di cui 140 risultarono con indice di saturazione superiore a 45% (100 maschi e 40 femmine). A tutti fu inviata la lettera di invito ad eseguire, ad almeno un mese di distanza, un secondo prelievo di controllo, a digiuno. Di essi, 128 donatori si sono presentati per il controllo di sideremia, transferrina, ferritina, VES ed indice di saturazione, seguiti dalla ricerca delle mutazioni del gene HFE.

La ricerca delle mutazioni di questo gruppo è stata eseguita presso il Laboratorio di Biologia molecolare del Dipartimento di Scienze Cliniche e Biologiche dell’Ospedale San Luigi di Orbassano (Torino), diretto dalla Prof.ssa Camaschella, coordinatrice del progetto regionale, con la stessa metodica del gruppo 1.

I calcoli statistici di confronto tra i vari gruppi studiati sono stati eseguiti mediante il test chi-quadro in tavole 2x2 con relativo calcolo della significatività.

**Risultati**

**Gruppo 1: Screening in base alla tipizzazione HLA sui donatori di midollo osseo**

Dei 1.825 donatori inseriti nello studio, 146 sono risultati HLA A3-B7. Di questi, 96 hanno risposto all’invito ad effettuare la tipizzazione HFE, di cui 49 maschi e 47 femmine.

La ricerca delle mutazioni del gene HFE nei 96 donatori ha evidenziato 39 donatori (41%) positivi per una o più mutazioni, 19 maschi e 20 femmine, di cui 1 omozigote C282Y, 6 eterozigoti composti C282Y/H63D, 19 eterozigoti C282Y, 2 omozigoti H63D, 11 eterozigoti H63D (Tabella I).

Le indagini biochimiche effettuate ai 39 donatori risultati positivi per una mutazione hanno individuato 7 donatori (5 maschi e 2 femmine) con indice di saturazione della transferrina >45% e più precisamente: una donatrice omozigote C282Y, 2 donatori eterozigoti composti C282Y/H63D, 2 donatori, un maschio ed una femmina, eterozigoti C282Y, 1 donatore H63D omozigote ed un donatore H63D eterozigote.
ordinator of the regional project. The method used for identifying any gene mutations was the same as that applied to group 1. Statistic correlation between the studied groups were performed using $c^2$ test in 2x2 tables, computing the level of significance.

**Results**

**Group 1: Screening of bone marrow donors based on HLA typing**

Of the 1,825 donors recruited into the study, 146 had the HLA A3-B7 haplotype. Of these 146 individuals, 96 (49 males and 47 females) responded to the invitation to undergo HFE studies. The HFE gene mutation analysis in these 96 donors revealed 39 (41%) who were positive for one or more mutations (19 males and 20 females). Of these 39 bone marrow donors, 1 was a C282Y homozygote, 6 were C282Y/H63D compound heterozygotes, 19 were C282Y heterozygotes, 2 were H63D homozygotes, and 11 were H63D heterozygotes (Table I).

The biochemical investigations carried out on the 39 donors who had at least one mutation identified 7 subjects (5 males and 2 females) with a transferrin saturation index $>$45%. In detail, there was one female C282Y homozygote, 1 male and 2 females C282Y/H63D compound heterozygotes, 1 male and 3 females C282Y heterozygotes, 1 female H63D homozygote, and 3 females H63D heterozygotes (Table I).

**Table I - HFE gene mutations found among HLA A3B7 bone marrow donors (group 1)**

<table>
<thead>
<tr>
<th>HLA-typed HLA A3B7 donors</th>
<th>HLA A3B7 donors typed for HFE</th>
<th>Donors with mutations of the HFE gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,825</td>
<td>146</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>C282Y +/-</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>C282Y/H63D</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>C282Y +/- H63D</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>C282Y +/- H63D</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>C282Y +/- H63D</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>C282Y +/- H63D</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Other</td>
<td>57</td>
</tr>
</tbody>
</table>

