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Cloning and regional assignment of the human myosin heavy chain 12 (MYH12) gene to chromosome band 15q21

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Abstract. Sequences encoding 1,235 bp of the human myosin heavy chain 12 (MYH12) gene have been cloned from a human brain cDNA library by PCR amplification. The human sequence is 95.8% identical to the mouse sequence at the amino acid level, indicating that the MYH12 gene has been evolutionarily well conserved. Somatic cell hybrid analysis and in situ hybridization place the MYH12 gene on human chromosome 15, at band q21, and extend distally the known region of chromosome 15 linkage homology on mouse chromosome 9.

The recessive murine *dilute* coat-color mutation is an old mutation of the mouse *fancy* that has been incorporated into many inbred strains of mice. The dilute phenotype results from the adendritic morphology of dilute melanocytes, which leads to an abnormal release of melanosomes into the developing hair shaft (reviewed by Silvers, 1979). The *dilute* gene has been shown to encode a novel myosin heavy chain (Mercer et al., 1991), designated myosin heavy chain 12 *(Myh12)*. The *Myh12* gene has also been cloned from chicken (Espindola et al., 1992; Espreafico et al., 1992; Sanders et al., 1992), where it has been designated myosin V, and a related myosin, *MYO2*, has been cloned from yeast (Johnston, 1991).

Both myosin V and *MYO2* proteins contain an actin-binding *N*-terminal head domain; a "neck" region that has six imperfect tandem repeats, which in the case of myosin V has been shown to bind calmodulin (Espindola et al., 1992); an alpha-helical coiled-coil region that promotes dimerization (Cheney and Mooseker, 1992); and a globular *C*-terminal tail of unknown function. The myosin V protein has been shown to act as a nonfilamentous dimer that binds to and motivates F-actin (Cheney et al., 1993). The overall amino acid homology between the murine *Myh12* and chicken myosin V genes is 91%, although when subdivided by regional domains, this homology can be as high as 94% (Espreafico et al., 1992). When compared to yeast *MYO2*, the strongest homology is to the myosin head domain, being 52% identical. The tail domain is less well conserved, being 28% identical (Espreafico et al., 1992).

Recently, sequencing of a cDNA selected from a human fetal brain expression library using a monoclonal antibody raised against the variable region of the N-*ras* protein revealed that the cDNA encoded part of the human homolog of mouse *Myh12* (Engle and Kennett, 1994). The overall homology at the amino acid level between mouse *Myh12* and human MYH12 in these studies was 95%. This cDNA was mapped to human chromosome 15 by somatic cell hybrid analysis (Engle and Kennett, 1994).

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In the studies described here we have used PCR primers based on the mouse *Myh12* sequence to amplify MYH12 sequences from human brain and to more precisely localize MYH12 on human chromosome 15. This mapping information allows us to further refine the regions of homology between mouse Chromosome 9 and human chromosome 15. The mapping studies also allow us to determine whether mutations in MYH12 are associated with human genetic diseases.

Materials and methods

Library screening

Human MYH12 sequences were isolated from a 2-yr-old female human cerebellum cDNA library cloned in lambda ZAP (Stratagene). Initial screening of the library was done by PCR amplification. Primer pairs were designed solely from the mouse sequence (Mercer et al., 1991). The PCR conditions used were 30 cycles of denaturization at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 74°C for 2 min. PCR products, which conformed to the expected size, were isolated from the PCR reactions, run over a Centricon 30 spin dialysis column (Amicon, Danvers, MA), and sequenced, using kinased oligomers, according to the manufacturer's (Sequenase) directions. One of the PCR products, Pcr3, was then used to probe plaque lifts of the plated human cDNA library. Two million plaques were screened, and eight positive clones obtained. Of these eight clones, seven were identical. The two different clones were sequenced using the Sequenase Version 2.0 sequencing kit (United States Biochemical).

Human MYH12 genomic clones were obtained from a λ DASH library, which was a gift from Dr. G. Vande Woude (ABL-Basic Research Program, Frederick, MD). Five genome equivalents were screened, and four clones were obtained. The clones were restriction endonuclease mapped relative to one another, and unique sequence probes were isolated in order to rescreen the library. Another four clones were obtained in this manner. In all, 47.5 kb of continuous MYH12 genomic DNA was obtained.

Mouse mapping

Interspecific backcross (IB) mice were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as previously described (Copeland and Jenkins, 1991). Genomic DNAs were isolated from the spleens of backcross mice as described by Jenkins et al. (1982). Backcross DNAs were digested with 8 U *Eco*RI (New England Biolabs) per microgram of DNA in a high-salt buffer containing 5 mM spermidine. The digested DNAs were electrophoresed through 0.8% agarose gels and processed as described earlier (Jenkins et al., 1982), except that Zetabind (AMF Cuno) was substituted for nitrocellulose. Hybridization conditions were as described (Jenkins et al., 1982), except a temperature of 60°C was used for both hybridization and washing.

