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SHORT REPORT

A 6p22 reference map of leukocyte DNA: exclusion of rearrangement in four cases of atypical haemochromatosis

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We describe a 4 Mb reference map of the haemochromatosis gene region in leukocyte DNA from seven controls and four atypical haemochromatosis patients. Three patients had normal coding sequence for HFE, the candidate gene for genetic haemochromatosis (GH). The fourth patient had classical GH but was heterozygous for Cys282Tyr with otherwise normal coding sequence. The genomic DNA was mapped by pulsed-field gel electrophoresis (PFGE) using five rare-cutting enzymes. Seventeen probes including HFE were positioned on the map. Despite proximity to the highly polymorphic major histocompatibility complex (MHC), no polymorphism was observed in the control group with these telomeric probes. Furthermore, major rearrangement of the HFE region was excluded as a mutation contributing to iron overload in these atypical patients. Maps of cloned DNA are linked through genes and other probes to this reference map of the HFE region in uncloned genomic DNA.

Keywords: haemochromatosis; HFE; 6p22; physical map; pulsed-field gel electrophoresis

Introduction

Genetic haemochromatosis (GH) is an autosomal recessive disorder of iron metabolism. It is common, affecting approximately 1 in 300. The gene was originally mapped close to the MHC by an association between GH and HLA-A3. This indicated a founder GH mutation on an ancestral chromosome. Although the MHC is highly polymorphic, no GH-specific rearrangements were detected in a PFGE study of haemochromatosis chromosomes, using HLA-A as the most telomeric probe.[1] More recently a cytogenetic rearrangement has been demonstrated in a GH family, where a chromosome 6 paracentric inversion segregated with the disease.[2]

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Table 1a Probes and single digest restriction fragment sizes

	Size of restriction enzyme fragments (kb)				
Probe name	*Not I (N)*	*BssH II (B)*	*Nru I (R)*	*MIu I (M)*	*Sfi I (F)*
SSADH–5′	1800	230	LM	LM	125
SSADH–3′	1800	230	LM	LM	125
p44S	1800	440	820	1300	500
p44T	1800	380	820	1300	380
RoRet	1800	380/510	820	1300	380
HFE	560	130/200	180	200	270
BTN	560	380	320	260	200
pY138	650	380	320	450	100/170
pY147	650	380	100	450	30/50
717b (D6S1260)	650	50	800	50	30
700	400	100	800	50	30
cDNA1	400	100	800	400	100
cDNA3	400	100	800	400	100
pY117	400	100	800	400/450	100/210
ZNF184	400	130	–	400	100/225
820	650/750	130/400	150	230	100
825	650/750	25	150	230	125
RFP	825	60	50	150	120

Restriction enzymes used were: N, Not I; B, BssH II; R, Nru I; M, Mlu I; F, Sfi I. LM, limiting mobility. –, probe not hybridized. Where two values for restriction fragment size are shown, these represent the cognate and partial digestion fragments.

A candidate gene (HFE) for GH has been isolated.[3] Most patients are homozygous for a missense mutation Cys282Tyr associated with the founder haplotype. Juvenile haemochromatosis (JH) is not linked to the HFE region,[4] although there may be overlap in the age of onset and presentation with GH.

In order to test for chromosomal rearrangements in haemochromatosis patients with either normal HFE coding sequence or heterozygous for Cys282Tyr, and to characterise the region in uncloned DNA, we describe a physical map of the HFE region in leukocyte DNA.

Patients and Methods

Patients

Four atypical haemochromatosis patients and seven controls were studied. Two males had juvenile-like haemochromatosis (patients 1 and 2)[5]. A third patient had borderline iron overload (5 g of iron removed by quantitative phlebotomy; liver iron index = 1.7 μmol/g/y), coexistent with colonic polyps which may have resulted in blood loss. These three patients had normal HFE coding sequence. The fourth patient had classical GH presentation[5] but was only heterozygous for Cys282Tyr.

Pulsed-field Gel Electrophoresis of Leukocyte DNA

Nine gene probes were used (Table 1): NAD^+-dependent succinic semialdehyde dehydrogenase-5' (SSADH-5') and SSADH-3'; RoRet; HFE; bovine butyrophilin B-30 domain (BTN); cDNA1 and cDNA3, zinc finger cDNAs[6]; ZNF 184; Ret finger protein (RFP). Additional single copy probes were subcloned from PACs, or as described.[7] PFGE of leukocyte DNA was performed.[8] The Cys282Tyr and His63Asp mutations of HFE were analysed as described.[5]

Results

The results for hybridisation of 18 probes on rare-cutting digests of leukocyte DNA are given in Table 1, and displayed as a 4 Mb reference map of the HFE region in Figure 1. Seventeen probes have been linked, eight of which correspond to genes. No polymorphisms or rearrangements in the size of restriction fragments

Table 1b Key double digest restriction fragment sizes

	Size of restriction enzyme fragments (kb)									
Probe name	*N/B*	*N/R*	*N/M*	*N/F*	*B/R*	*B/M*	*B/F*	*R/M*	*R/F*	*M/F*
SSADH–5′	230	230/600	230	80	230	230	80	LM	125	125
p44S	–	820	–	500	440	–	440	820	450	500
p44T	380	820	1300	380	380	380	370	820	370	380
HFE	120/190	170	200	270	130	–	120	130	170	200
BTN	340	190	260	200	230	260	200	190	–	200

