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Chromosomal Assignment of the Genes for Human Aldehyde Dehydrogenase-1 and Aldehyde Dehydrogenase-2

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SUMMARY

Chromosomal assignment of the genes for two major human aldehyde dehydrogenase isozymes, that is, cytosolic aldehyde dehydrogenase-1 (ALDH1) and mitochondrial aldehyde dehydrogenase-2 (ALDH2) were determined. Genomic DNA, isolated from a panel of mouse-human and Chinese hamster-human hybrid cell lines, was digested by restriction endonucleases and subjected to Southern blot hybridization using cDNA probes for ALDH1 and for ALDH2. Based on the distribution pattern of ALDH1 and ALDH2 in cell hybrids, *ALDH1* was assigned to the long arm of human chromosome 9 and *ALDH2* to chromosome 12.

INTRODUCTION

Two major and at least two minor aldehyde dehydrogenase isozymes exist in human and other mammalian livers. One of the major isozymes, designated as ALDH1, or E_1, is of cytosolic origin, and another major isozyme, designated as ALDH2 or E_2, is of mitochondrial origin. The two isozymes are different from each other with respect to their kinetic properties, sensitivity to disulfiram inactivation, and protein structure [1–5]. Remarkable racial differences between Caucasians and Orientals have been found in these isozymes. Approximately 50% of Orientals have a variant form of ALDH2 associated with diminished activity, while virtually all Caucasians have the wild-type active ALDH2

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[6, 7]. Some Orientals (1%–10%) have an inactive variant form of ALDH1 [8]. The very high incidence (50%–80%) of acute alcohol intoxication in Orientals in comparison with Caucasians (about 10%) is attributed to the absence of the active, wild-type enzyme and the accumulation of acetaldehyde in alcohol-sensitive Orientals [6].

Recently, the gene locus for a minor human ALDH isozyme, ALDH3, which is strongly expressed in lung and stomach and mainly oxidizes benzaldehyde, was assigned to chromosome 17 [9].

Chromosomal locations of *ALDH1* and *ALDH2* genes in humans have not yet been determined. Since the isozymes are not expressed in fibroblast cultures, segregation analysis of the human enzymes in rodent-human hybrid cell lines is not feasible. Recently, cDNAs for human ALDH1 and ALDH2 were cloned [10]. We report here chromosomal assignments of *ALDH1* and *ALDH2* loci determined by Southern blot hybridization analysis of DNA samples obtained from rodent-human hybrid cell lines using the cDNA probes.

MATERIALS AND METHODS

Cell Hybrids

A panel of 16 independent somatic cell hybrids (prefix CF84) were isolated from polyethylene glycol-mediated fusion of mouse B82 (GM0347A) cells and human male fibroblasts (IMR91) using standard procedures. These mouse-human hybrids do not retain human chromosome 9 under nonselective conditions. Therefore, the following rodent-human hybrids containing human chromosome 9 or a portion of it were also used: GL8 is a mouse-human hybrid selectively retaining a human X/9 translocation chromosome (Xqter→Xq12::9p24→9qter) and no other human chromosome; GL8R is a subclone of GL8 that contains no human chromosomes; CF11-4 is a Chinese hamster-human hybrid retaining a second X/9 translocation chromosome (Xqter→Xq13::9q34 →9pter) and no other human chromosome [11]; CF17-22 and 17-24 are Chinese hamster-human hybrids derived from the fusion of human cells containing a 17/9 translocation [(t(9;17)(9p17q:9q17p)], 17-22 retaining both products of the translocation and 17-24 retaining only t(9p/17q); CF57-1 is a Chinese hamster-human hybrid retaining the same X/9 translocation as in GL8; and CF43-8 is a Chinese hamster-human hybrid retaining a deleted human 9 (9qter→9p22:) [12]. Cells of these clones were grown up for DNA extraction, and chromosome analysis was done on a sample of the cell pellet; at least 30 metaphases were analyzed in detail per clone with the aid of Q-banding.

cDNA Probes for Human ALDH1 and ALDH2

The probes for ALDH1 were a cDNA (1.579 kilobases [kb]) that encodes 340 amino acid residues of the COOH-terminal part of human ALDH1 and an *Eco*RI-*Sau*3AI fragment of the cDNA (0.285 kb). The probes for ALDH2 were a cDNA (1.243 kb) that encodes 399 amino acid residues of the COOH-terminal part of human ALDH2, an *Eco*RI-*Hinc*II fragment (0.35 kb) of the cDNA, and a *Hin*dIII-*Eco*RI fragment (0.42 kb) that corresponds to the 3′-noncoding region of the cDNA [10]. These probes were labeled with ^{32}P by nick-translation, using [α-^{32}P]dCTP to give a specific activity of 2–5 $\times$ 10^8 cpm/μg [13].

