Assignment of human ferritin genes to chromosomes 11 and 19q13.3→19qter

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Summary. Extracts of hamster–human and mouse–human hybrids, some with translocations involving chromosome 19, have been assayed for both human spleen ferritin (rich in L subunits) and human heart ferritin (rich in H subunits). Hybrid lines retaining part of the long arm of chromosome 19 including the region 19q13.3→19qter produced human “L” type ferritin. This confirms the previous assignment of the “ferritin gene” to chromosome 19 (Caskey et al. 1983). However, lines retaining chromosome 11 were found to contain human “H” type ferritin suggesting that the gene for the “H” subunit is on this chromosome. The presence of chromosome 6 was not necessary for the expression of either “H” or “L” type human ferritin. It thus seems unlikely that the gene for idiopathic haemochromatosis is a ferritin gene.

Introduction

The iron storage protein ferritin is found in all cells and its structure is well understood (Harrison et al. 1980; Wustefeld and Crichton 1982). However, its role in the regulation of iron metabolism and any part played in disorders of iron metabolism remain obscure. The principal inherited disorder of iron metabolism is idiopathic haemochromatosis (Powell and Holiday 1980; Simon et al. 1980; Edwards et al. 1981), an autosomal recessive disease in which excessive iron absorption leads to considerable accumulation of iron in the liver and other organs. The haemochromatosis gene is linked to the HLA region on chromosome 6. Recently, Caskey et al. (1983) have assigned the “ferritin gene” to chromosome 19 and have concluded that it is unlikely that abnormalities in the structural gene for human ferritin can explain idiopathic haemochromatosis. They described the application of a radioimmunoassay for human spleen ferritin to extracts of hybrid cells from Chinese hamster ovary and human lymphocytes or fibroblasts, and concluded that the assay could “specifically measure a broad spectrum of human isoferritins without detecting any Chinese hamster ferritin present in the hybrid cells”. Our studies with immunoassays for spleen, heart, and HeLa cell-type ferritins (Jones and Worwood 1978; Jones et al. 1980) show that ferritins containing human H but not L subunits would not be readily detected with antibodies to human spleen ferritin but would be detectable with antibodies to heart ferritin. It is therefore possible that Caskey et al. (1983) have only described the chromosome localisation of one of two or more ferritin genes. The possibility remains that an abnormality in the gene for the H subunit is the cause of idiopathic haemochromatosis. We have measured the concentrations of both spleen (“L” type) and heart (“H” type) ferritin in hybrid cells in order to confirm the assignment of a ferritin gene to chromosome 19, to search for a gene for the H subunit, and so provide a more complete answer to the question: is the “haemochromatosis” gene a ferritin gene?

Materials and methods

Hybrid cell lines. Characteristics of the human–rodent somatic cell hybrid lines studied are given in Figs. 1 and 2. Lines G1711B, G175AoXIB, G24B2AMB, and G24B2TGB were hybrids of human lymphocytes and mouse RAG cells. Line G24B2TG was derived from G24B2AM by back selection in thioguanine. G35F3B and G35C1B were hybrids of human lymphocytes and Chinese hamster E36 cells. All the above and the parent lines RAG and E36 were supplied by Dr. Gail Bruns. Several of these (G1711B, G175AoXIB, G24B2AMB, G35F3B, and G35C1B) contained translocated portions of human chromosome 19 as did line no. GM89A99c7B (Mohanadas et al. 1980). Line AMIR2X1B (Goodfellow et al. 1983) contained an intact chromosome 19. Cell lines WA1, WA3, WA9, and WA14 were independent clones, isolated from a fusion of human peripheral blood mononuclear cells with a mutant cell line Wg3-h derived from the Chinese hamster DON line (Westerveld et al. 1971) and maintained at the Sir William Dunn School of Pathology, University of Oxford. Hybrid cells were selected in HAT medium (Littlefield 1964) according to standard procedures (Goss and Harris 1977). Karyotyping, enzyme and ferritin assays were carried out on cells or extracts from the same passage.

Preparation of cell lysates. Confluent cells from a 75 cm² flask were trypsinized, suspended in phosphate-buffered saline (PBS), and collected by centrifugation. The cells were suspended in 400 μl PBS, transferred to a microfuge tube, and collected by centrifugation for 15 s in a microcentrifuge (MSE “Micro Centaur”). The supernatant was removed and the cell pellet resuspended by addition of 30 μl of lysis buffer (van...
Nevertheless showed concordance between presence of chromosome 11 and "H" type ferritin in early passage but it was no longer present cytogenetically following amplification and no information from enzyme assays or DNA probes was available. The results shown in this figure were supported in four other lines for which complete karyotypes were not available but which never-theless showed concordance between presence of chromosome 11 and "H" type ferritin.

