Etiologic and clinical studies in primary sclerosing cholangitis
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Chapter III

CASE REPORT

A family with hereditary hemochromatosis, ulcerative colitis, and primary sclerosing cholangitis

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SUMMARY

This report describes a family in which the rare combination of hereditary hemochromatosis, ulcerative colitis, and primary sclerosing cholangitis was found. The gene encoding for hereditary hemochromatosis is located on the short arm of chromosome 6, as are the HLA-DR genes, which are important candidate genes for genetic susceptibility for inflammatory bowel disease. Subsequent to the index patient, who had all three diseases, a screening was done in his parents and siblings including HLA-DR, HLA-DQ and HFE typing, ANCA 1 s, liver tests, and sigmoidoscopy with histology. On the basis of HLA and HFE typing three probable haplotypes could be distinguished. The youngest brother was found to have the same haplotypes as the index patient, and had biochemical iron overload. One sister had ulcerative colitis. The genetics of inflammatory bowel disease and hereditary hemochromatosis are discussed.
INTRODUCTION

The pathogeneses of ulcerative colitis (UC) and primary sclerosing cholangitis (PSC), which is in about 75% of cases associated with the former, are not known. Considerable evidence suggests that a common immune dysbalance underlies both diseases (1-6). Epidemiological and animal studies have shown a genetic predisposition for inflammatory bowel disease, including UC (7-14). For PSC the evidence is less abundant, but familial occurrence of PSC has been reported (15-18).

Candidates for a role in genetic susceptibility for IBD and PSC are the HLA class II genes, since their products play a central role in the immune response. A recent meta-analysis of studies on associations of HLA haplotypes and inflammatory bowel disease reported that UC was positively associated with HLA-DR2(15) (odds ratio 1.65, CI 1.22-2.25) and HLA-DR9 (odds ratio 1.54, CI 1.06-2.24), and negatively with HLA-DR4 (odds ratio 0.54 CI 0.43-0.68) (14). HLA-DR2(15) also conferred an increased risk for UC in a group of 70 Dutch patients (odds ratio 1.8 CI 1.1-3.0) (14). In PSC a 100% association with the HLA-DR52a antigen was initially reported, but subsequent studies reported that about 50% of PSC patients are HLA-DR52a positive, which was not significantly different from the normal population prevalence of 28% (19-21). In a series of 96 PSC patients from our own institution, the prevalence of DR52a was 42%. In addition, positive associations with HLA-DR3 have been reported (22, 23).

The HLA-DR locus is situated on the short arm of chromosome 6, which is not far from the locus of the gene (called HFE) for hereditary hemochromatosis (HH) (24). HFE encodes a major histocompatibility complex class 1-like molecule that requires interaction with β2-microglobulin for expression on the cell membrane. Two missense mutations have been identified, one resulting in a change in cysteine at position 282 to tyrosine (Cys282Tyr) and one resulting in an exchange of histidine at position 63 to aspartate (His63Asp). Patients with a typical HH phenotype have been found to be homozygous for the Cys282Tyr mutation in 82-100% (24-27).

We here report the combination of HH, UC and PSC within one family, which allowed to delineate a HFE, HLA-DR and HLA-DQ genotype.

FAMILY CASE

The index case was a male patient of 44 years, who was referred by his family physician because of chronic fatigue and abnormal liver tests. He also complained of
increased loose stool frequency and five kilogram weight loss. No abnormalities were found on physical examination and the liver was not enlarged. Laboratory examination showed an ESR of 44 mm/hr, the aspartate amino transferase was 74 U/L (N < 47), the alanine amino transferase was 160 U/L (N < 37), the alkaline phosphatase was 206 U/L (N 26-103), the γ-glutamyl transpeptidase was 276 U/L (N < 68), the transferrin saturation was 1.04. Subsequently, he was found homozygous for the cysteine282tyrosine missense mutation in the HFE gen. A percutaneous liver biopsy showed increased iron load, but also signs of pericholangitis with mononuclear portal infiltration and onion skin lesion, which is a pathognomonic sign of PSC. Mild inflammation of the left colon was found at sigmoidoscopy compatible with ulcerative colitis, which was corroborated by histology. A small bowel series was normal.

