HFE genotyping demonstrates a significant incidence of hemochromatosis in undifferentiated arthritis

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Abstract

Objective

Hereditary hemochromatosis is a common autosomal recessive disorder of iron metabolism. Among Northern Europeans the carrier frequency is estimated to be 1 in 10, while up to 1 in 200 is affected by the disease. Arthropathy is one early clinical manifestation of this disease, but the articular features are often misdiagnosed. In this study the two frequent mutations of the HLA-linked hemochromatosis gene (HFE) were investigated in a rheumatology clinic population.

Methods

Two hundred and six consecutive patients (mean age 57.7 years; 38 male/168 female) attending a rheumatology clinic over a period of 14 months were screened for HFE mutations (C282Y and H63D). All standard diagnostic procedures were used to identify the aetiology of the arthropathy. Mutations were evaluated by separation on PAGE of digested PCR amplificates of DNA (by SnapI and Bcl-I, for C282Y and H63D, respectively) obtained from PBMCs.

Results

The C282Y and H63D allele frequencies were 4.5 and 12.8 in patients with rheumatic diseases. Five patients were homozygote for H63D (2.4%), and one for C282Y (0.5%). Five patients were compound heterozygous (2.4%). The observed C282Y allele frequency in rheumatic patients with undifferentiated arthritis was 12.9 and exceeded that of healthy subjects ($p = 0.01$).

Conclusions

Determination of the HFE genotype is clinically useful in patients with arthritis of unknown origin, to allow early diagnosis of hemochromatosis.

Key words

HFE mutation, C282Y, H63D, hereditary hemochromatosis, rheumatologic patient.

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Introduction

Hereditary hemochromatosis (HH) is a common autosomal recessive disorder of iron metabolism. It is characterised by excess accumulation of iron in various organs, leading to arthropathy, liver cirrhosis, diabetes or cardiomyopathy. Arthropathy develops in 25% to 50% of patients (1) and its clinical manifestations range from classic painful polyarthropathy, characteristically involving the metacarpophalangeal (MCP) and proximal interphalangeal joints (PIP) (1), to classic polyarthritis (2). The association of HH with chondrocalcinosis (3) is well known and can cause acute attacks of pyrophosphate arthropathy (3). Except in the early stages of the disease the standard treatment for HH – phlebotomy – neither alleviates the joint complaints nor prevents the progression of joint disease (4).

Thus, early diagnosis by screening for the HFE gene in rheumatologic patients might help to prevent the subsequent irreversible joint damage. Two missense mutations, C282Yand H63D, in the HFE gene responsible for HH have been identified so far (5). The C282Y point mutation of the HFE gene plays a key role in the pathogenesis of HH (6). The clinical relevance of the H63D mutation is not yet fully understood. The prevalence of homozygotes is estimated to be 1: 250-400 in Northern Europe, and the carrier frequency is about 1:8 to 1:10 (7). In this study we analysed the frequency of the HFE gene mutations in patients referred to a rheumatology department and compared them with healthy subjects.

Patients and methods

Patients

One hundred and ten patients attending our rheumatology department (22 male/88 female, mean age: 62.12 ± 1.27; mean ± SD) and 96 cases examined in the Department of Rheumatology, University of Vienna (16 male/80 female, mean age: 52.81 ± 1.50) were investigated between September 1999 and November 2000. The subjects included 134 patients with rheumatoid arthritis (RA) (according to the criteria of the American Rheumatism Association [8]; 15 male/119 female, mean age: 60.4±1.2) and 21 patients with psoriatic arthritis (PsA, according to accepted criteria [9]; 8 male/13 female, mean age: 51.8±2.5) Twenty-four suffered from osteoarthritis (OA) (2 male/22 female, mean age: 61.7 ± 2.6).

In 27 patients a specific disease classification was impossible (6 male/21 female, mean age: 45± 2.9). An exact history, precise primary examinations, radiological investigations and laboratory tests were performed in all patients. A history of recent infections preceding the beginning of joint symptoms could be excluded in all patients. Reactive arthritis triggered by *Chlamydia trachomatis* or enteric bacteria such as yersinia, salmonella, *Campylobacter jejuni* or shigella were excluded by examining standardized immunoserologic procedures and stool cultures. Human leukocyte antigen (HLA)-B 27 was positive in two cases, there was no clinical or radiographic evidence of sacroilitis, and furthermore no patient had a specific family history of ankylosing spondylitis or related spondyloarthropathies.