**Table II - New blood donors undergoing biochemical screening (group 2)**

<table>
<thead>
<tr>
<th>New donors</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,285</td>
<td>Males 705</td>
</tr>
<tr>
<td>140</td>
<td>Females 580</td>
</tr>
<tr>
<td>11%</td>
<td>Males 14%</td>
</tr>
<tr>
<td>128</td>
<td>Females 7%</td>
</tr>
<tr>
<td>48</td>
<td>Males 45%</td>
</tr>
<tr>
<td>37%</td>
<td>Females 19%</td>
</tr>
<tr>
<td>128</td>
<td>Males 91</td>
</tr>
<tr>
<td>128</td>
<td>Females 37</td>
</tr>
</tbody>
</table>

**Gruppo 2: screening biochimico sui nuovi donatori di sangue**

Dei 1,285 donatori inseriti nello screening e selezionati in base alla tipizzazione HLA A3-B7, sono stati individuati 39 donatori portatori di una o più mutazioni del gene HFE, pari al 2,14% della popolazione sottoposta a screening.

**Screening for HFE mutations in blood and marrow donors**

Dei 1,285 nuovi donatori di sangue inseriti nello studio, 140 sono risultati con indice di saturazione della transferrina superiore a 45% al primo screening. Di questi si sono sottoposti al secondo controllo 128 donatori, di cui 91 maschi e 37 femmine. Dei maschi, 41 donatori, pari al 45% si sono confermati con indice di saturazione della transferrina $>$45%, mentre delle femmine solo 7, pari al 19% (Tabella II). In tutti i 128 donatori sono state ricercate le mutazioni del gene HFE; di essi sono risultati positivi per una o più mutazioni 59 donatori (46%), 44 maschi e 15 femmine di cui 1 omozigote C282Y, 6 eterozigoti composti C282Y/H63D, 11 eterozigoti C282Y, 7 omozigoti H63D, 34 eterozigoti H63D (Tabella III).

Dei 1,285 donatori inseriti nello screening e selezionati in base alla percentuale di saturazione della transferrina confermata $>$45% sono stati individuati 26 donatori portatori di una o più mutazioni del gene HFE, pari al 2,02% della popolazione sottoposta allo screening iniziale. Stratificando i dati in base alla conferma dell'indice di
2 male C282Y/H63D compound heterozygotes, 2 C282Y heterozygotes (one male and one female), 1 male H63D homozygote and 1 male H63D heterozygote.

Overall, of the 1,825 donors who underwent screening and selection on the basis of an HLA A3-B7 haplotype, 39 carriers of one or more mutations of the HFE gene were identified, which is equivalent to 2.14% of the population that was screened.

Group 2: Biochemical screening of new blood donors

Of the 1,285 new blood donors included in the study, 140 were found to have a transferrin saturation index exceeding 45% at the first screening. Of these, 128 donors (91 males and 37 females) underwent a second control test.

The transferrin saturation index was confirmed to be >45% in 41 of the males (45%) but in only 7 of the females (19%) (Table II).

Mutational analysis of the HFE gene was carried out in all 128 donors and was positive for one or more mutations in 59 of them (46%), 44 males and 15 females. One was a C282Y homozygote, 6 were compound heterozygotes for C282Y/H63D, 11 were C282Y heterozygotes, 7 were H63D homozygotes, and 34 were H63D heterozygotes (Table III).

Among the 1,285 donors screened and selected on the basis of a transferrin saturation confirmed to be >45%, 26 carriers of one or more mutations of the HFE gene were identified, which is equivalent to 2.02% of the population that underwent the initial screening.

Stratifying the data on the basis of confirmed high transferrin saturation index and HFE mutations, it was found that, of the 48 donors with a confirmed saturation index >45%, 26 had mutations of the HFE gene (23 males and 3 females). In more detail, there was one female C282Y homozygote, 4 male compound heterozygotes for C282Y/
H63D, 6 male C282Y heterozygotes, 3 male H63D homozygotes and 12 donors (9 males and 3 females) who were H63D heterozygotes.

Among the 80 subjects whose second transferrin saturation test did not confirm an index >45%, 33 (22 males and 11 females) did in fact have HFE gene mutations: 2 were compound heterozygotes for C282Y/H63D, 5 were C282Y heterozygotes, 4 were H63D homozygotes and 22 were H63D heterozygotes (Table III).

