

Reverse cascade screening of newborns for hereditary haemochromatosis: a model for other late onset diseases?

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Background: Genetic testing can determine those at risk for hereditary haemochromatosis (HH) caused by *HFE* mutations before the onset of symptoms. However, there is no optimum screening strategy, mainly owing to the variable penetrance in those who are homozygous for the *HFE* Cys282Tyr (C282Y) mutation. The objective of this study was to identify the majority of individuals at serious risk of developing *HFE* haemochromatosis before they developed life threatening complications.

Methods: We first estimated the therapeutic penetrance of the C282Y mutation in people living in la Somme, France, using genetic, demographic, biochemical, and follow up data. We examined the benefits of neonatal screening on the basis of increased risk to relatives of newborns carrying one or two copies of the C282Y mutation. Between 1999 and 2002, we screened 7038 newborns from two maternity hospitals in the north of France for the C282Y and His63Asp (H63D) mutations in the *HFE* gene, using bloodspots collected on Guthrie cards. Family studies and genetic counselling were undertaken, based on the results of the baby's genotype.

Findings: In la Somme, we found that 24% of the adults homozygous for the C282Y mutation required at least 5 g iron to be removed to restore normal iron parameters (that is, the therapeutic penetrance). In the reverse cascade screening study, we identified 19 C282Y homozygotes (1/370), 491 heterozygotes (1/14) and 166 compound heterozygotes (1/42) in 7038 newborns tested. The reverse cascade screening strategy resulted in 80 adults being screened for both mutations. We identified 10 previously unknown C282Y homozygotes of whom six (four men and two women) required venesection. Acceptance of neonatal screening was high; parents understood the risks of having HH and the benefits of early detection, but a number of parents were reluctant to take the test themselves. Neonatal screening for HH is straightforward. Reverse cascade screening increased the efficiency of detecting affected adults with undiagnosed haemochromatosis. This strategy allows almost complete coverage for HH and could be a model for efficient screening for other late onset genetic diseases.

Hereditary haemochromatosis (HH) caused by mutations in *HFE* is a common autosomal recessive disorder of iron metabolism in people of northern European extraction. Often, middle aged patients present with early clinical symptoms of general fatigue, arthralgia, and arthritis, which are not specific to HH. Liver disease, diabetes, and impotence are complications that arise later.¹ In northern France, about 85% of patients with HH are homozygous for the Cys282Tyr (C282Y) mutation in *HFE*.² The role of a second mutation, the substitution of histidine by aspartic acid at position 63 (H63D) is unclear.³ There are a number of arguments favouring preventative screening for haemochromatosis in northern European populations: (a) 2–5 of every 1000 individuals in the North of Europe are homozygotes for the C282Y mutation (genotype HH/YY), (b) normal life expectancy can be restored if iron is removed by venesection in the pre-cirrhotic stage; and (c) premature death results if HH remains undetected for too long.⁴ Despite the fact that HH can be considered as a model genetic disorder for screening and disease prevention, there is no consensus regarding the optimal screening strategy.^{5–7} The challenge is how to identify the majority of individuals at serious risk of developing iron overload before they develop life threatening complications.

The diagnostic utility of a single measurement of iron status, (such as percentage transferrin saturation (%T_{sat}) or unbound iron binding capacity test) varies with the age of testing.^{8,9} Disease is accompanied by increased serum ferritin levels, which unfortunately are also associated with a

number of other conditions. Repeated biochemical testing is recommended, but this is costly.^{7,10} Although men show signs of the disease earlier than women, there is a wide age range associated with the onset of symptoms in both sexes.¹¹ For these reasons, the optimum age for screening adults using serum iron parameters has yet to be established.

Genetic screening is an alternative but it raises ethical, political, and economic issues. In particular, the incomplete penetrance of the C282Y mutation in homozygotes raises problems as to the definition of the disease and when to treat.^{12–17} Other genotypes are occasionally associated with the disease.¹⁸ There are a number of reports describing low levels of clinical penetrance in C282Y homozygotes.^{15,17} These findings in particular have suggested that it is not cost effective to undertake population screening for haemochromatosis. On the other hand, there are other reports suggesting that there are much higher levels of mutation penetrance elsewhere.¹⁹ Should the definition of penetrance depend upon abnormal biochemical parameters or should it also include clinical disease? One of the main reasons for instigating a screening programme for haemochromatosis is that disease prevention and hence, any screening strategy, needs to identify patients in the early pre-clinical phase before irreversible end organ damage has occurred. If widespread population screening is to be cost effective for

Abbreviations: CF, cystic fibrosis; HH, hereditary haemochromatosis; PKU, phenylketonuria

haemochromatosis, it is important to understand the nature of disease penetrance in that particular population.