No patient had evidence of metabolic (HbA1c < 6.0%), renal or thyroid disease or malignancies. Human immunodeficiency virus (HIV) infection was excluded by enzyme-linked immunosorbent assay (ELISA).

Previously published groups of 487 healthy blood donors served as controls (13). The control group consisted of 487 randomly selected healthy blood donors from eastern Austria aged 18–45 years.

All participants signed the written informed consent form.

Laboratory determinations

Gene analysis. HFE gene analysis was performed in the patients and controls as described earlier (10). Briefly, PCR-amplified DNA obtained from PBMC was digested with the restriction enzymes SnapI (for detection of C282Y) and Bcl-I (for detection of H63D), and analyzed by polyacrylamide gel electrophoresis (PAGE).

Iron status. Iron status was evaluated by measuring the serum iron concentration using the ferrozine method (nor-

mal value: 40-150 µg/dl), serum transferrin by nephelometry (BNA; Behring, Marburg, Germany; normal: 200-360 mg/dl) and serum ferritin by turbidimetry on a BM-Hitachi automatic analyzer (Boehringer Mannheim, Germany; normal: 18-440 µg/L in men, 8-120 µg/L and 30-300 µg/L in pre- and postmenopausal women, respectively). Transferrin saturation was calculated (serum iron 70.9 / serum transferrin; [normal, 16%-45%]).

Radiographs and liver function tests. Radiographs of the hands, wrists and knees were obtained in nearly all patients. Additionally, other symptomatic joints were radiographed when appropriate. In all patients with undifferentiated arthropathy, the liver enzymes were determined.

Statistical analysis

95% confidence limits (CL) were calculated by assuming that the number of HFE mutations is a Poisson variable. For the statistical analysis, an unpaired two-sided Student's t-test or a χ^2-test was used, as appropriate.

Results

HFE frequency and iron status

Results of genotype analysis for the C282Yand H63D mutations performed on 206 patients with rheumatic diseases are summarized in Table I. The allele frequencies (%) for the C282Y and H63D mutations were 4.50 and 12.80 in patients and 4.83 (n.s.) and 11.09 (n.s.) in a control group of 487 healthy blood donors (10), respectively. Six patients were homozygotes (1 C282Y [0.5%] and 5 H63D [2.4%]), 5 were compound heterozygotes (2.4%) and 49 heterozygotes (12 C282Y [5.7%] and 37 H63D [18.0%]). The prevalence of the C282Ymutation was significantly higher in patients with arthritis of unknown origin compared to the control group ($p=0.01$) and to patients with OA($p = 0.009$) or RA($p = 0.037$). The frequency of the H63D mutation in patients with PsA and RA (allele frequencies (13.9%) was higher compared

Table I. Distribution of HFE gene mutation and serum ferritin levels and transferrin saturation index in 206 rheumatologic patients.

	No WT	C282Y/	H63D/ WT	C282Y/ H63D	H63D/ H63D	C282Y/ C282Y	WT/WT	Ferritin (mg/dl); mean ± SD(range)	Transferrin saturation index (%); mean ± SD (range)
Osteoarthritis	24	0	3	0	0	0	21 *2	120.8 ± 30.7 (15.4 - 464)	22.4 ± 2.1 (5.3 - 33.6)
Undifferentiated arthropathy	27	3*1 (11.1)	2 (7.4)	2 (7.4)	1 (3.7)	1 (3.7)	18 *1 (66.6)	121.4 ± 59.3 (5.7 - 1390)	24.2 ± 4.9 (2.4 - 100)
Rheumatoid arthritis	134	8 (6.0)	26 (19.4)	2 (1.5)	4 (3.0)	0	94 (70.1)	106.9 ± 11.9 (3.0 - 791.8)	19.1 ± 1.1 (1.8 - 66.4)
Psoriatic arthritis	21	1	6	1	0	0	13	117.5 ± 22.7 (12.6 - 884.0)	26.3 ± 4.3 (3.5 - 92.5)
Total	206	12 (5.8)	37 (18.0)	5 (2.4)	5 (2.4)	1 (0.5)	146 (70.9)	113.9 ± 12.1 (5.7 - 1390)	20.7 ± 1.1 (1.8 - 100)
Healthy controls°	487	39 (8.0)	88 (18.1)	8 (1.6)	6 (1.2)	0	346 (71.0)		

° From ref. 10. The figures in brackets give the percentages of the numbers, WT: wild type.
* Number of patients with chondrocalcinosis.