Southern Blot Hybridization Analysis

DNA was prepared from various cell lines as described [14]. Twenty micrograms each of the DNA samples was digested with *Eco*RI, 3U/μg DNA, for 15 hrs at 37°C. DNA

fragments were separated by electrophoresis in 0.9% agarose gels and transferred onto a nitrocellulose filter, as described by Southern [15]. Prehybridization was carried out in a solution containing 50% formamide, 3 × SSC, 1 × Denhardt's solution [16], and denatured salmon sperm DNA (500 μg/ml) at 42°C overnight; hybridization was carried out in the same solution plus 5% dextran sulfate and ^{32}P-labeled cDNA probe (10^6 cpm/ml) at 42°C for 24 hrs. The filters were quickly rinsed twice in 0.1 × SSC, 0.1% SDS at room temperature and then washed twice at 53°C for 1 hr.

RESULTS

Chromosomal Assignment of ALDH1 *Gene*

The hybridization pattern of *Eco*RI-digested human DNA with 1.58 kb ALDH1 cDNA probe showed six strong hybridization-positive fragments of approximately 15 kb, 6 kb, 4.3 kb, 2.9 kb, 1.9 kb, and 1.6 kb. In addition, two weakly positive bands were visible, particularly in the case of heavy sample loading (fig. 1). The *Eco*RI-digests of mouse DNA and that of Chinese hamster DNA also showed several hybridization-positive fragments with various lengths and strengths (fig. 1). The 15-kb, 6.0-kb, and 1.9-kb fragments of human DNA were readily distinguishable from the positive bands originating from DNAs of mouse. The results of the segregation analysis on 19 rodent-human hybrid cell lines indicate that the 15-kb, 6.0-kb, and 1.9-kb bands segregated only with human chromosome 9 (table 1).

Figure 1 also demonstrates that the 6.0-kb, 2.9-kb, and 1.9-kb fragments of human DNA were distinguishable from positive bands of Chinese hamster and were present in the cell hybrid CF 17-22 (fig. 1, lane 5) and absent in the cell hybrid CF17-24 (fig. 1, lane 6). When the *Eco*RI-*Sau*3AI fragment (0.285 kb of

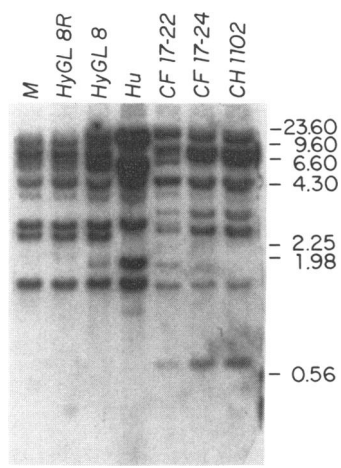

FIG. 1.—Hybridization of nick-translated human ALDH1 cDNA probe (1.58 kb) with *Eco*RI-digested genomic DNA. *M* = mouse B82/GM347 DNA; *HyGL8R* = mouse-human cell hybrid GL8R DNA; *HyGL8* = mouse-human cell hybrid GL8 DNA; *Hu* = human IMR 91 DNA; *CF17-22* = Chinese hamster-human cell hybrid CF17-22 DNA; *CF17-24* = Chinese hamster-human cell hybrid CF17-24 DNA; *CH1102* = Chinese hamster 1102 DNA. Hybrids GL8 and CF17-22 are positive for human *ALDH1* sequences.