Probes

The murine *Myh12* cDNA probe, pdA68, is a 2.45-kb cDNA isolated from a B16 melanoma cell line. The sequence starts at position 2,208 bp in the published murine sequence (Mercer et al., 1991). The second murine probe, pdC23, is a 2.35-kb cDNA, which overlaps with pdA68, but extends 5′ by an additional 565 bp.

The two human MYH12 cDNA clones isolated in this study are referred to as pHdil3 and pHdil6. The pHdil6 clone is 700 bp in size and is entirely contained within pHdil3, which is 1,235 bp in size. For somatic cell hybrid analysis, an 855-bp subfragment of pHdil3 was used as hybridization probe. This was necessary because high background hybridization was seen when the full-length pHdil3 was used as a hybridization probe. The region spanning 781 to 981 bp of pHdil3 was later found to have some regions of high homology with human *Alu* sequences. The 1,235-bp *Eco*RI insert of pHdil3 was isolated and cut with *Xho*I. The resultant fragments were run on a 1% agarose gel, and an 855-bp fragment was isolated. This fragment was used directly as a probe for somatic cell hybrid analysis.

Unique sequence human genomic MYH12 probes were identified by probing restriction endonuclease digested and blotted human MYH12 genomic lambda clones with nick-translated total human genomic DNA. Any band that did not hybridize was subsequently subcloned into Bluescript KS (Stratagene) and used as probe against restriction endonuclease-digested and blotted total human genomic DNA. Those subclones that gave discrete bands were used in subsequent analyses. Two such subclones, pHud5R1.9 and pHud2R1.2, were identified in this manner (Fig. 3). pHud5R1.9 is a 1.9-kb *Eco*RI subclone derived from the 5′ end of one of the clones. Consequently, the 5′ *Eco*RI site of pHud5R1.9 is from the vector and is not present in genomic DNA. pHud2R1.2 is a 1.2-kb *Eco*RI subclone.

Somatic cell hybrid analysis

Human chromosomal mapping of MYH12 was accomplished by hybridizing the ^{32}P-labeled 855-bp human cDNA fragment derived from pHdil3 to Southern blots of *Eco*RI-digested genomic DNA of normal human and Chinese hamster control and 14 human × Chinese hamster somatic cell hybrids from six different fusion experiments (Francke et al., 1986). For regional chromosomal assignment, a panel containing human × Chinese hamster somatic cell hybrids that retained defined portions of human chromosome 15 (Brissenden et al., 1986), also digested with *Eco*RI, was used. These hybrids were generated from human fibroblasts carrying a balanced reciprocal translocation, t(15;22)(q14;q13.3) (Oliver et al., 1978).

Somatic cell hybrid studies performed with human MYH12 genomic clones were done using both the pHud5R1.9 and pHud2R1.2 probes. The Chinese hamster × human somatic hybrid cell line (15A) carrying only human chromosomes 15 and 1p (Tasset et al., 1988) was a gift from Dr. F.-T. Kao (Eleanor Roosevelt Institute, Denver, CO). The cell lines with derivatives of the t(15;17)(q22;q11) translocation were gifts from Dr. D. Ledbetter (National Institutes of Health, Bethesda, MD). The der(17) cell line carries the translocation derivative 17pter→17q11::15q22→15qter. The der(17), der(15) cell line carries this and the reciprocal translocation derivative 15p→15q22::17q11→17qter and the following chromosomes: 1, 3, 5, 7, 11, 13, 16, 18, 20, and 21. Fifteen to twenty-five percent of the cells also carry one or multiple copies of the following chromosomes: 6, 12, 15, 19, 22, and X. Two human × mouse somatic cell hybrid cell lines were also examined: 1750-4 carried human chromosomes 8 and 17 (25% of cells also had chromosome 11), and 1750-11 carried human chromosomes 4, 6, 7q, 8, 12, 17, and 18 (a few of the cells also carried one or more of the following chromosomes: 10, 14, and X).

In situ hybridization

Human metaphase spreads were prepared from phytohemagglutinin-stimulated peripheral blood lymphocytes. Cells in the exponential growth phase were synchronized by treatment with 5-bromodeoxyuridine (0.18 mg/ml, Sigma) for 16 h, and then the cells were released from the block by incubation in fresh medium containing thymidine (2.5 μg/ml) for 6 h (Fan et al., 1990). Metaphase cells were harvested and chromosome spreads prepared according to standard procedures.

