**Review**

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# Hunting the Hemochromatosis Gene: Progress and Problems

**Key Words**
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**Abstract**
Hereditary hemochromatosis (HFE) is an inherited recessive disorder which causes progressive iron overload. Homozygotes for the affected gene develop symptoms of parenchymal organ damage and especially liver cirrhosis in midlife. Early diagnosis is important in order to prevent symptoms. The protein responsible for the increased iron absorption is unknown. The tight association of the disease gene with HLA-A has been known for nearly 20 years, but its precise localization remains uncertain. Linkage and linkage disequilibrium analyses in different populations have focussed on two possible locations of the gene either very close to HLA-A, or at the telomeric site of 6p in the vicinity of the D6S105 marker.

## Introduction

Hereditary hemochromatosis (HFE) is an inborn error of iron metabolism which causes progressive iron overload in adult life. Iron is toxic to cells and leads to impairment of organ function resulting in liver cirrhosis, cardiomyopathy, diabetes mellitus, endocrinopathies and arthropathies in the fifth–sixth decades of life. Susceptibility to hepatocellular carcinoma is greatly increased in HFE patients with liver fibrosis and cirrhosis [1]. Since the iron may be effectively removed by a simple treatment based on periodic phlebotomies, early diagnosis is extremely important to prevent disease complications [1, 2].

HFE is a common disorder among Caucasians, especially in North American and European populations. It is characterized by an autosomal recessive pattern of inheritance [3]. Heterozygotes may have abnormalities of iron parameters but the full clinical picture usually develops only in homozygotes [4]. The estimated gene frequency is 0.05–0.07. Ho-

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**Table 1.** Biochemical tests used for HFE diagnosis

| | Males | Females |
|---|---|---|
| Transferrin saturation | >62% | >50% |
| Serum ferritin, ng/ml | >325 | >150 |
| Hepatic iron | | |
| Grade | 3–4 | |
| Distribution | hepatocellular | |
| Concentration (μmol/g dry weight) | >100 | |
| Hepatic iron index[1] | >2 | |

[1] Hepatic iron concentration/age (in years).

mozygote frequencies are reported to be 3–10 per 1,000 [1–4]. Several factors influence disease expression. Clinical symptoms occur more frequently in males than in females. The latter, although genetically affected, may not express the disease during their fertile years due to physiological iron losses. Diet composition, especially alcohol intake, pharmacological iron or coexisting chronic blood losses may also influence genotype expression. The large diffusion of this gene among Caucasians is explained by the hypothesis that HFE carriers had a nutritional advantage in ancient times characterized by an iron-poor environment, since their duodenal mucosa provided more iron [5, 6].

Criteria for the diagnosis of HFE are based on biochemical tests including serum iron, transferrin and ferritin (table 1). Transferrin saturation values greater than 62% predict the affected genotype in >90% of males [7]. Lower transferrin saturation values (>50%) have been proposed to screen women [7, 8]. Increased serum ferritin is less accurate since it predicts only 71% of the affected genotypes. Liver biopsy is required to confirm the diagnosis and to assess the degree of iron overload. A protocol for early hemochromatosis screening has been suggested recently [8].

Criteria to diagnose heterozygous carriers and to discriminate them from normals are much more undefined, since iron parameters in the two groups show a remarkable degree of overlap [7].

## The Biochemical Defect: Current Hypotheses

The biochemical defect in HFE is still unknown, but the deregulation of intestinal iron absorption is currently the most accepted hypothesis. Notwithstanding remarkable progress in the knowledge of intracellular iron control [9, 10], the mechanism of inorganic iron transport from the intestinal lumen into the duodenal cell still remains speculative. All the known proteins involved in iron metabolism have been ruled out as candidates for the primary defect in HFE, since their corresponding genes have been mapped on chromosomes other than 6. These include transferrin, the transferrin receptor, ferritin and the recently discovered iron-regulating-element-binding protein (IRE-BP) or iron-regulating factor (IRF), which has an important role in intracellular iron regulation [9, 10]. Mobilferrin [11], an integrin-like membrane protein [12] and a membrane iron-binding protein (MBPI) [13] have been proposed as iron carriers in the duodenal mucosa cells. However their role, if any, in HFE, as well as the structure and chromosomal localization of the corresponding genes are still to be defined.

As alternatives to an increased amount of a hypothetical iron carrier in the membrane [13, 14] a macrophage defect, leading to the release of too much iron to the circulating transferrin [15], as well as a defect in the hepatocytes which accumulate the metal, have been proposed.