If the degree of penetrance is high, then this provides a justification for some form of population screening. Genetic screening is not age dependent and could be particularly cost effective if it could be incorporated with DNA screening for other diseases such as cystic fibrosis (CF). The ability to follow a cohort over time and see if and when the members develop clinical disease will also help in understanding penetrance in haemochromatosis when presentation is so variable. Another advantage of a neonatal screening programme for haemochromatosis is that it also permits the application of reverse cascade screening.

Cascade screening is used to identify asymptomatic individuals who are at risk of developing a disease because they have an affected relative. Reverse cascade screening identifies the asymptomatic individual first and uses this information to identify undiagnosed affected relatives.

We analysed the number of C282Y homozygotes identified in the Somme département of France over a 4 year period, and identified those who required the removal of more than 5 g of iron by quantitative phlebotomy, using the iron burden to define therapeutic penetrance. In a population in which 50% of C282Y homozygotes express disease, then 40% of homozygotes should be detected by screening first to third degree relatives of C282Y homozygotes.²⁰ On the other hand, if penetrance is lower, with 25% of the C282Y homozygotes showing signs of iron burden, the number of at risk individuals detected by reverse cascade screening falls to 24%.²⁰ This then reduces the number of people to be tested to identify a single at risk individual.

Therefore, as an alternative to phenotypic screening, we investigated the advantages of neonatal screening for HH followed by screening for the variants in parents and other relatives when the neonate was identified as heterozygous or homozygous for the at risk genotype (that is, "reverse" cascade screening). The advantage of this strategy is that it identifies a target population of relatives who have an increased risk of HH.

MATERIALS AND METHODS

Penetrance of the C282Y homozygous genotype in the adult population

Available data regarding the Somme district were extracted from the Institut National des Statistiques et des Études Économiques and from the Institut National des Études Démographiques, Paris, France. We estimated the penetrance of the C282Y mutation during the period 1996–2000 using demographic, biochemical, genetic, and phlebotomy records in patients having the HH/YY genotype in our département. We defined a fully penetrant genotype as one requiring the removal of a minimum of 5 g of iron to return serum iron parameters to normal. Using this information, we instigated a neonatal screening study for HH.

Neonatal study design

Both local and national ethics permission were obtained, with the following qualifications: the national ethics committee asked that all parents should have access to their child's results, and the local research ethics committee restricted the length of the study to 3 years. The work was approved by the Ministry of Health (registered no. DGS 2002/0366) and insured as required for research programmes involving genetic testing of no immediate benefit (Biomedicinsure no. 200300035; Gerling Co., France). This insurance policy protects the hospital against claims of negligence that might be filed at a later date by the parents.

Neonatal screening was conducted at two maternity hospitals in Picardie (northern France, including the

Somme département) between 1999 and 2002. Medical staff (paediatricians and nurses) attended a series of seminars on neonatal screening and HH with 6 monthly updates.

When each baby was 1 day old, parents were given a four page leaflet describing the genetics of HH and its complications, supplemented on the second day by verbal information including a question and answer session. The time taken (5–30 minutes) depended on the parents' understanding of genetics. Participation in the programme was entirely voluntary, with a clear explanation that the results of the screen would initially benefit parents and relatives. Implications of the at risk genotype (HH/YY) and its ramifications for their baby were explained to parents.

Consent was modified as new genes involved in haemochromatosis were described. Initially, however, it was made clear to parents that screening was restricted to the C282Y and H63D mutations in the *HFE* gene and that the study did not include a test for neonatal haemochromatosis. Parents were given 2 days to accept or refuse the genetic test for their baby. When informed consent from both parents was obtained, on the third day after birth blood was spotted onto a Guthrie card that forms part of the routine screening for phenylketonuria (PKU), hypothyroidism, and CF. Local general practitioners and paediatricians practising in the private sector also received written information from the local social security system concerning this 3 year research programme for HH neonatal screening. Three annual continuing medical education conferences on haemochromatosis, its management, and reverse cascade screening were organised in the medical school for all local clinicians.

