A RECOMBINATION EVENT CLOSE TO HFE GENE IN HEREDITARY HEMOCHROMATOSIS

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ROETTO A., SBAIZ L., BOSIO S., PIPERNO A., FARGION S., CARELLA M., TOTARO A., GRIFA A., GASPARINI P., CAMASCHELLA C. – A recombination event close to HFE in Hereditary Hemochromatosis.
Ann Génét, 1997, **40**, n° 3, 150-153.

SUMMARY : A candidate gene for Hereditary Hemochromatosis (HFE) has been recently cloned from a region 4 cM telomeric to HLA-A on the short arm of chromosome 6. This gene, defined HFE, is mutated in a high proportion of HFE patients. Positional cloning of HFE has been difficult, because of the extended region of linkage disequilibrium observed around the gene and of the rarity of recombination events in this DNA area. Here we describe a crossover in an Italian HFE patient which occurred close to the HFE gene in a restricted interval between D6S2221 and D6S2240-D6S2238 markers. The molecular analysis of this event and the segregation of the HFE mutations in the family are consistent with the position of the HFE gene telomeric to D6S2221.

KEY-WORDS : Hemochromatosis. – Iron. – Recombination. – Microsatellites. – Gene mapping.

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RÉSUMÉ : Un gène pour l'hémochromatose héréditaire (HFE) a été cloné récemment à partir d'une région de 4 cM télomérique à HLA-A sur le bras court du chromosome 6. Le gène, appelé HFE, est muté chez une grande proportion de patients HFE. Le clonage positionnel de HFE a été difficile à cause d'une grande région en déséquilibre de liaison autour du gène et de la rareté des recombinaisons dans cette région d'ADN. Les auteurs décrivent un crossing-over chez un patient HFE Italien qui est survenu près du gène HFE. Les limites de la recombinaison sont réduites à un interval de moins de 200 Kb entre les marqueurs D6S2221 et D6S2240-D6S2238. L'analyse moléculaire de cet événement et la ségrégation des mutations HFE dans cette famille sont en accord avec la position du gène HFE télomérique à D6S2221.

MOTS-CLÉS : Hémochromatose. – Fer. – Recombinaison. – Microsatellites. – Localisation génétique.

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Manuscript submitted for publication April 8, 1997.
Accepted in revised form September 15, 1997.

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INTRODUCTION

Hemochromatosis (HFE) is an autosomal recessive disorder characterized by an abnormally high intestinal iron absorption and a progressive increase of total body iron. Iron overload results in midlife in clinical complications including cirrhosis, cardiopathy, diabetes, endocrine dysfunctions, arthropathy and susceptibility to liver cancer. The disease is common among Caucasians [9]. The nature of the metabolic defect is unknown. Linkage to HLA class I antigens, placed the gene on the short arm of chromosome 6 [25]. However, precise localization of the HFE locus by positional cloning has been difficult since no cytogenetic abnormality was observed in patients, linkage disequilibrium was recorded in an unusually extended region [5, 11, 13, 14, 24] and the few recombinants described [7, 8, 12, 23] were either incorrect or useless to localize the gene [11]. The recombinant most telomeric to HLA-A occurred close to HLA-F, 300 Kb distal to HLA-A [3]. Recently an HLA class I-like gene, initially named HLA-H but now referred as HFE [21], has been cloned about 4 cM telomeric to the major histocompatibility complex [10]. This gene was found to be mutated in a high proportion of HFE patients. In particular, a substitution changing cysteine at position 282 with tyrosine (C282Y) in exon 4 accounts for 83-100% of HFE alleles of Northern European origin [10, 15, 16] and for about 70% of HFE alleles in Southern Europe [1, 6]. An additional mutation, changing histidine at position 63 to aspartic (H63D) has an uncertain role in the pathogenesis of the disease.

In the present paper we describe a recombination event in an Italian HFE patient, which occurred in a restricted interval between D6S2221 and D6S2240-D6S2238 markers. This event establishes that the HFE locus is distal to D6S2221 marker, which maps within 200 Kb proximal to HFE gene. This event is the HFE gene closest recombination so far reported.

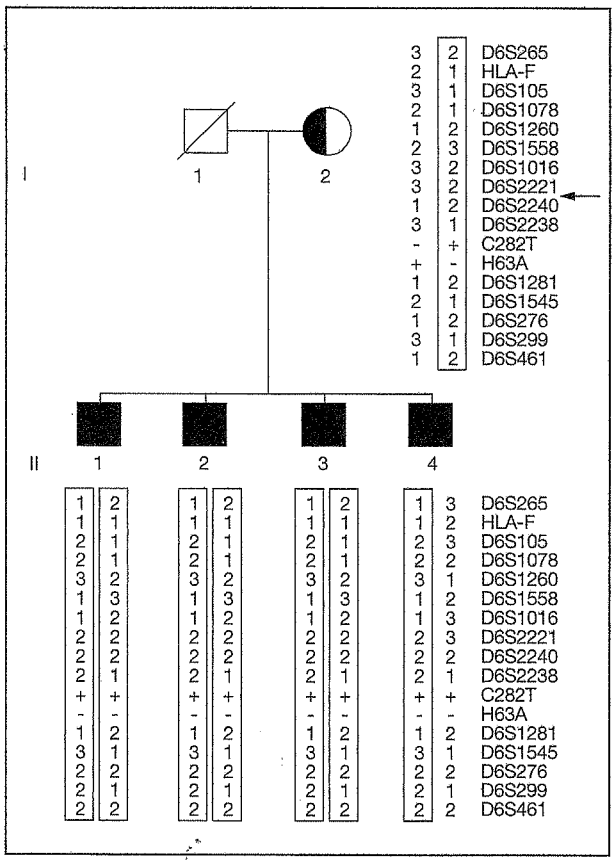

Fig. 1. – Pedigree of the family, The position of the polymorphic markers is represented from the top (centromere) to the bottom (telomere). Only the informative markers are reported. Alleles are numbered according to molecular weight with the smallest designated 1. The affected chromosomes are boxed. The position of the recombination in I-2 is indicated by the arrow.

PATIENTS AND METHODS

A previously reported HFE family with four affected siblings was studied [22]. The pedigree is reported in figure 1. At the time of diagnosis the proband (II-1) was 36 years old and his three affected siblings were aged 33, 30 and 28 respectively. Transferrin saturation was > 80% and serum ferritin ranged between 850 and 4200 µg/L in the patients. Liver biopsy performed in II-1, II-2 and II-3, showed siderosis of grade IV (Scheuer et al. 1992) and hepatic iron index was largely greater than 2. II-4 refused liver biopsy, however he underwent weekly phlebotomies achieving iron depletion after removal of 10 g of iron.

I-1 died aged 51 for an acute infectious disease. I-2 was a 63 years old woman at the time of diagnosis. Serum ferritin was 63 µg/L and transferrin saturation was 40%. These values remain unchanged during 6 year follow up.

HLA serological typing of the family members is reported elsewhere [22].

Molecular studies in patients

DNA was obtained by peripheral blood buffy coats, according to standard protocols.

Markers studied were the following: D6S265, HLA-F, D6S306, D6S105, D6S464, D6S1002, D6S1078, D6S1260, D6S1558, D6S1016, D6S2231, D62220, D6S2221, D6S2238, D6S2240,

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