Since there is still speculation about the biochemical defect, the candidate gene ap-

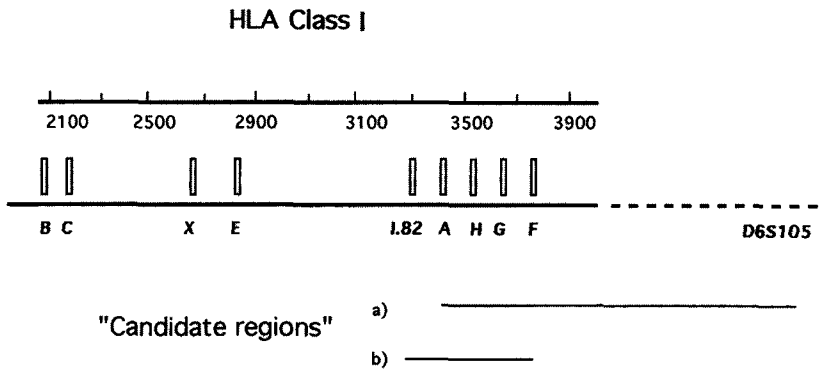


**Fig. 1.** Upper: schematic representation of the HLA class I region. Numbers (kb) in the upper line are from Campbell and Trowsdale [24]. HLA genes and probes mentioned in the text are indicated. The heavy dashed line indicates a region that has not been physically mapped. Lower: most likely HFE gene localizations (candidate regions) according to Australian (a) and Italian-French (b) data. Localizations are based both on linkage and linkage disequilibrium analyses [for details see text and ref. 25, 26–30].

proach to isolate the HFE gene is at present unfeasible, but the disease is suitable for a positional cloning strategy.

## Positional Cloning

*Linkage Analysis*

Positional cloning of the gene responsible for HFE is favored by the tight linkage of the disease with the HLA-A locus of the major histocompatibility complex (MHC) which lies in the distal portion of the 6p21.3 band [5, 6, 16–20]. Siblings who share identical HLA haplotypes with affected brothers are considered to be homozygotes for the HFE gene and at risk for developing the disease. The iron overload syndrome present in Africa, in Bantu populations, is a different disorder, not HLA associated, with both genetic and nutritional components [21]. Whether the same syndrome occurs sporadically in non-Bantu populations is unknown.

Until recently, the paucity of markers mapped on 6p precluded further linkage studies in HFE families and attempts to isolate the responsible gene. Two major advances have contributed to significant progress: first, the cloning of much of the region containing HLA class I genes in YAC vectors [22–24], and second, the availability of new markers in the region, especially short tandem-repeat sequences. Multipoint linkage analysis of highly informative markers of 6p in Italian HFE families indicated the region containing I.82, an anonymous marker 100 kb centromeric to HLA-A (fig. 1) as the most likely candidate area [25]. In contrast, a similar analysis in Australian families provided high lod score values at a recombination fraction of 0 both with HLA-A and D6S105 [26] (fig. 1), thus extending the candidate area on the telomeric side of HLA-A. D6S105 is a microsatellite, mapped at approximately 3.3 cM from HLA-A in CEPH families [27].

The existence of linkage disequilibrium between specific HLA-A and -B serotypes and the disease is well known [16–18]. The HLA-A3 serotype is reported in approximately 70% of HFE patients and in 25% of normals. B7 is present in 45% of patients versus 20% of normals and B14 accounts for 20 versus 10%, respectively [17]. Linkage disequilibrium values obtained by molecular studies apparently produced conflicting results. A strong allelic association was documented between the disease locus and the anonymous marker I.82 in a French study [28, 29]. The association was not maintained centromeric to I.82 [28], in agreement with the stronger linkage of the disease with HLA-A than -B [17]. Moreover, a large study of linkage disequilibrium of HFE and normal chromosomes from the Brittany population using polymorphic biallelic markers suggested that the linkage disequilibrium zone extends for approximately 400 kb telomeric to I.82 [30]. On the other side, a stronger allelic association was demonstrated with allele 8 of the D6S105 microsatellite (see above) in Australian patients [26]. These data are supported by a similar study in a Welsh population [31]. The degree of association with the same allele is not reported in other populations; from preliminary results, it seems to be lower in the Italian population [unpubl. data].

The difficulty in correctly localizing the HFE locus indicates that the linkage disequilibrium data must be considered with caution for defining gene location. There is not always a direct correlation between the physical distance of two markers and the degree of association between them, the lack of correlation being explained by genetic mutation, drift or selection [32]. The discrepancy of Australian versus Italian results could be related to the lack of recombination events between HLA-A and D6S105 in the former sample. The presence of a prevalent mutation linked to a specific chromosome haplotype might explain the Australian results. From the lesson derived by the study of other inherited disorders such as cystic fibrosis [33] or phenylketonuria [34], the degree of genetic heterogeneity is expected to be striking in Italy even for HFE, making the pool of HFE chromosomes more heterogeneous than in other populations. To restrict the candidate region, a heterogeneous population could be more informative than a uniform one for analysis.