Genetic studies

DNA was extracted from a Guthrie card spot with the QIAamp Blood kit (Qiagen SA, Courtaboeuf, France) using the specified protocol for dried blood. As previously described,^{21,22} 10 µl of the eluted DNA (75–100 ng) were used for genotyping the C282Y and H63D mutations with appropriate controls.

We calculated allele and genotype frequencies for both mutations in neonates. Expected genotype frequencies were estimated according to the Hardy-Weinberg equilibrium. We performed χ^2 tests to verify Hardy-Weinberg equilibrium for all genotypes and to compare genotype and allele frequencies in different groups.

Parents received the results through the post and were invited in for free genetic counselling if their newborn had the genotype HH/YY. They were informed that, although the HH/YY genotype was a risk factor for developing iron overload in later life, it did not affect the health of the baby, but that family screening was advisable. If there was no response, the parents received a second letter and if there was no reply to this, a telephone call. The couples themselves were responsible for informing other family members. Genetic counselling was arranged at the request of the parents, during which it was stressed that the baby did not need any treatment or a special diet, but regular serum iron measurements as an adult was advised. Transferrin saturation and serum ferritin were not measured in the neonates.

RESULTS

The population of the Somme département is 551 479, of whom 84% are white. Using the Hardy-Weinberg equilibrium, and knowing that C282Y allele has an allele frequency in this part of France of $q = 0.0577$, we found that within the white subset, about 1542 people are expected to be homozygous for the C282Y mutation in the *HFE* gene.^{7,23} Because of age and sex dependency of the manifestations of the symptoms, corrections were made using demographic data. In the white population, 21.1% of the women are aged

≥45 years and 22.5% of the men are aged ≥35 years, therefore we estimated that in this population 347 men and 325 women are C282Y homozygotes at risk of developing symptoms for HH. From 1996 to 2000, 288 patients fulfilled these age criteria and were identified as C282Y homozygotes (176 men; 112 women). Of the men, 107 have had at least 5 g of iron removed, giving a therapeutic penetrance of 30% (107/347), while 54 women were treated, giving a therapeutic penetrance of 16.6% (54/325). This gives an overall penetrance of 24% (161/672), a figure that, based on the calculations of Krawczak *et al.*,²⁰ suggested that cascade screening was likely to be effective.

To see whether reverse cascade screening would be a potential and efficient screening strategy for haemochromatosis, we used Laplace-Bayes's theorem to estimate the risk of parents having the homozygous genotype (HH/YY) if their child was either homozygous or heterozygous for the C282Y mutation. Using an average Y allele frequency (q) already known in the white population from previous studies (q = 0.0577), corresponding to a prevalence of about 1/300, we calculated that if an HH/YY newborn is identified, the probability for each parent being HH/YY is q, which corresponds to a relative risk of 1/q compared with the general population—that is, they have a 17 fold greater risk. In the case of a C282Y heterozygote, the probability of each parent being HH/YY is q/2, corresponding to a relative risk of 1/2q, or an 8.5 fold greater risk compared with that of the general population. Probability for both parents to be homozygous for the C282Y mutation when a child is homozygous is q².

We then carried out a pilot study to estimate the take up rate and to plan the logistics of neonatal screening study for haemochromatosis. Of those who first received an explanatory leaflet about the HH screening programme in neonates, 85% (105/123) agreed to neonatal genetic testing and family studies. Based on this result, we decided to organise neonatal screening for HH in Picardie.

We screened 7038 of 8280 babies born during the study period for the C282Y and H63D mutations. We identified 19 C282Y homozygous babies from 18 families; two were brothers. Results for all genotypic groups for all ethnic groups are presented in table 1. Of 7038 newborns tested, 694 (9.8%) had at least one non-white parent. There were no C282Y homozygotes in this group. Frequency of the C282Y allele in newborns with one or two non-white parents was 0.0200 compared with an allele frequency of 0.0526 in the group in which both parents were white (p<0.001). The H63D allele frequency was 0.140 in the neonates with one or two non-white parents compared with 0.185 in the neonate group in which both parents were white (p = 0.05). Genotype frequencies for C282Y and H63D mutations followed Hardy-Weinberg equilibrium for five genotypes but not for compound heterozygotes (HD/CY) (p<0.001). This discrepancy in HD/CY had also been observed in a previous study of healthy adults from the same region.²³

Screening of families with C282Y homozygous neonates

Of 18 couples, 11 registered for genetic counselling with consent from both parents. Seven families did not respond to the letters they were sent, nor did they contact any medical staff. Counselling was performed 3–6 months after their baby was identified as a C282Y homozygote. Screening of these 22 parents identified five family members from four different families as C282Y homozygotes; in one family, the homozygotes were a parent and the baby's 7 year old brother, and in each of the other three families it was a parent. None of these families had a history of haemochromatosis.