**Cytogenetic analysis.** Chromosome preparations were obtained using standard techniques and G.T.G. banded (ISCN 1978) with a method modified from that of Seabright (1971). All cell lines were karyotyped by direct microscopic examination. Mitotic figures from cell lines G24B2AMB, G24B2TGB, G35C1B, G35F3B, G1711B, G175A0X1B, GM89A99e7B, and AMIR2X1B were analysed by identification of human chromosomes only. However, since cell lines WAl–WA14 had not previously been characterised cytogenetically, a more detailed analysis was undertaken. Cells from the parental Wg3-h line were karyotyped and a detailed karyogram was constructed. The hybrid cell lines WA1–WA14 were then karyotyped with reference to the karyogram, 16 to 24 cells being analysed fully and several cells partially analysed in each cell line. A chromosome was described as being present (Fig. 1) if it was seen in at least one third of the cells analysed. Cytogenetic analysis was complicated by the frequency of marker chromosomes: from 38% of cells in WA9 to 79% in WA3. These were derived in most cases from identifiable Wg3-h chromosome rearrangements. No marker chromosome was detected in more than one cell line. The human chromosome complement of the cell lines is shown in Fig. 1.

**Identification of human enzymes in hybrid cells.** Enzymes were detected in lysates of the cells by Cellogel electrophoresis. The general procedure was that described by Meera Khan (1971). Electrophoresis was carried out in a Shandon tank at room temperature. The following enzymes were assayed (human chromosome locations are given for each enzyme).

**Fumarate hydratase (chromosome 1).** Fumarate hydratase was assayed as described by van Someren et al. (1974) and Tolley and Craig (1975). It was necessary to incubate parallel strips with and without fumarate but with all other reagents in order to distinguish bands due to fumarate hydratase from those due to lactate dehydrogenase.

**Phosphoglucomutase (PGM1 chromosome 1).** The method of van Someren et al. (1974) only permitted separation of human and Chinese hamster enzymes.

**Superoxide dismutase (SOD 2, chromosome 6 and SOD 1, chromosome 21).** Superoxide dismutase was assayed as described by van Someren et al. (1974).

**Lactate dehydrogenase (LDH A, chromosome 11 and LDH B, chromosome 12).** Lactate dehydrogenase was assayed as described by Meera Khan (1971).

**Pyruvate kinase (chromosome 15).** Pyruvate kinase was assayed as described by van Someren et al. (1974).

**Glucose phosphate isomerase (chromosome 19).** The separation buffer was that described by Meera Khan (1971) and the stain by Nichols and Ruddle (1973).

**Adenosine deaminase (chromosome 20).** Adenosine deaminase was assayed by the method of van Someren et al. (1974).

**DNA probes.** In several cases DNA probes were used to confirm the presence or absence of particular human chromosomes: for chromosome 2, immunoglobulin k variable (Malcolm et al. 1982); for chromosome 4, G8 (Gussella et al. 1983); chromosome 5, L1–7 (P. Pearson, personal communication); chromosome 13, 7F12 (Cavenee et al. 1984); and chromosome 14, pAW101 (de Martinville et al. 1982). Isolation of DNA, probe labelling bynick-translation, Southern blotting, and hybridisation were carried out by standard procedures (Maniatis et al. 1982).

**Assays for human ferritin.** Ferritins rich in L subunits were detected using the immunoradiometric assay for spleen ferritin (Worwood 1980). The more acidic isoferritins (rich in H subunits) were detected with the immunoradiometric assay for heart ferritin (Jones and Worwood 1978) except that the antibody used for coating the tubes was a monoclonal antibody (24A) to human heart ferritin (Cavanna et al. 1983) kindly

![Fig. 1. Human chromosomes were identified cytogenetically. ■, chromosome present; □, chromosome absent. The presence or absence of a particular chromosome was confirmed by the assay of marker enzymes (chromosomes 1, 6, 11, 12, 15, 19, 20, and 21) or by the use of DNA probes (chromosomes 2, 4, 5, 13, and 14). DNA probes were not applied to lines WA1, 3, 9, and 14. The cell line originally contained this chromosome in early passage but it was no longer present cytogenetically following amplification and no information from enzyme assays or DNA probes was available. The results shown in this figure were supported in four other lines for which complete karyotypes were not available but which nevertheless showed concordance between presence of chromosome 11 and "H" type ferritin](image-url)