In situ hybridization with radiolabeled probes was performed as described by Harper and Saunders (1981). Metaphase spreads were hybridized at 5 and 20 ng of probe per milliliter of hybridization mixture. In the first experiment, pHud2R1.2 was nick translated and used as a probe. In the second experiment, the 1.2-kb *Eco*RI insert was isolated from pHud2R1.2 and random primed (Prime-it, Amersham). Hybridized slides were washed at 40°C. Autoradiographs were exposed for 1–8 d at 4°C and then developed.

Fluorescence in situ hybridization (FISH) was carried out according to Pinkel et al. (1986), with minor modifications (Testa et al., 1992). Nonisotopically labeled pHud5R1.9 was prepared by nick translation of the entire plasmid, using biotin-11-dUTP, which was then denatured in 70% formamide in 2 × SSC at 70°C for 2 min and hybridized to chromosome preparations (40 μl hybridization mixture containing 20 ng of probe per slide) at 37°C overnight. Hybridization was detected with fluorescein isothiocyanate (FITC)-conjugated avidin. Chromosomes were counterstained with propidium iodide and 4′,6-diamidino-2-phenylindole (DAPI) and observed with a Zeiss Axiophot fluorescence microscope. Propidium iodide and FITC signals were observed simultaneously through Zeiss filter combination 487709, whereas DAPI banding of the same field was observed separately with Zeiss filter combination 487701. Metaphase spreads were photographed using Kodak Ektachrome ASA 400 film.

Results and discussion

A high level of evolutionary conservation was seen when a 2.45-kb murine *Myh12* cDNA probe, pdA68, was used on Southern blots of *Eco*RI-digested DNA from a variety of species, including other rodents (rat, guinea pig, Syrian hamster), other mammals (mink, rabbit, dog, cat, sheep, human), and birds (chicken, pheasant) (Fig. 1). This suggested that it might be possible to clone human MYH12 sequences by PCR amplification using primers based on the mouse sequence. Ten oligomer primer pairs that collectively span base pairs 3,212 to 5,523 of the published murine *Myh12* sequence (Mercer et al., 1991) were used to PCR amplify the homologous sequence from a human brain cDNA library. Two primer pairs gave appropriately sized PCR products. These products were sequenced directly using kinased oligomers as primers and found to contain *Myh12*-related sequences (data not shown).

One PCR product, which spans base pairs 3,820 to 3,988 of the murine sequence, was then used to screen the human brain cDNA library. Two clones were obtained and sequenced (Fig. 2). The smaller of the two cDNA clones (pHdil6) (base pairs 38 to 738 in Fig. 2) was completely contained within the larger clone of 1,235 bp (pHdil3). Comparative translations of the human and murine sequences across this region are shown

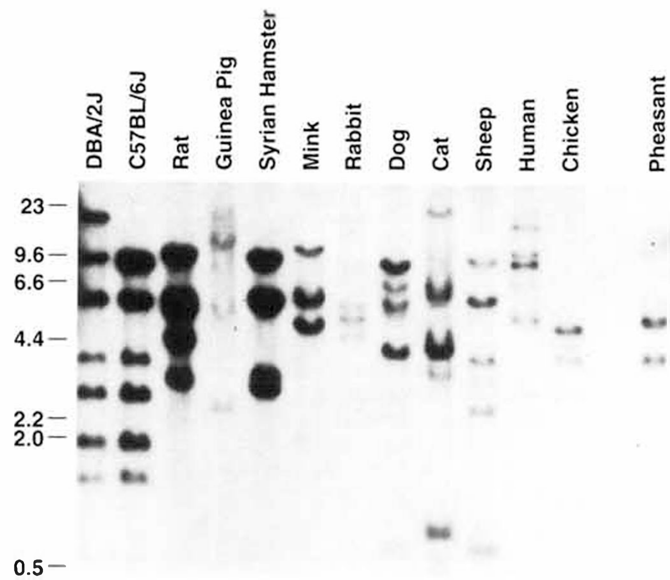

Fig. 1. The *Myh12* gene is evolutionarily well conserved. Genomic DNAs from two inbred mouse strains, DBA/2J and C57BL/6J, as well as other mammalian and avian species, were digested with *Eco*RI. Southern blotted, and hybridized with a ^{32}P-labeled murine *Myh12* cDNA probe, pdA68. The molecular weight, in kilobases, of *Hin*dIII-digested lambda DNA is shown on the left.