Twenty of these 33 subjects did not have abnormal levels of iron or ferritin.

Since measurements of ferritin and serum iron are among the obligatory annual examinations that periodic donors undergo, we calculated how many donors would be selected for mutational analysis and be found to have HFE mutations if only these two parameters were used to direct the selection and not the at least twice confirmed transferrin saturation index > 45%. The threshold values used were 150 mg/dL for serum iron and 150 ng/mL for ferritin.

Considering only ferritin, 34 donors, all males, were found to have values exceeding 150 ng/mL, (the mean ferritin among all the donors was 55 ng/mL, while the mean among the 108 donors selected for screening because of a saturation index >45% at the first test was 105 ng/mL).

Of these 34 donors, 18 had mutations of the HFE gene: 1 was a homozygous for C282Y, 3 were compound heterozygotes, 5 were C282Y heterozygotes, 3 were H63D homozygotes and 6 were H63D heterozygotes. When both tests were considered, 65 donors were found to have iron and/or serum iron levels above the respective thresholds.

Of these, 36 had one or more mutations of HFE: 1 was a C282Y homozygote, 4 were C282Y/H63D compound heterozygotes, 9 were C282Y heterozygotes, 5 were homozygous for H63D, and 17 were H63D heterozygotes (Table IV). Two donors with all iron parameters raised did not have any mutations.

**Table IV** - HFE gene mutations in new blood donors with STf >45% at the first donation according to values of ferritin, sideraemia and iron saturation index.

<table>
<thead>
<tr>
<th>Donors typed for HFE</th>
<th>Donors typed for HFE</th>
<th>Donors with mutations of the HFE gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>STf &gt;45% confirmed</td>
<td>C282Y+/+</td>
</tr>
<tr>
<td>128</td>
<td>48</td>
<td>1</td>
</tr>
<tr>
<td>Ferritin &gt;150 ng/mL</td>
<td>34</td>
<td>1</td>
</tr>
<tr>
<td>Ferritin &gt; 150 ng/mL</td>
<td>and/or sideraemia &gt;150mg/dL</td>
<td>65</td>
</tr>
</tbody>
</table>

STf = transferrin saturation

**Discussione**

Da quando, nel 1996, Feder e colleghi hanno individuato il gene HFE dell'emocromatossi, malattia caratterizzata da una lunga fase asintomatica, fatale se non trattata, ma facilmente identificabile con test di screening biochimici e genetici e la cui terapia con salassi ripetuti, se iniziata precocemente, è risolutiva, si è ipotizzato da più parti la necessità di sottoporre a screening per le mutazioni del gene HFE le singole popolazioni, soprattutto quelle in cui l'HH risulti frequente.

Come evidenziato anche da altri autori, il beneficio di identificare soggetti con mutazioni del gene HFE ha una ricaduta, oltre che medica, anche sulla qualità di vita e sulla produttività del singolo, con la probabilità di individuare altri familiari predisposti a sviluppare HH.

Per giustificare uno screening generalizzato è necessario però tenerne in debito conto il costo ed il beneficio, individuando quale popolazione sottoporre allo screening e gli eventuali indicatori utili per la selezione dei soggetti da tipizzare.

Lo scopo del nostro studio è stato quello di verificare l'utilità di uno screening, ipotizzato da alcuni degli autori in studi precedenti, tipizzando per HFE i soggetti già tipizzati precedentemente per il sistema HLA e risultati HLA A3-B7, rispetto ad uno screening basato su dati biochimici, in particolare l'indice di saturazione della transferrina confermato >45% in due donazioni successive in nuovi donatori di sangue. Entrambi gli screening risultano a basso costo in quanto usufruiscono, come indicatori, di test già eseguiti per l'iscrizione al Registro Donatori di Midollo Osseo o per la donazione di sangue, quali la tipizzazione.
Table V - HFE gene mutations found among HLA A3B7 bone marrow donors (group 1) and new blood donors with confirmed STf>45% (group 2)