The patient was diagnosed as suffering from a combination of hereditary hemochromatosis, primary sclerosing cholangitis, and ulcerative colitis. He was treated with repetitive phlebotomies and mesalamine 1000 mg thrice daily, upon which his fatigue and bowel complaints resolved completely.

A detailed family history revealed a sister with ulcerative colitis and a father with prostate carcinoma. His mother and three brothers were apparently in healthy condition.

Both parents and four siblings were screened for the presence of HH, PSC, and UC by careful history taking, transferrin saturation, HLA-H2 mutation analysis, antinuclear cytoplasmic antibodies (ANCA) as a subclinical marker of UC/PSC, alkaline phosphatase and γ-glutamyl transpeptidase, and sigmoidoscopy with histology. The latter examination was not performed in the elderly parents.

ANCAs detection was performed by indirect immunofluorescence on ethanol fixed human lymphocytes. Detection of a missense mutation of nucleotide 845 from G to A in the HFE = HLA-H2 gene was performed with an oligonucleotide ligation assay as previously described (28).

HLA-DR and HLA-DQ genotyping for the HLA-DRB1, HLA-DRB3, HLA-DRB5, and HLA-DQB1 loci was performed on lymphocytes. Because both parents carried the HLA-DR52 antigen and HLA-DQ3 which can be further subdivided, PCR-subtyping into HLA-DRB3*0101 (HLA-DR52a), HLA-DRB3*0202 (HLA-DR52b), and HLA-DRB3*0301 (HLA-DR52c) was performed. Further subtyping of HLA-DQ3 was not done, as HLA-DR11, for which both parents were also positive, is usually in linkage disequilibrium in Caucasians with the HLA-DQ3 subtype HLA-DQ7.

The results of the family screening are shown in figure 1. No family member had
liver test abnormalities. The youngest brother had a high transferrin saturation and was consequently found homozygous for the Cys282Tyr mutation in the HFE gene, conform the index case.

Using this information, three probable haplotypes could be distinguished within this family as shown in fig 1.

![Family tree showing the three probable haplotypes and the various diseases present in the offspring.](image)

**C282T** = HFE mutation  
**WT** = wild type HFE

**DISCUSSION**

This family report describes the occurrence of the rare combination of ulcerative colitis, primary sclerosing cholangitis and hereditary hemochromatosis. Three probable chromosome 6 haplotypes derived from five loci could be identified within this family. Of the HLA-DR phenotypes that are known to be associated with UC, in this family the index patient carried HLA-DR15, but his sister who was also diagnosed as suffering from UC did not. Conversely, two other siblings carried the DR15 phenotype but did not have signs of UC. The index patient also carried both
HLA-DR phenotypes that have been implicated in PSC, *i.e.* HLA-DR3 and HLA-DR52a. Two siblings carried the HLA-DR3 and HLA-DR52a alleles without signs of PSC. Notably, the youngest brother had the same haplotypes as the index patient but suffered only from HH.

These observations underscore the notion that the genetics of IBD and PSC are complex and not only involve incomplete penetrance but probably also oligogenic inheritance and/or genetic heterogeneity. The former concept implies a limited number of different genes acting together to result in the disease, in the latter model IBD is the result of multiple disease processes which could involve different genes and modes of inheritance (29, 30).

In contrast, hemochromatosis is inherited as a recessive trait and is one of the commonest monogenic diseases in Caucasians with a prevalence of 1:200 to 1:400 in the US (31-33). Recent haplotype and linkage disequilibrium analysis estimated the single ancestral mutation to have occurred about 62 generations ago, and interestingly a conserved genomic region inherited from the disease founder of 6.5 Mb is found in 9% of HH-patients’ chromosomes (34). This 6.5 Mb region does not encompass the HLA-DR locus. To our knowledge (confirmed by an extensive MedLine search), there are no epidemiological data in the literature linking HH to UC or vice versa.

In this family, the distribution of the three probable haplotypes did not correspond with the presence of either PSC or UC in 4 of the 5 siblings. Although it is not excluded that either PSC or UC might become apparent later in life, this is not likely, since both diseases have their clinical peak onset at a relatively young age, and all three unaffected individuals had negative ANCAs. It is more likely that the discordance between haplotypes and disease phenotypes reflects incomplete penetrance and oligogenic inheritance. These data further confirm the limited clinical relevance of testing for HLA haplotypes for the diagnosis of either UC or PSC.

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