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Human     1 ATTAGAAGAAACGAAACAACTGGAACTCGACCTTAATGATGAAAGGCTGAGATATCAGAACCTTCTGAATGAGTTCAGTCGCCTGGAAGAAAGATATGATGACCTCAAGGAAGAGATGAC  120
Human     1 L E E T K Q L E L D L N D E R L R Y Q N L L N E F S R L E E R Y D D L K E E M T   40
Mouse  1059 V . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1098

Human   121 CCTTATGGTGCATGTGCCTAAGCCTGGACACAAGAGAACAGACTCCACCCACAGCAGCAACGAGTCTGAATATATCTTTAGCTCTGAAATTGCAGAAATGGAAGACATTCCATCAAGGAC  240
Human    41 L M V H V P K P G H K R T D S T H S S N E S E Y I F S S E I A E M E D I P S R T   80
Mouse  1099 . . L N . . . . . . . . . . . . . . . . . . . . T . . . . F . . T . . . A P . .   1138

Human   241 AGAGGAACCAAGTGAGAAGAAGGTACCTCTGGACATGTCATTGTTCCTTAAGCTCCAGAAGCGGGTCACAGAGCTGGAGCAGGAGAAGCAGGTGATGCAGGATGAGCTGGAGCGCAAGGA  360
Human    81 E E P S E K K V P L D M S L F L K L Q K R V T E L E Q E K Q V M Q D E L E R K E   120
Mouse  1139 . . . I . . . . . . . . . . . . . . . . . . . . . . . . . . L . . . . . D . . .   1178

Human   361 GGAGCAGGTGCTCCGCAGCAAGGCCAAGGAAGAAGAAAGACCACAAATTAGAGGTGCAGAACTGGAATATGAGTCACTCAAGCGTCAAGAACTAGAATCAGAAAACAAAAAACTGAAGAA  480
Human   121 E Q V L R S K A K E E E R P Q I R G A E L E Y E S L K R Q E L E S E N K K L K N   160
Mouse  1179 . . . F . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1218

Human   481 TGAGCTAAATGAGTTGCGCAAGGCCCTCAGTGAGAAAAGTGCCCCAGAGGTGACCGCCCCAGGTGCACCTGCCTACTGTGTCCTCATGGAGCAGCTGACCTCTGTGAGCGAGGAGCTTGA  600
Human   161 E L N E L R K A L S E K S A P E V T A P G A P A Y C V L M E Q L T S V S E E L D   200
Mouse  1219 . . . . . . . . . . . . . . . . . . . . . . . . . R . . . . . . . . . . . . . .   1258

Human   601 TGTCCGCAAGGAGGAAGTCCTCATCTTAAGGTCTCAACTGGTGAGCCAAAAAGAAGCCATCCAACCCAAGGATGACAAGAATACAATGACAGATTCCACAATACTTTTGGAAGATGTACA  720
Human   201 V R K E E V L I L R S Q L V S Q K E A I Q P K D D K N T M T D S T I L L E D V Q   240
Mouse  1259 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1298

Human   721 AAAAATGAAAGATAAAGGTGAAATAGCACAAGCATACATTGGTTTGAAAGAAACAAATAGGCTCCTGGAATCCCAGCTGCAGTCACAGAAGAGGAGCCATGAGAATGAGGCCGAGGCCCT  840
Human   241 K M K D K G E I A Q A Y I G L K E T N R L L E S Q L Q S Q K R S H E N E A E A L   280
Mouse  1299 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1338

Human   841 CCGTGGGGAGATCCAGAGCCTGAAGGAGGAGAACAACCGACAGCAGCAGCTGCTGGCCCAGAACCTGCAGCTGCCCCCAGAGGCCCGCATTGAGGCCAGCCTGCAGCACGAGATCACCCG  960
Human   281 R Q E I Q S L K E E N N R Q Q Q L L A Q N L Q L P P E A R I E A S L Q H E I T R   320
Mouse  1339 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1378
                              ↓
Human   961 GCTGACCAACGAAAACTTGGATTTGATGGAACAACTTGAAAAACAGGATAAGACGGTCCGTAAACTGAAAAAACAACTGAAAGTATTTGCCAAAAAAATTGGCGAACTAGAAGTGGGCCA  1080
Human   321 L T N E N L D L M E Q L E K Q D K T V R K L K K Q L K V F A K K I G E L E V G Q   360
Mouse  1379 . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . . .   1444

Human  1081 GATGGAGAACATATCCCCAGGACAGATCATTGATGAACCCATCCGACCAGTCAACATTCCCAGGAAAGAAAACGATTTCCAAGGGATGCTGGAATACAAGAAGGAGGATGATCAAAAACT  1200
Human   361 M E N I S P G Q I I D E P I R P V N I P R K E N D F Q G M L E Y K K E D D Q K L   400
Mouse  1445 . . . . . . . . . . . . . . . . . . . . . . . K . . . . . . . . . R . . E . . .   1484