*Recombinants*

Analysis of recombination events in affected kindreds is useful in defining candidate regions for gene location. Recombinants have rarely been reported in HFE and their study has produced contradictory results locating the gene either centromeric [35] or telomeric with respect to HLA-A [20]. The major problem in these analyses is the variable penetrance of the disease, which complicates the discrimination between true recombinants and subjects who did not express the disease. At least one recombinant has been explained by an error in HLA typing [35]. We have recently reported on a possible recombinant describing a HFE patient HLA-A, -B identical to the affected brother, but discordant with respect to HLA-F and D6S105 microsatellite alleles [25]. Although this is a single observation it would localize the gene centromeric to HLA-F (fig. 1).

*Physical Map of the Candidate Region*

The region containing HLA class I extends through approximately 1,700 kb and contains several distinct gene sequences. Besides classical (A, B, C) and nonclassical (E, F, G) HLA genes, there are several HLA pseudogenes [23]. Recently, novel coding sequences were isolated from this region, not structurally related to HLA class I, but probably related to the immune response genes [36, 37].

Much of the DNA in this region is now isolated on a YAC contig [22, 23]. Long-range

physical maps of the region are available, based on pulse field electrophoretic studies [38–40]. These studies have shown that variation in length of the segments both centromeric and telomeric to HLA-A may occur in different HLA haplotypes [40, 41]. A certain degree of genetic instability characterizes this region and makes its study complex. YACs and cosmids that contain HLA-A and -H are unstable and rearrange in laboratory propagation [23]. These findings could be explained by the presence of a recombination hot spot possibly located between the HLA-A and HLA-F loci. There is indeed a discrepancy between the physical (approximately 340 kb) [39] and the genetic distance reported in different studies (from 0 to 8 cM) [22, 25, 26, 40] between these two loci. Since a 50-kb deletion close to HLA-H was reported in an HLA-A3/HLA-A24 heterozygote, the deleted area has been excluded as a localization of the HFE gene [41].

The D6S105 marker, which shows the strongest allelic association with HFE in Australians, lies outside the HLA class I region. YACs containing this marker do not contain HLA class I. Using radiation hybrids, D6S105 was localized close to the histone gene H1.5 [27, 42]. Attempts to develop a YAC contig from D6S105 to HLA class I are in progress [27].

*Candidate Genes*

A large number of novel genes have recently been isolated from the class I region [24]. The majority are HLA pseudogenes or genes probably related to the immune response [36, 37]. Screening the YAC B30H3, which contains an insert of approximately 320 kb in the HLA-A region, with a cDNA library from the duodenal mucosa, seven HFE candidate genes were isolated and mapped in single or multiple copies both centromeric and telomeric to HLA-A [43]. Some of these genes are expressed exclusively in the duodenum; others are much more widely expressed. It is likely, but unproven, that the gene involved in HFE is expressed in duodenal mucosa. Studies are in progress to ascertain if patients affected by HFE show mutations at the level of these transcripts [44]. Other expressed sequence tags (ETS) from this region have been recently isolated [27, 45]. Thus the HLA class I segment shows a high density of genes, similar to that of HLA class II and III. It is remarkable that all the genes isolated from this region up to now are either HLA related or involved in the immune response.

So far, no transcripts have been reported from the region containing the D6S105 microsatellite.

*Carrier Detection and Presymptomatic Diagnosis*

At present the most reliable method for diagnosing hemochromatosis is by evaluation of iron status or diagnosis of presymptomatic disease in families at risk by HLA typing [8]. The isolation of the HFE gene will facilitate the diagnosis, allowing early detection of at-risk subjects and even population screening. Informative probes could be studied as alternatives to HLA typing to assess the risk in relatives of patients, and specific haplotypes could be used in well-studied populations. Preliminary data in Italian families [unpubl. results] suggest that at least a specific haplotype is restricted to affected chromosomes. If data are confirmed on extended samples it is not unreasonable to suggest the use of restricted haplotypes in a clinical setting to assist the diagnosis of uncertain cases.

It has also been suggested that the extent of liver iron overload in HFE is mainly determined by genetic factors, on the basis of the concordance of the iron status in the liver of HFE siblings [46]. The phenotypic expression of specific HFE alleles will certainly be clarified by identification of the abnormal gene.

It is also to be expected that the protein sequence will provide additional insights into the complex topic of mechanisms of iron absorption and redistribution.


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