Table 1 HFE genotypes among the neonate population (all ethnic groups)

Genotype	Observed (n = 7038)	Expected	p
HH/CC	4195 (59.61)	4173 (59.30)	0.6
HD/CC	1952 (27.74)	1962 (27.90)	0.76
HH/CY	491 (6.97)	531 (7.54)	0.07
DD/CC	215 (3.05)	230 (3.25)	0.35
HD/CY	166 (2.36)	125 (1.77)	<0.001*
HH/YY	19 (0.27)	17 (0.24)	0.61

Results are n (%). p value: χ^2 analysis for all genotypes. Y allele frequency is 0.0494, 95% CI 0.047 to 0.0548; D allele frequency is 0.181, 95% CI 0.172 to 0.190. Ethnic group was self reported. *Genotype frequencies for C282Y and H63D variants were each in Hardy-Weinberg equilibrium; however, increased frequency of double heterozygotes (HD/CY) was observed owing to partial linkage disequilibrium (p<0.001) between these variants.

Screening of families with C282Y heterozygous neonates

Parents with a C282Y heterozygous baby could also request family screening. In this group, only 10 of 657 couples requested genetic testing. Five C282Y homozygotes from two families were identified: one parent and an aunt in one family; one parent, an uncle, and a grandmother in the other. Again, there was no family history of haemochromatosis.

Iron status in family members identified as C282Y homozygotes

A follow up was arranged for family members identified as C282Y homozygotes. Baseline (T₀) T_{sat} and serum ferritin levels were determined in all the 10 C282Y homozygotes with a follow up measurement (T₁) at 6 months (table 2). Subject V was immediately treated by venesection in view of the high T_{sat}. Subject II, following a second biochemical (T₁) estimation 3 months later, had elevated T_{sat}, and treatment was recommended. After the second estimation, treatment was recommended for subjects III, IV, VII, and IX. All these patients had normal C reactive protein levels. Subject IX, a woman aged 49 years, presenting with 88% T_{sat} and a serum ferritin of 100 was not treated originally, as her serum ferritin fell within the normal range (50–200 µg/l). Subsequently her serum ferritin has risen to 226 µg/l (table 2) and she is now receiving treatment. In total, all of the four men and two of the five women now require venesection. We aim to maintain their serum ferritin at ≤50 µg/l and T_{sat} levels at ≤25%. Of the five HH/YY women, two women are being treated for haemochromatosis. Further questioning revealed that individuals II and V had symptoms of asthenia. An x ray analysis showed a loss of joint space at metacarpophalangeal joints in individual II. All the other individuals were symptomless.

Serum iron parameters in family members identified as compound heterozygotes

There were 14 people (seven men, seven women; 10 of whom were grandparents) from 11 families identified with the genotype HD/CY. Only one, a grandfather (aged 50 years) had an increased serum ferritin level (378 µg/l) with a normal T_{sat} (39%) on initial testing. Six months later, his T_{sat} was 54% and serum ferritin 648 µg/l, following which one 400 mL venesection was performed; following venesection, T_{sat} and serum ferritin values were 25% and 150 µg/l, respectively. Twelve months later, his serum iron parameters remain within the normal range.