Human  1201 TGTTAAGAACCTGATTCTGGAACTGAAGCCACGTG   1235
Human   401 V K N L I L E L K P R   411
Mouse  1485 . . . . . . . . . . .   1495
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Fig. 2. Amino acid sequence comparison of human and murine MYH12. The human MYH12 cDNA sequence determined in this study is shown on the top line. The second line shows the amino acid translation of this human sequence, and the third line shows the translation of a corresponding murine *Myh12* brain cDNA sequence (Mercer et al., 1991). Only those amino acids that differ from the human sequence are indicated for mouse. The arrow indicates the location of a 25-amino acid insertion in the mouse brain cDNA sequence that was not found in the human brain cDNA sequence. The mouse and human sequences have been aligned by omitting this sequence from the mouse. A comparison of this partial human cDNA sequence to that reported by Engle and Kennett (1994) is described in the text.

Fig. 3. A partial genomic restriction endonuclease map of the human MYH12 gene. Genomic regions that hybridize to various human and mouse MYH12 cDNAs are indicated as boxes below the map. The first line of boxes represents those fragments that hybridize with the mouse *Myh12* cDNAs, pdA68 and pdC23. The second line of boxes represents those fragments that hybridize with the partial human MYH12 cDNA, pHdil3. The location of unique sequence genomic probes, pHud5R1.9 and pHud2R.2, are also shown. The direction of MYH12 transcription is indicated by an arrow. R = *Eco*RI; X = *Xba*I; H = *Hin*dIII. Restriction sites indicated by a dot are derived from the cloning vector.

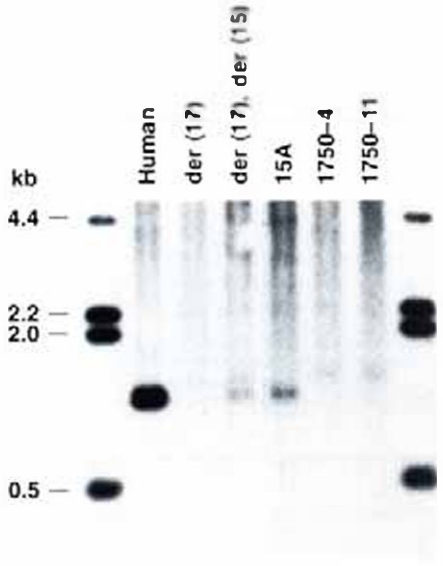

Fig. 4. Southern blot of *Eco*R1-digested genomic DNA from human and the somatic cell hybrid lines; der(17); der(17), der(15); 15A; 1750-4; and 1750-11 probed with pHud2R1.2. The 1.2-kb band specific for MYH12 seen in the human control lane is also seen in the lanes from the cell lines der(17), der(15), and 15A.

in Fig. 2. The two sequences are 95.8% identical, and 98.3% similar at the amino acid level, suggesting that these cDNA clones contain sequences from the human MYH12 gene. One notable difference in the two sequences was a stretch of 25 amino acids that was present in the mouse brain cDNA, but not in the human sequence. (The position of the insert is shown by the arrow in Figure 2.) The murine 25-amino acid insert was also not observed in the chicken brain myosin V sequence (Espreafico et al., 1992) or in the human sequence reported by Engle and Kennett (1994). This 25-amino acid sequence is preceded by a good potential splice site (data not shown), raising the possibility that this coding difference results from alternative splicing (Shapiro and Senapathy, 1987). The human cDNA sequence reported by Engle and Kennet (1994) extends 421 amino acids more 5′ than the sequence of pHdil3. However pHdil3 extends 36 amino acids further 3′. Comparison of the sequence reported here and that reported in Engle and Kennett (1994) reveals two base-pair differences. We find codon 1 to be TTA(L) and codon 186 to be TGT(C); Engle and Kennett (1994) found GTA(V) and CGT(R), respectively. These differences may result from natural polymorphisms within the human population.

The murine chromosomal location of the locus encoding pHdil3 was determined by interspecific backcross analysis. No recombinants between this locus and the mouse *Myh12 (dilute)* locus were observed in 108 animals typed in common (data not shown), providing additional confirmation that pHdil3 contains sequences from the human MYH12 gene.

Genomic clones

Four human MYH12 genomic clones were obtained by screening a human λ DASH library with murine probe pdA68. Four additional genomic clones were obtained in a second round of library screening using two unique sequence genomic probes, pHud2R1.2 and pHud5R1.9, generated in the first round of library screening (Fig. 3). Restriction endonuclease mapping of these eight clones produced a 45.7-kb genomic map of a portion of the human MYH12 gene (Fig. 3). Regions that hybridize to the murine and human MYH12 cDNAs are indicated by boxes below the genomic map.