<table>
<thead>
<tr>
<th>Donors included in the initial screening</th>
<th>Donors typed for HFE</th>
<th>Donors with mutations of the HFE gene</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n°</td>
<td>%</td>
</tr>
<tr>
<td>Group 1</td>
<td>1,825</td>
<td>96</td>
</tr>
<tr>
<td>Group 2</td>
<td>1,285</td>
<td>48</td>
</tr>
</tbody>
</table>

STf= transferrin saturation

Discussion

Since 1996, when Feder and colleagues identified the HFE gene of haemochromatosis, a disease characterised by a long, asymptomatic period, fatal if untreated but easily identified by genetic and biochemical screening and which can by cured by early institution of therapy with repeated phlebotomy, various groups have raised the suggestion of screening certain populations, particularly those in which HH is frequent, for mutations of the HFE gene.

As already shown by some researchers, the benefit of identifying subjects with mutations of the HFE gene has implications, beyond the purely medical ones, for the individual's quality of life and productivity as well as for the probability of identifying other family members predisposed to developing HH.

However, in order to justify general screening, the costs and the benefits must be analysed, identifying which populations to submit to screening and any indicators that could be useful for selecting the subjects to undergo typing.

The aim of our study was to verify the usefulness of a screening strategy suggested by some authors in previous studies, determining the HFE genotype of subjects who had already undergone HLA typing and been found to have the HLA A3-B7 haplotype, compared to screening based on biochemical data, that is, a confirmed transferrin saturation index >45% in two successive samples from new blood donors.

Both screening strategies were cheap since the indicators used were results of tests already carried out for enrolment in the Register of Bone Marrow Donors or for donation of blood, that is HLA typing and some biochemical examinations, respectively. The results (Table V) show that screening based on HLA A3-B7 haplotype identified HFE mutations in 2.14% of bone marrow donors, with a slightly higher prevalence of C282Y mutation (1.4% of subjects) than of H63D mutation (1.04%), due to the fact that the C282Y mutation is in linkage disequilibrium with the 7.1 ancestral haplotype, while the H63D mutation correlates more strongly with HLA A29. In fact, biochemical screening based on a confirmed transferrin saturation index HLA or other esami biochimici. I risultati ottenuti (Tabella V) indicano che lo screening in base all'aplotipo HLA A3-B7 ha permesso di individuare soggetti con mutazioni HFE nel 2,14% dei donatori di midollo osseo, con una prevalenza leggermente più elevata della mutazione C282Y (1,4% dei soggetti), rispetto alla mutazione H63D (1,04%), dovuta al fatto che la mutazione C282Y è in linkage disequilibrium con l'aplotipo ancestrale 7.1, mentre la mutazione H63D correla maggiormente con l'HLA A29. Infatti lo screening biochimico basato sulla conferma dell'indice di saturazione della transferrina >45% ha evidenziato mutazioni HFE nel 2,02% dei nuovi donatori di sangue, ma con prevalenza maggiore della mutazione H63D (1,5% dei soggetti sottoposti a screening) rispetto alla mutazione C282Y (0,8%), differenze statisticamente non significative rispetto al primo gruppo (p=0,15).

Dall'analisi delle frequenze alleliche della mutazione C282Y, ristretta ai soggetti HLA A3-B7 del gruppo 1 (96 donatori) ed ai soggetti del gruppo 2 con indice di saturazione confermato >45% (48 donatori) (Tabella V), risulta che nel primo la frequenza allelica della mutazione C282Y è 0,1406 e nel secondo è 0,1250. Nel gruppo di controllo (soggetti del gruppo 1) la stessa frequenza è 0,0480, altamente significativa se paragonata rispettivamente al gruppo 1 (p=0,0025) ed al gruppo 2 (p=0,0302).

La stessa cosa non è valida per la mutazione H63D la cui frequenza allelica in entrambi i gruppi non risulta significativamente diversa rispetto alla stessa popolazione di controllo.

Pur essendo presenti nella popolazione della Provincia di Cuneo mutazioni del gene HFE diverse dalle due mutazioni classiche, in particolare la mutazione S65C e la mutazione rara E168Q, in tutti i gruppi sono state riscontrate solo le mutazioni C282Y ed H63D.