DISCUSSION

The penetrance of a genotype can be defined as the proportion of individuals with that particular genotype who

Table 2 Iron status in family members identified as C282Y homozygotes

Subject no.	Sex	Age (years)	T _{sat} (%) T ₀ /T ₁	Ferritin (µg/l) T ₀ /T ₁	Amount of iron removed (g)*
I	M†	7	39/42	190/220	NA
II	M†	34	63/73§	430/680§	6.5
III	M†	32	47/59	342/525	5.25
IV	M‡	41	48/75	150/542	6.0
V	M‡	24	79/ND	672/ND	6.5
VI	F†	30	25/33	15/46	NA
VII	F‡	27	78/89	137/634	5.5
VIII	F†	23	10/22	12/55	NA
IX	F‡	49	88/92	100/226	0.5
X	F‡	27	65/68	32/82	NA

*To maintain to T_{sat} ≤ 25% and ferritin ≤ 50 µg/l. Subject identified through: †C282Y homozygous newborn; ‡C282Y heterozygous newborn. Transferrin saturation (T_{sat} %) and serum ferritin (µg/l) at genotyping (T₀); T₁ is 6 months or §3 months past T₀. NA, not applicable.

have the associated phenotype. If these individuals then present with clinical symptoms, this can then be regarded as clinical penetrance. Based on questionnaire data or clinical observation, the prevalence of symptoms associated with haemochromatosis is common in both C282Y homozygotes and individuals with the wildtype genotype. Therefore, the clinical penetrance of the HH/YY genotype with regard to these symptoms is low.^{15–17} Biochemical penetrance, the finding of increased serum iron indices, is much higher than the clinical penetrance. Indeed, most C282Y homozygotes display a common biochemical phenotype, namely an elevated transferrin saturation level that is found with an increased serum ferritin level in up to 77% of men and 56% of women.²⁴ Heterogeneity in the presentation of HFE associated haemochromatosis, together with the fact that biochemical presentation is both age and gender specific, present difficulties in comparing studies involving the HH/YY genotype.²⁵ Because of this, the penetrance of the HH/YY genotype has proved difficult to establish. Therefore, in our study we took a different approach by examining therapeutic penetrance, by referring it to the actual iron burden. The assumption is that if a genetic disease is treated, thus preventing complications, the genotype giving rise to the disease is fully penetrant. Rather than waiting for massive accumulation of iron, the demonstration of increased iron stores was chosen as the indication for treatment. Although different definitions cannot give rise to comparisons, the removal of at least 5 g of iron in order to restore normal iron biochemical parameters seems a useful definition of penetrance, particularly, as this has long been part of the definition of the disease, yet more recent reports have not considered this factor in describing what is and what is not haemochromatosis.

In this study, we identified 288 of a possible 672 haemochromatosis patients who had first been referred to consultants because they had high serum iron parameters and/or clinical manifestations including arthralgia and/or fatigue. If the definition of disease is based on quantitative phlebotomy, the removal of 5 g of iron over a period of less than a year (that is, the “therapeutic penetrance”), 161 patients met this criterion, but 127 of the 288 C282Y homozygotes did not. It is possible that among the remaining predicted 384 C282Y homozygotes, some may have undiagnosed haemochromatosis, thus the observed therapeutic penetrance of the HH/YY genotype of 24% (161/672) may well be an underestimate. Nevertheless, in a population with this condition, widespread population screening for haemochromatosis may be advantageous.

Neonatal screening for HH has been undertaken by other groups purely as a means of population screening to establish the frequencies of the C282Y and H63D alleles.^{26–27} Our strategy combines population wide and reverse cascade screening of C282Y homozygous newborns to enable early detection of disease in parents and relatives. The neonatal period is an excellent time to access family members of different generations and arguably an ideal time to detect early haemochromatosis, as the majority of the parents of the newborns were <35 years of age in this study.

Initially, this study was designed to screen for the C282Y and H63D mutations where both parents were whites, but was later expanded as universal screening at the request of non-white parents. The percentage of newborns were missed was 15%; 12% due to staff forgetfulness, and 3% to parents declining the test. Thus, the acceptance rate for neonatal screening for C282Y and H63D was high; the parents were aware of the implications for themselves and their families. Traditionally, population screening for haemochromatosis has had no psychosocial impact on anxiety, mental health, or physical health status.²⁸ Anxiety was never given as a reason for refusal of neonatal screening, possibly because haemochromatosis is treatable.²⁹ Both parents were unanimous in their decision to accept or refuse neonatal testing. In France, current law prevents disclosure of genetic testing results to insurance companies, banks, or employers, and hence this is unlikely to be a reason for refusal. To obtain a high acceptance rate for genetic screening programmes elsewhere, it may be necessary to enact local law to prevent disclosure of genetic information to limit discrimination by insurance companies, employers and banks. Currently, neonatal screening for PKU, hypothyroidism, haemoglobinopathies, and CF does not require parental permission, as these are not DNA based tests. However if a result justifies DNA analysis (for example, in CF) parental consent is required.