Somatic cell hybrid analysis

A human MYH12 cDNA probe (see Materials and methods) detected five human-specific fragments of 10, 7.6, 5.4, 5.0, and 2.6 kb (the 2.6-kb fragment could not be scored in hybrids because it comigrated with a Chinese hamster fragment) when hybridized to *Eco*RI-digested normal human and human × Chinese hamster somatic cell hybrid DNAs containing a human chromosome 15. There were at least two discordances for all other human chromosomes (data not shown). This result is in full accordance with the localization of human MYH12 to human chromosome 15 (Engle and Kennett, 1994).

When a chromosome 15 regional panel was hybridized with the same sequence, human-specific fragments were detected in hybrids that retained an intact human chromosome 15 as well as in a hybrid that contained the distal region of human chromosome 15, specifically 15q14→qter. These fragments were not observed in a hybrid that contained the proximal region of human chromosome 15 (15pter→q14) (data not shown).

The unique sequence genomic probes pHud2R1.2 and pHud5R1.9 were also used as probes on somatic cell hybrid DNAs. pHud2R1.2 detected a 1.2-kb *Eco*RI fragment, and pHud5R1.9 detected a 4.0-kb *Eco*RI fragment, when hybridized to normal human DNA. pHud2R1.2 also hybridized faintly to a 1.4-kb fragment in mouse and Chinese hamster DNA. pHud5R1.9 did not hybridize to rodent DNA. Neither probe hybridized to the mouse × human somatic cell hybrid lines 1750-4 or 1750-11, excluding human chromosomes 3, 4, 6, 7p, 8, 12, 17, and 18 for the human MYH 12 locus. Both probes hybridized to DNA from a human × Chinese hamster cell hybrid (15A), which contains only human chromosomes 15 and 1p. Additionally, both probes hybridized to DNA from the

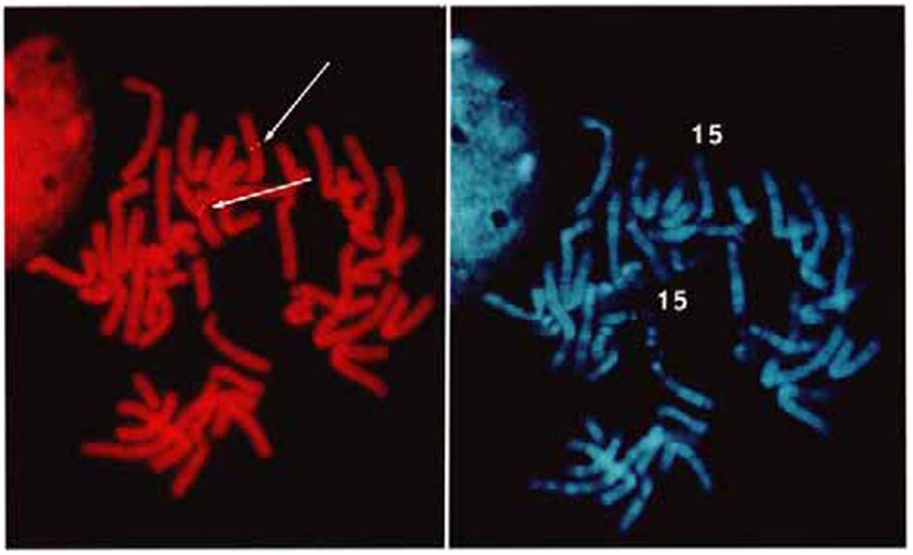

Fig. 5. Localization of MYH12 to human metaphase chromosomes by FISH. Chromosomes were stained simultaneously with propidium iodide and DAPI. Left, propidium iodide staining, showing FITC hybridization signals on both chromosome 15 homologs at band 15q21 (arrows). Right, DAPI staining of the same metaphase spread demonstrating a Giemsa-like banding pattern.

mouse × human somatic cell hybrid der(17), der(15) that contains both translocation derivative chromosomes, 17pter→17q11::15q22→15qter, and the reciprocal 15pter→15q22::17q11→17qter but not to DNA from a somatic cell hybrid der(17) that carries only the der(17) translocation derivative 17pter→17q11::15q22→15qter. Figure 4 shows the hybridization of pHud2R1.2 to human genomic DNA and the following somatic cell hybrid cell lines: der(17); der(17), der(15); 15A; 1750-4; and 1750-11.

Collectively, these data indicate that the human MYH12 locus maps to chromosome 15 in the region q14→q22.

In situ hybridization

Initially, radiolabeled pHud2R1.2 was used to map the MYH12 gene on human chromosomes by in situ hybridization. A total of 220 metaphase spreads were examined to determine the distribution of silver grains among the chromosomes. Of these 220 cells, 50 (23%) showed labeling on chromosome 15. Among 645 labeled sites, 55 (9%) grains were located on chromosome 15. Thirty-five of these 55 grains (64%) clustered to bands 15q14→q22. The largest number of grains (16) was located at band 15q21.