Se si valutano gli stessi indici biochimici del gruppo 2 dei donatori di midollo osseo HLA A3-B7 con mutazioni HFE, ben 32 soggetti su 39 presentano un indice di saturazione della ferritina <45%, a parziale conferma della penetranza incompleta del genotipo HFE mutato.
>45% led to the identification of HFE mutations in 2.02% of new blood donors, but in this case there was a higher prevalence of the H63D mutation (1.5% of screened subjects) than of the C282Y mutation (0.8%), a not statistically significant difference with respect to the former group (p=0.15). An analysis of the allele frequencies of the C282Y mutation, restricted to HLA A3-B7 subjects, in group 1 (96 donors) and in group 2 subjects with a confirmed saturation index >45% (48 donors) (Table V), showed that the allelic frequency of the C282Y mutation was 0.1406 in the former group and 0.1250 in the latter.

The frequency in a random sample of the population of Cuneo was 0.0480, very significantly different from that in group 1 (p=0.0025) and in group 2 (p=0.0302).

The same thing did not hold true for the H63D mutation, whose allele frequency in the two groups selected for genotyping was not significantly different from that in the random control population. Although various other HFE gene mutations, besides the two classical ones, are present in the Province of Cuneo, in particular the S65C mutation and the rare E168Q mutation, only the C282Y and H63D mutations were found in the groups of this study.

When the biochemical indicators examined in group 2 were evaluated in HLA A3-B7 bone marrow donors with HFE mutations, it was found that 32 out of the 39 had a transferrin saturation index <45%, partially confirming the incomplete penetrance of the mutated HFE genotype, this incomplete penetrance was also manifested in the 20 blood donors in group 2 who, despite having one or more mutations of the HFE gene, did not have any abnormalities of iron metabolism.

Since measurement of ferritin concentration is one of the obligatory annual tests for periodic blood donors, we wanted to evaluate whether the ferritin concentration alone could be an adequate indicator for selecting donors to undergo genetic tests for haemochromatosis, thus enabling the transferrin test, which is not part of the obligatory tests, to be avoided. This strategy would have identified 18 donors with HFE gene mutations, which is 1.4% of the donors recruited in the study: one was homozygous for C282Y, 3 were C282Y/H63D compound heterozygotes, 5 were C282Y heterozygotes, 3 were H63D homozygotes and 6 were heterozygous for H63D.

Nineteen donors would have been missed: 1 C282Y/H63D compound heterozygote, 3 C282Y heterozygotes, 3 H63D homozygotes and 12 H63D heterozygotes. If serum iron concentration, another obligatory test to evaluate iron status in donors, was also evaluated, more carriers of HFE mutations were identified (2.8% in our

Conclusion

I due screening studiati in questo lavoro, hanno permesso di individuare la stessa percentuale di soggetti con mutazioni HFE.

Lo screening basato sulla tipizzazione HLA A3-B7 permette di individuare una quota maggiore di soggetti con mutazioni C282Y, dovuto al fatto che questa mutazione e gli alletti A3-B7 sono in linkage disequilibrium con l'aplotipo ancestrale 7.1, ma presenta l'inconveniente di individuare donatori con mutazioni HFE senza accumulo di Fe, inconveniente dovuto alla possibile penetranza incompleta del gene mutato. Le mutazioni HFE da sole non sarebbero quindi sufficienti per sviluppare la malattia, la diagnosi richiede comunque una conferma con altri parametri dell'accumulo di ferro, quali ferritina e indice di saturazione della transferrina elevate. Non essendo la differenza rispetto allo screening basato sui valori biochimici statisticamente significativa, si ritiene consigliabile applicare quest'ultimo in quanto, oltre ad evitare l'errore dovuto alla penetranza incompleta, può essere applicato facilmente in tutti i SIMT. Infatti tutti hanno a disposizione i risultati di test di laboratorio sufficienti per effettuare lo screening sui propri donatori, a differenza di uno screening basato sulla tipizzazione HLA, i cui dati sono disponibili solo per quei SIMT che siano contemporaneamente Centri Donatori del
study), but the specificity of the identification of subjects to genotype decreased.