Although acceptance of neonatal screening was high, only 11 of the 18 families (61%) with homozygous newborns accepted further testing. The take up rate for further testing in families with C282Y heterozygous babies was much lower compared with those with homozygous newborns; only 10 of 657 families (1.6%) responded despite all the information and support provided. In these families, increased personal contact with parents might have increased the response rate,³⁰ but this would have been time consuming, and required more staff and resources. However, despite the poor response, reverse cascade screening identified five C282Y homozygotes from the 10 families with C282Y heterozygote newborns. These results demonstrate that parents were willing for their baby to be screened, yet unwilling to be screened themselves. Our screening strategy benefits both parents equally (table 2). A previous study showed that it was difficult to recruit young men, the very group most likely to benefit from screening programmes for HH.³¹ If this lack of willingness to participate in screening programmes is prevalent among the adult population, it has wide implications for preventative screening in a wide range of diseases. Screening for several diseases at once is the most cost effective approach, and neonatal screening is the most cost effective time. It also lends itself to the creation of a centralised register, allowing longitudinal studies on disease penetrance, not just for HH but also for other diseases that might be incorporated later into disease surveillance programmes.

Using this strategy of reverse cascade screening, we have identified 10 previously unknown C282Y homozygotes, of whom six (four men and two women) are now being treated by regular venesection, thus preventing complications of the disease and restoring normal life expectancy. Annual follow ups have been proposed for the other three women who are

C282Y homozygotes. Close monitoring has been proposed for one individual, the 7 year old son of subject II (table 3), whose serum ferritin rose by more than 20% in 1 year. Of 14 compound heterozygotes, one required treatment, despite the mean (SD) age of this group being 49 (2.3) years.

It should be noted that all four HH/YY men identified in this study had elevated serum iron parameters 1 year after diagnosis. It is important to appreciate the significance of raised serum iron parameters in the absence of clinical disease. In particular, arthritis due to joint damage associated with haemochromatosis is not always reversible.³² It should be noted that the youngest patient identified was aged 24 years and the oldest 49 years. Increased serum iron parameters in one so young indicates that such individuals are likely to develop haemochromatosis unless treated. Therefore, our approach allows the early identification of HH with the aim of preventing clinical disease. The large number of relatives requiring treatment for haemochromatosis suggests that our figure for therapeutic penetrance of 24% may well be an underestimate for this part of France.

This study raises several questions and issues. When do we actually treat someone with HH/YY genotype with raised a T_{sat} % but normal ferritin levels? How often do we repeat serum ferritin level measurements? It has also been questioned whether families with heterozygous newborns should be investigated. An unexpected finding was the identification of five homozygotes from 10 of such families, a number similar to that identified through reverse cascade screening of homozygous newborns. Although the number of such families tested is low, it shows that enrichment in HH/YY genotypes in families from heterozygous babies has to be taken into account in a future screening strategy. All family members diagnosed were very grateful for the early detection of their haemochromatosis.

Reverse cascade screening offers the possibility of early clinical intervention, preventing morbidity and mortality associated with HH. Cost effective preventive measures include regular blood donation and reduction of alcohol intake. A major difficulty with any neonatal screening programme is making sure that information is retained for the future. Screening newborns is not just for the benefit of their families, provided their genotypes are not lost. A register is vital. Setting up a local haemochromatosis register could play a key role in coordinating a multidisciplinary approach to the management of patients, and facilitate family and longitudinal studies for assessment of penetrance of the HH/YY genotype. Childless couples are excluded in the initial screening step, but may be screened through being a relative. For reverse cascade screening to be successful, effective genetic counselling and universally available medical information sheets are vital.

In summary, reverse cascade screening for HH is very effective in identifying previously unknown affected individuals. Reverse cascade screening from a neonate who is HH/CY or HH/YY identified 10 homozygotes from 80 parents and relatives (1/8), which is a high percentage compared with random screening.²⁰

ELECTRONIC DATABASE INFORMATION

Accession numbers and URLs for data in this article: Genbank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for haemochromatosis gene (HFE)); and Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/omim/> (for HH (OMIM #235200)).

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