TABLE 1

SEGREGATION OF HUMAN *ALDH1* AND *ALDH2* GENES WITH HUMAN CHROMOSOMES ON RODENT-HUMAN HYBRIDS

	HUMAN CHROMOSOMES																								
CLONE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y	ALDH1	ALDH2
CF84-2 ...	+	+	−	+	+	+	+	+	−	−	(−)	+	−	+	+	+	+	+	+	+	−	+	−	−	−	+
CF84-3 ...	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−	−	−
CF84-4 ...	+	+	+	+	−	+	+	+	−	+	−	−	+	+	+	−	+	+	+	+	−	−	−	−	−	−
CF84-5 ...	−	+	+	−	+	(+)	−	+	−	−	−	−	(+)	+	+	−	+	−	+	−	+	+	+	+	−	−
CF84-7 ...	−	−	+	+	−	+	(−)	(+)	−	−	+	(+)	−	+	−	−	+	−	−	+	−	−	−	−	−	+
CF84-20 ..	−	+	+	+	(+)	−	+	+	−	−	+	+	+	+	(−)	+	+	+	−	+	+	+	+	−	−	+
CF84-21 ..	−	−	−	−	−	−	+	−	−	+	−	+	−	+	−	−	+	−	−	+	+	−	−	(+)	−	+
CF84-25 ..	−	−	−	+	+	+	−	+	−	+	+	+	−	−	−	−	+	−	(+)	−	+	−	−	−	−	+
CF84-26 ..	+	−	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+
CF84-27 ..	−	+	−	−	+	+	−	+	−	−	+	+	−	−	+	−	+	+	−	+	+	−	−	−	−	+
CF84-30 ..	−	−	+	+	(+)	+	−	−	−	+	−	(+)	+	+	−	−	+	+	−	(+)	+	+	−	−	−	+
CF84-34 ..	−	−	−	+	−	+	+	+	−	+	+	−	−	+	−	−	+	−	(+)	+	−	+	−	−	−	−
CF84-35 ..	−	−	+	+	−	+	+	+	−	−	−	+	−	+	+	−	+	+	−	−	−	−	−	−	−	+
CF84-37 ..	−	−	−	+	+	+	+	(+)	−	−	+	+	−	+	−	−	+	−	−	+	+	+	−	−	−	+
CF84-38 ..	+	−	+	+	−	+	+	+	−	+	−	+	+	+	+	−	+	+	+	−	−	+	−	−	−	+
CF84-39 ..	−	−	+	−	−	−	+	+	−	−	−	−	−	−	+	−	+	−	−	−	−	−	−	−	−	−
GL8.....	−	−	−	−	−	−	−	−	+*	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
GL8R	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
CF11-4 ...	−	−	−	−	−	−	−	−	+†	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−
	Discordancy (#/19)																								
ALDH1...	7	8	12	14	10	15	13	16	0	10	8	13	9	15	11	5	18	11	10	13	10	10	4	5		
ALDH2...	10	11	9	5	5	6	8	7	13	9	7	0	10	6	10	8	5	6	11	6	5	7	11	10		

NOTE: "+" indicates presence of the chromosome in > 30% of analyzed cells; (+), 10%–30%; (−), 5%–10%; and "−", not detected.

* Contains the translocation chromosome Xqter→Xq12::9p24→9qter exclusively.

† Contains the translocation chromosome Xqter→Xq13::9q34→9pter exclusively.

ALDH1 cDNA) was used as a hybridization probe, two human bands (2.9 kb and 1.9 kb) were both distinguishable from Chinese hamster bands of 2.7 kb and 1.6 kb (fig. 2). The Chinese hamster-human hybrid cell line CF17-22/7, which contained both products of a reciprocal translocation between chromosomes 9 and 17 [t(9;17)(9p17q)(9q17p)], had the 2.9- and 1.9-kb bands, while CF17-24/9, which had only 17q/9p human chromosome and was missing the long arm of chromosome 9, had lost both bands (fig. 2). Based on these results, the human *ALDH1* gene can be assigned to the long arm of chromosome 9. Clone CF11-4 contains an X/9 translocation chromosome (9pter→9q34::Xq13 →Xqter) and was positive for the presence of *ALDH1*. Previous studies have shown that the locus for adenylate kinase-1 (AK1) assigned to 9q34 is missing from this chromosome [11]. Therefore, *ALDH1* can be excluded from the very distal end of the long arm of chromosome 9.