**Conclusions**

The two screening strategies evaluated in this study identified the same percentage of subjects with *HFE* mutations. The screening based on HLA A3-B7 haplotype detected a higher proportion of subjects with *C282Y* mutations, because this mutation and the A3-B7 alleles are in linkage disequilibrium with the 7.1 ancestral haplotype, but has the drawback of identifying donors with *HFE* mutations without increased iron stores, a problem perhaps caused by incomplete penetrance of the mutated gene. *HFE* mutations alone do not, therefore, appear to be sufficient for the disease to develop; the diagnosis nevertheless requires confirmation by other findings of iron accumulation, such as a high ferritin concentration and elevated transferrin saturation index. Given that the difference from the screening based on biochemical values was not statistically significant, it is recommended that this latter strategy is used since, besides avoiding errors due to incomplete penetrance, it can be applied easily in all SIMT. In fact, all SIMT have the results of enough laboratory tests to be able to screen their own donors, whereas the data for screening based on HLA typing are only available in those SIMT which are also Donor Centres for the Italian Registry of Bone Marrow Donors. Furthermore, the frequency of the ancestral haplotype 7.1, which is associated with the *C282Y* mutation, varies from Region to Region and also within single Regions; for example, the frequency of A3-B7 is 3.38% in the Province of Cuneo (Menardi, unpublished data) whereas the Italian mean is 2.26%. It remains to be established whether screening based only on ferritin values is sufficient to identify subjects to undergo *HFE* typing. In order to do this, it is thought that periodic female donors with ferritin values constantly >150 ng/mL and male donors with values constantly >300 ng/mL should be invited to undergo *HFE* typing; this represents 0.5% of our female periodic donors and 1.0% of our male periodic donors.

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Registro Italiano Donatori di Midollo Osseo. Inoltre la frequenza aplotipica dell’aplotipo ancestrale 7.1, cui è associata la mutazione *C282Y*, varia da Regione a Regione e anche all’interno delle singole Regioni, con una frequenza di HLA A3-B7 per la Provincia di Cuneo di 3,38% (Menardi, dati non pubblicati) rispetto alla media italiana di 2,26%.

Rimane da accertare se uno screening basato solo sui valori di ferritina sia sufficiente ad individuare i soggetti da sottoporre a tipizzazione *HFE*. A questo scopo si pensa di invitare ad effettuare la tipizzazione *HFE* i donatori periodici con valori di ferritina stabilmente >150 ng/mL per le donatrici e >300 ng/mL per i donatori, che rappresentano rispettivamente lo 0,5% dei nostri donatori di sesso femminile e l’1,0% dei nostri donatori periodici maschili.

Parte di questa ricerca, relativa agli esami specifici sui nuovi donatori di sangue, è stata finanziata dall’Assessorato alla Sanità della Regione Piemonte.

**Riassunto**

L’emocromatosi ereditaria (HH) è una delle più comuni alterazioni che colpiscono i popoli di origine nord-europea. L’individuazione del gene *HFE*, con le sue mutazioni *C282Y* e *H63D*, consente la diagnosi genetica di un gran numero di casi.

Tuttavia, sono state riscontrate diverse frequenze delle mutazioni in differenti popolazioni di pazienti affetti da HH. Si dovrebbero realizzare screening delle mutazioni del gene *HFE* nei Paesi ad alta incidenza di HH. In questo studio vengono presentati due diversi programmi di screening per HH, il primo riguardante semplicemente lo studio biochimico del metabolismo del Fe su 1.285 nuovi donatori e una secondo basato sulla tipizzazione dell’aplotipo HLA A3-B7 di 1.825 candidati donatori di midollo osseo iscritti al Registro Italiano Donatori di Midollo (IBMDR) e arruolati presso il Dipartimento di Medicina Trasfusionale della Provincia di Cuneo.

Con entrambi i programmi è stato possibile identificare soggetti portatori di mutazioni *HFE*, con preferenza per lo screening biochimico facilmente utilizzabile in tutti i SIMT.

**Parole chiave:** emocromatosi, gene *HFE*, genetica di popolazione
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