Table II. Patients with arthritis of unknown origin and C282Ymutations.

Patient number	Age/sex	mutation	Clinical presentation	Ferritin/Trf saturation (%)	Other hemochromatosis manifestations
1	59/m	C282Y/C282Y	Arthritis in MCP, PIP, DIP	1390/101.9	Elevated LFP
2	44/f	C282Y/H63D	Arthritis in MCP, PIP, DIPand hip	13.8/ 16.4	Palpable liver
3	46/f	C282Y/H63D	Arthritis of hand and knees	9.6/ 29.78	-
4	31/f	C282Y/WT	Arthritis in hands and feet	25.8/ 25.93	-
5*	61/f	C282Y/WT	Arthritis of hand, knees and hip	115.4/ 43.32	-
6	55/f	C282Y/WT	Arthralgia in MCP, PIP, DIP	59.5/13.21	-

* Patient with chondrocalcinosis.
MCP: metacarpophalangeal joint; PIP: proximal interphalangeal joints; DIP: distal interphalangeal joint.

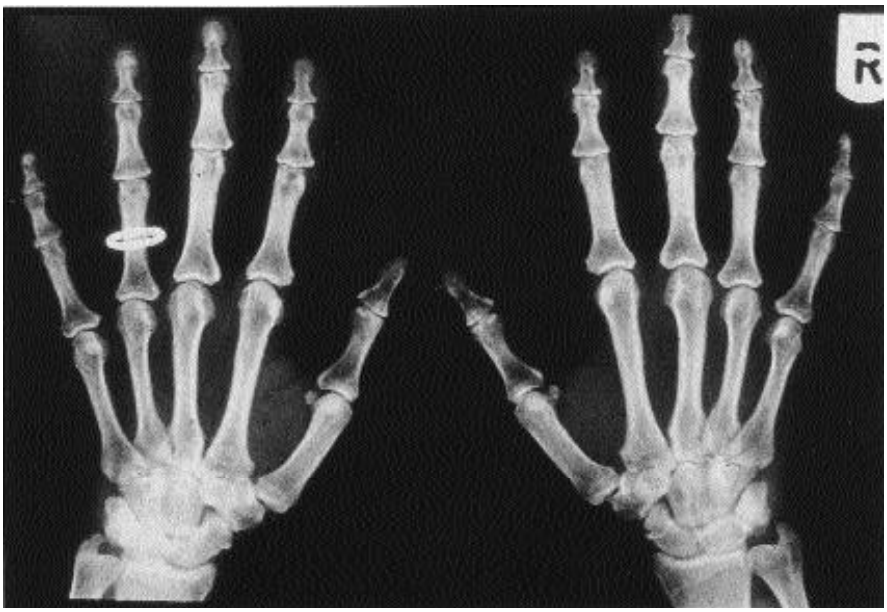

Fig. 1. X-ray of the hands in patient no. 6 showing cyst formation, sclerosis, and small osteophytes in the second and third PIPjoints.