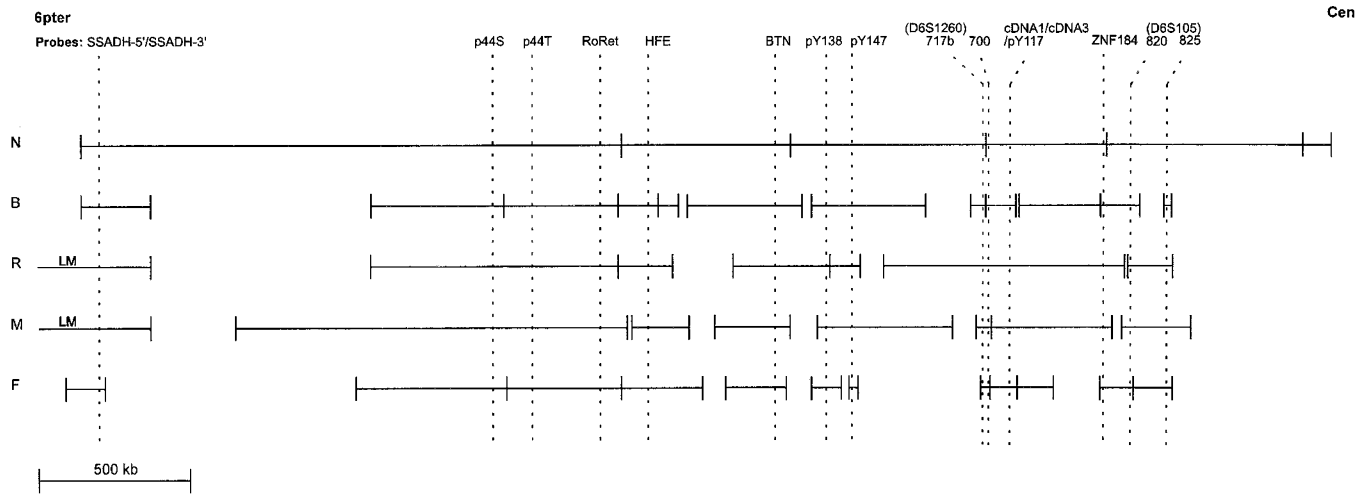

Figure 1 *A reference physical map of the HFE region in leukocyte genomic DNA. The probes are shown mapping to their restriction fragments indicated by horizontal bars. Partial digestion fragments are shown where they could be accurately positioned. Restriction enzymes as in Table 1.*

were observed in either control or atypical haemochromatosis DNA.

Discussion

Four atypical haemochromatosis patients and seven controls were analysed to construct the reference genomic map. Analysis of primary genomic DNA avoids problems of clone instability, rearrangement and chimaerism. We observed good general agreement between this map of primary leukocyte DNA and the PFGE map of a monosomy 6 cell line.[7] However, all Not I fragments and pY117 fragments were discordant, possibly reflecting differences in haplotype or ethnic origin (Caucasian/East African) of the individuals studied.[7] The 1.1 Mb PAC contig of Ruddy *et al*[9] includes RoRet–BTN, in the centre of this map. Analysis of 250 kb genomic sequence[9] demonstrated a cluster of potential Not I, BssH II, Nru I and Sfi I sites between RoRet and HFE, consistent with the CpG island we observed. The eight gene probes link this genomic map to the transcript and clone maps of the region. Despite the extensive polymorphism of the MHC, these telomeric 6p22 probes displayed no polymorphism in control DNA.

Two patients had presentation similar to JH (patients 1 and 2)[5]. Although JH is unlinked to HFE,[4] there may be overlap in the age of onset and presentation of JH and GH. Further genetic studies are required to facilitate their accurate diagnosis. The third atypical patient had clinical features of GH but normal coding sequence. The fourth patient had classical features of GH, but was only heterozygous for Cys282Tyr, with otherwise normal HFE coding sequence. The aetiology of disease in these patients is unclear. Major rearrangement of the HFE region was excluded as a mutation contributing to iron overload in these atypical patients.

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References

1 Lord DK, Dunham I, Campbell RD, Bomford A, Strachan T, Cox TM: Molecular analysis of the human MHC class I region in hereditary haemochromatosis. A study by pulsed-field gel electrophoresis. *Hum Genet* 1990; **85**: 531–536.
2 Venditti CP, Seese NK, Gerhard GS *et al*: 46,XX, inv(6)(p21.1p23) in a pedigree with hereditary haemochromatosis. *J Med Genet* 1997; **34**: 24–27.
3 Feder JN, Gnirke A, Thomas W *et al*: A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet* 1996; **13**: 399–408.
4 Camaschella C, Roetto A, Cicilano M *et al*: Juvenile and adult hemochromatosis are distinct genetic disorders. *Eur J Hum Genet* 1997; **5**: 371–375.

5 Worwood M, Shearman JD, Wallace DF *et al*: A simple genetic test identifies 90% of UK patients with haemochromatosis. *Gut* 1997; **41**: 841–844.
6 Partridge J, Wallace DF, Robertson A *et al*: Cloning and molecular characterisation of a cross-homologous zinc finger locus ZNF204. *Genomics* 1998; **50**: 116–118.
7 Volz A, Albig W, Doenecke D, Ziegler A: Physical mapping of the region around the large histone gene cluster on human chromosome 6p22.2. *DNA Sequence* 1997; **8**: 173–180.
8 Ho MF, Monaco AP: PFGE in physical mapping, In: Monaco AP (ed). *Pulsed Field Gel Electrophoresis: a Practical Approach*. Oxford University Press: Oxford, 1995, pp 21–43.
9 Ruddy DA, Kronmal GS, Lee VK *et al*: A 1.1-mb transcript map of the hereditary hemochromatosis locus. *Genome Res* 1997; **7**: 441–456.