The hybridization detection efficiency was considerably higher in subsequent FISH experiments using a nonisotopically labeled pHud5R1.9 probe. Fluorescent signals were detected on chromosome 15 in 22 (63%) of 35 metaphase cells. Fluorescent signals on chromosome 15 were distributed as follows: one chromatid (seven cells), two chromatids (nine cells), three chromatids (three cells), four chromatids (three cells). Labeling on both homologs was observed in nine cells. Forty-four of 46 signals on chromosome 15 were located at band q21 (Fig. 5).

Comparative mapping

The nearest proximal marker to *Myh12 (dilute, d)* on mouse Chromosome 9, which has been mapped in humans, is pyruvate kinase 3 *(Pk3)* (Kingsley et al., 1989). PKM2 (the human homolog of *Pk3*) maps to human chromosome band 15q24→q25 (Popescu and Cheng, 1990). The mapping of MYH12 to chromosome band 15q21 extends distally the known region of homology between mouse Chromosome 9 and human chromosome 15. The nearest distal marker to *Myh12* which has been mapped in humans is bone morphogenetic protein 5 *(Bmp5)*. BMP5 has been assigned to human chromosome 6 (Hahn et al., 1992). *Bmp5* has also been shown to be encoded by the *short-ear* mutation (Kingsley et al., 1992), which resides 0.16 cM distal of *dilute (Myh12)* (Russell, 1971). Thus, the mapping of MYH12 to band 15q21 narrows to 0.16 cM the interval within which the break in homology between human chromosomes 15q and 6 must occur on mouse Chromosome 9.

MYH12 and human disease

Finally, it is of interest to ask whether MYH12 could be associated with any human disease syndromes. The only human diseases known to map on human chromosome 15q that have any phenotypic similarity to the *dilute* mouse are the Prader-Willi (PWS) and Angelman syndromes (AS), which sometimes produce a hypopigmented phenotype. However, the MYH12 locus appears to map distal to the region (15q11→15q13) associated with PWS and AS (Kaplan et al., 1987; Knoll et al., 1989; Magenis et al., 1990), and it has recently been shown that the hypopigmentation phenotype associated with PWS and AS is due to mutations in the human P gene (Rinchik et al., 1993).

Many other tyrosinase-positive albinism syndromes in man have also been recognized, but have not yet been mapped. These syndromes, whose phenotypes bear a resemblance to mouse *dilute*, include Cross syndrome, Elejalde syndrome, and Griscelli syndrome (Witkop, 1984, Engle and Kennett, 1994). As more is understood about the cell biology and gene mapping of such syndromes, it may become possible to associate MYH12 with a human genetic disease.

References

Brissenden SE, Page DC, de Martinville B, Trowsdal D, Botstein D, Francke U: Regional assignments of three polymorphic DNA segments on human chromosome 15. Genet Epidem 3:231–239 (1986).

Cheney RE, Mooseker MS: Unconventional myosins. Curr Opin Cell Biol 4:27–35 (1992).

Cheney RE, O'Shea, MK, Heuser JE, Coelho MV, Wolenski JS, Espriafico EM, Forscher P, Larson RE, Mooseker MS: Brain myosin-V is a two-headed unconventional myosin with motor activity. Cell 75:13–23 (1993).

Copeland NG, Jenkins NA: Development and applications of molecular genetic linkage map of the mouse genome. Trends Genet 7:113–118 (1991).

Engle LJ, Kennett RH: Cloning, analysis, chromosomal localization of myoxin (MYH12), the human homologue to the mouse *dilute* gene. Genomics 19: 407–416 (1994).

Espindola FS, Espreafico EM, Coelho MV, Martins AR, Costa FRC, Mooseker MS, Larson RE: Biochemical and immunological characterization of p190-calmodulin complex from vertebrate brain: a novel calmodulin binding myosin. J Cell Biol 118: 359–368 (1992).

Espreafico EM, Cheney RE, Matteoli M, Nascimento AAC, De Camilli PV, Larson RE, Mooseker MS: Primary structure and cellular localization of chicken brain myosin-V (p190), an unconventional myosin with calmodulin light chains. J Cell Biol 119:1541–1557 (1992).

Fan Y-S, Davis LM, Shows TB: Mapping small DNA sequences by fluorescence in situ hybridization directly on banded metaphase chromosomes. Proc natl Acad Sci, USA 87:6223–6227 (1990).

Francke U, Yang-Feng TL, Brissenden JF, Ullrich A: Chromosomal mapping of genes involved in growth control. Cold Spr Harb Symp quant Biol 51:855–866 (1986).