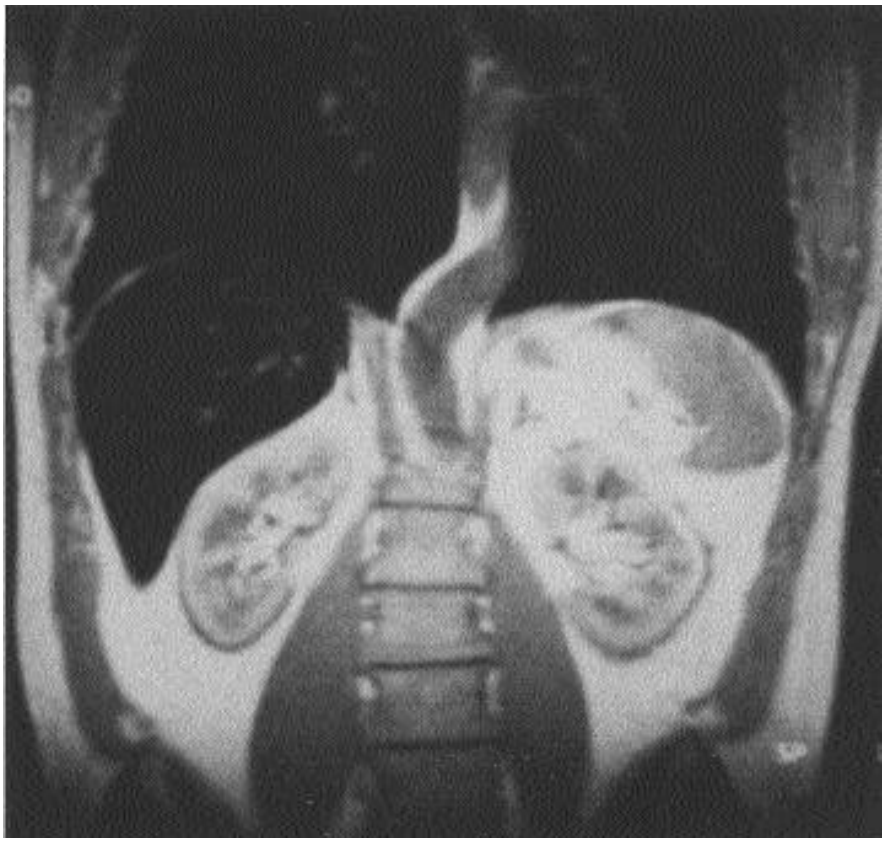

Fig. 2. The magnetic resonance of the liver in the Index patient (pt. no. 1). MRI showed decreased signal intensity in the whole liver and this finding is pathognomonic of iron overload.

with healthy controls (11.1%, p = 0.2). There was no overlap in serum ferritin levels and the transferrin saturation index in patients with different diagnoses (Table I).

Patients with arthritis of unknown origin and C282Y mutations

In the subgroup of 27 patients with arthritis of unknown origin, 6 patients were C282Y positive. The clinical features of these six patients are summarized in Table II. In pt. no. 6 (Fig. 1) cystic changes and sclerosis of subchondral bones and small osteophytes of the second and third PIP joints in both hands were present. In one patient (pt. no. 5) radiographs showed calcium deposition in cartilage.

The Index Patient

The patient homozygous for the C282Y mutation (pt. no. 1) presented with synovitis of the MCP and PIP joints in the right and left third digits. The rest of the physical examination was normal.

X-ray examination of involved joints revealed only reduced joint space in the DIP and some cystic formation in the carpal bones.

Blood studies showed a mild elevation of serum ALT 29 U/liter (normal < 22), a rising serum ferritin level to 1390 µg/l (normal: 18-440 µg/L), and an increasing transferrin saturation of 101.9 (normal: 16%-45%). For further evaluation, a magnetic resonance of the liver was done and indicated a decreased signal intensity in the whole liver, such as excess hepatocellular iron generally causes (Fig. 2). A liver biopsy was refused by the patient.

In order to reduce the iron overload, phlebotomies were started shortly after the diagnosis at a rate of 300 ml/week and are currently performed at 3-month intervals.

Six months after beginning treatment, we found clinical improvement with a disappearance of the synovitis in both hands. A family screening could be obtained for one previous and the two following generations (Fig. 3). His father died at the age of 56 years due to liver failure. A cousin of the index patient (60 years old) underwent frequent small phlebotomies but refused any further investigations. The index patient's daughter was heterozygous for C282Y and his granddaughter was compound heterozygous. At the time of diagnosis both were without clinical abnormalities.

Discussion

HH appears to be an important cause of arthropathy in patients with arthritis of

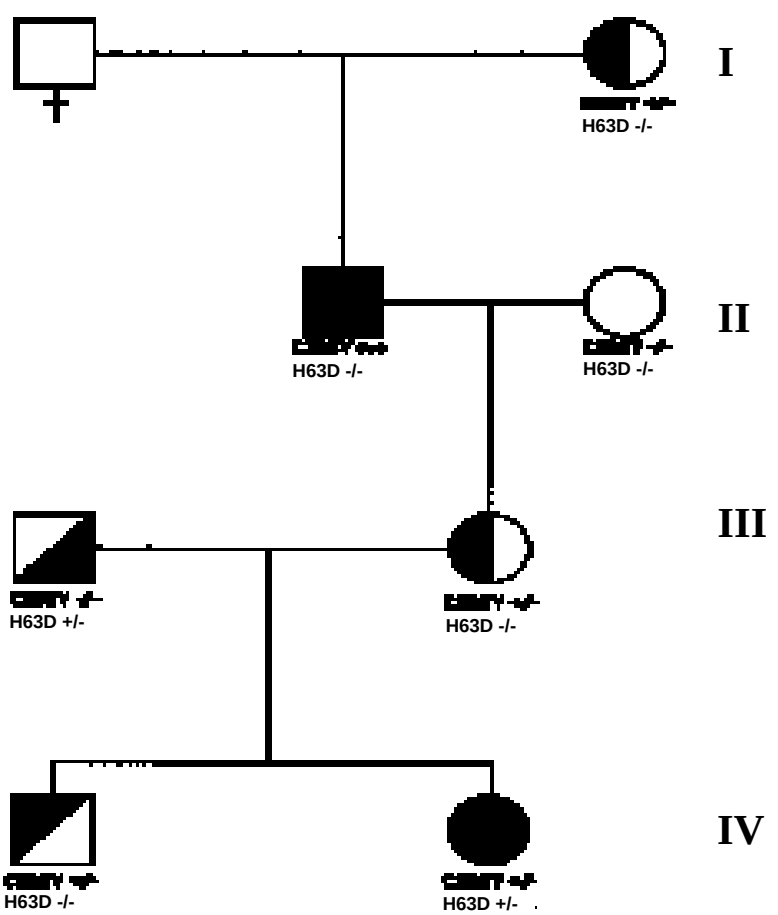

Fig. 3. Pedigree of all family members.
Row 1: Parents of the index patient: Father not available (†) mother heterozygous for C282Y;
Row 2: Index patient (C282Y/C282Y) and his wife homozygous for WT(WT/WT);
Row 3: Daughter heterozygous for C282Y (C282Y/WT) and her husband heterozygous for H63D (WT/H63D);
Row 4: Daughter's children: Boy heterozygous for C282Y (C282Y/WT), daughter compound heterozygous (H63D/C282Y).

unknown origin. In these patients the C282Y allele frequency (12.9%) exceeds that of healthy controls (4.9%). One patient was a homozygous carrier of the C282Y mutation; two were C282Y/H63D compound heterozygotes and thus had HFE-associated HH. Three additional patients were C282Y heterozygotes.

Arthritis occurs in about half of all patients with HH (1) and may be the first manifestation of the disease. Arthritis in HH may however be confused with RA, PsA and OA. Clinical manifestations range from mere arthralgia to acute pseudogout (11); sometimes the disease follows a course with symmetric arthritis resembling rheumatoid arthritis. Chondrocalcinosis is often associated with hemochromatosis; in our study it was observed in one C282Y positive patient.

Although HH is rare in patients presenting with arthropathy, the correct etiologic classification is essential to initiate appropriate therapy. Phlebotomy, the standard treatment for HH, does not improve the clinical symptoms of arthropathy but may prevent further irreversible joint damage (12).

The second finding of this study was that the frequency of H63D mutations tended to be higher in patients with RA and PsAthan in healthy controls. Similar observations have been reported by others (13). Associations of H63D were reported also in other diseases like in porphyria cutanea tarda (14) or in malignant gliomas (15). Although the clinical relevance of this mutation is unclear, these results suggest that the H63D mutation may play a role in the development of these diseases. The presence of this mutation may facilitate iron uptake or storage as in inherited anemias (16) or may simply be a marker of an HLA 1 allele associated with susceptibility to develop RA. These data need confirmation from larger studies.

In summary, HFE mutations may be related to the occurrence of arthritis. The determination of the HFE genotype, especially the C282Ymutation, is particularly useful in patients with arthritis of unknown origin and should be part of the routine diagnostic workup.

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