Hahn GV, Cohen RB, Wozney JM, Levitz CL, Shore EM, Zasloff MA, Kaplan FS: A bone morphogenetic protein subfamily: chromosomal localization of human genes for BMP5, BMP6, BMP7. Genomics 3:759–762 (1992).

Harper ME, Saunders GF: Localization of single copy DNA sequences on G-banded human chromosomes by in situ hybridization. Chromosoma 83: 431–439 (1981).

Jenkins NA, Copeland NG, Taylor BA, Lee BK: Organization, distribution, stability of endogenous ecotropic murine leukemia virus DNA sequences in chromosomes of *Mus musculus*. J Virol 43:26–36 (1982).

Johnston GC, Prendergast JA, Singer RA: The *Saccharomyces cerevisiae* MYO2 gene encodes an essential myosin for vectorial transport of vesicles. J Cell Biol 113:539–551 (1991).

Kaplan LC, Wharton R, Elias F, Mandell F, Donlon T, Latt SA: Clinical heterogeneity associated with deletions in the long arm of chromosome 15: report of 3 new cases and their possible genetic significance. Am J med Genet 28:45–53 (1987).

Kingsley DM, Bland AE, Grubber JM, Marker PC, Russell LB, Copeland NG, Jenkins NA: The mouse *short ear* skeletal morphogenesis locus is associated with defects in a bone morphogenetic member of the TGFβ superfamily. Cell 71:399–410 (1992).

Kingsley DM, Jenkins NA, Copeland NG: A molecular genetic linkage map of mouse chromosome 9 with regional localizations for the *Gsta, T3g, Ets-1* and *Ldlr* loci. Genetics 123:165–172 (1989).

Knoll JH, Nicholls RD, Magenis RF, Graham Jr JM, Lalande M, Latt SA: Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion Am J med Genet 32:285–290 (1989).

Magenis RE, Toth-Fejel S, Allen IJ, Black M, Brown MG, Budden S, Cohen R, Friedman JM, Kalousek D, Zonana J: Comparison of the 15q deletions in Prader-Willi and Angelman syndromes: specific regions, extent of deletions, parental origin, clinical consequences. Am J med Genet 35:333–349 (1990).

Mercer JA, Seperack PK, Strobel MC, Copeland NG, Jenkins NA: Novel myosin heavy chain encoded by murine *dilute* coat colour locus. Nature 349: 709–713 (1991).

Oliver N, Francke U, Pelligrino MA: Regional assignment of genes for mannose phosphate isomerase, pyruvate kinase-3, and β2-microglobulin expression on human chromosome 15 by hybridization of cells from a t(15;22)(q14;q13.3) translocation carrier. Cytogenet Cell Genet 22:506–510 (1978).

Pinkel D, Straume T, Gray JW: Cytogenetic analysis using quantitative, high-sensitivity, fluorescence hybridization. Proc natl Acad Sci, USA 83:2934–2938 (1986).

Popescu NC, Cheng SY: Chromosomal localization of the gene for a human cytosolic thyroid hormone binding protein homologous to the subunit of pyruvate kinase, subtype M2. Somat Cell molec Genet 16:593–598 (1990).

Rinchik EM, Bultman SJ, Horsthemke B, Lee S-T, Strunk KM, Spritz RA, Avidano KM, Jong MTC, Nicholls RD: A gene for the mouse pink-eyed dilution locus and for human type II oculocutaneous albinism. Nature 361:72–76 (1993).

Russell LB: Definition of functional units in a small chromosomal segment of the mouse and its use in interpreting the nature of radiation-induced mutations. Mutat Res 11:107–123 (1971).

Sanders G, Lichte B, Meyer HE, Kilimann MW: cDNA encoding the chicken ortholog of the mouse *dilute* gene product: sequence comparison reveals a myosin I subfamily with conserved C-terminal domains. FEBS Lett 311:295–298 (1992).

Shapiro MB, Senapathy P: RNA splice junctions of different classes of eukaryotes: sequence statistics and functional implications in gene expression. Nucl Acids Res 15:7155–7174 (1987).

Silvers WK: The Coat Colors of Mice, pp 83–90 (Springer-Verlag, New York 1979).

Tasset DM, Hartz JA, Kao F-T: Isolation and analysis of DNA markers specific to human chromosome 15. Am J hum Genet 42:854–866 (1988).

Testa JR, Taguchi T, Knudson AG, Hino O: Localization of the interferon-α gene cluster to rat chromosome bands 5q31 → q33 by fluorescence in situ hybridization. Cytogenet Cell Genet 60:247–249 (1992).

Witkop Jr CJ: Inherited disorders of pigmentation, in Clinics in Dermatology, Vol 2 (JB Lippincott Co, Philadelphia 1984).