Chromosomal Assignment of ALDH2 *Gene*

The hybridization patterns of *Eco*RI-digested human DNA with the ALDH2 cDNA probe (1.234 kb) showed five positive fragments of approximately 20 kb, 7.0 kb, 2.0 kb, 1.7 kb, and 1.0 kb (figure is not shown). The 7.0-kb, 2.0-kb, 1.7-kb, and 1.0-kb bands were unique in human DNA since *Eco*RI-digested mouse DNA exhibited three hybridization-positive fragments of 20 kb, 9.6 kb, and 0.8 kb. When the *Eco*RI-*Hin*cII fragment (0.35 kb) of ALDH2 cDNA was used as a probe, four hybridization bands, 7.0 kb, 2.0 kb, 1.7 kb, and 1.0 kb, were observed in human DNA, while only a 9.6-kb band was found in the mouse DNA (fig. 3). When the *Hin*dIII-*Eco*RI fragment of ALDH2 cDNA (noncoding 3′-region of 0.42 kb) was used as a probe, a hybridization band of 20 kb was found in human DNA, but no hybridization band existed in mouse DNA (figure is not shown). The unique human DNA bands always cosegregated with

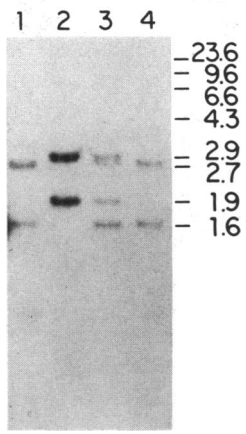

FIG. 2.—Hybridization of the nick-translated human ALDH1 cDNA fragment (0.285 kb) with *Eco*RI-digested genomic DNA. *Lane 1* = Chinese hamster 1102 DNA; *Lane 2* = human DNA; *Lane 3* = CF17-22 Chinese hamster-human cell hybrid DNA; *Lane 4* = CF17-24 Chinese hamster-human cell hybrid DNA. CF17-22 is positive for human *ALDH1* sequences.

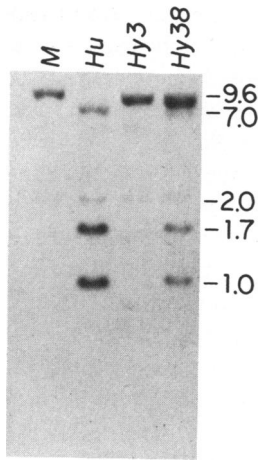

FIG. 3.—Hybridization of the nick-translated human ALDH2 cDNA fragment (0.35 kb) with *Eco*RI-digested genomic DNA. *Hu* = human IMR 91 DNA; *M* = mouse B82/GM347 DNA; *Hy3* = mouse-human hybrid CF84-3 DNA; *Hy38* = mouse-human hybrid CF84-38 DNA. Hy3 is negative and Hy38 is positive for human *ALDH2* sequences.

chromosome 12 in the hybrid cell lines examined (table 1). Thus, the human *ALDH2* gene can be assigned to chromosome 12.

DISCUSSION

Although the exon-sequences corresponding to the ALDH1 cDNA and ALDH2 cDNA probes do not contain an *Eco*RI site [10], multiple hybridization-positive bands were observed in Southern hybridization patterns of *Eco*RI-digested human DNA (figs. 1, 2, and 3). Several possibilities may account for the multiple hybridization bands: Intron-sequences of *ALDH1* and *ALDH2* genes would contain *Eco*RI-recognition sites, thus producing multiple fragments hybridizable with the cDNA probes. Multiple positive bands could be produced by ALDH-like genes, or pseudogenes, which might exist in the genome. Incomplete digestion or nonspecific digestion of DNA by *Eco*RI would cause the formation of multiple bands. In the present experiment, the digestions were always carried out using wild-type phage DNA as a control to ensure specific and complete digestion by *Eco*RI. The hybridization condition used has been considered as stringent [17], and the same pattern of multiple positive bands was also observed using higher washing temperatures (up to 58°C). Thus, the possibilities of incomplete and/or nonspecific digestion of the genomic DNAs, and cross-hybridization, are unlikely, but could not be ruled out entirely.

Human DNA digested by *Hin*dIII, *Kpn*I, *Bam*HI, *Hin*cII, and *Sph*I also showed complex hybridization patterns with the hybridization probes.

Homology between human ALDH1 and ALDH2 is 66% in the coding regions of their cDNAs and 69% at the protein level [10]. Hybridization patterns of *Eco*RI-digested human DNA with the two cDNA probes exhibited completely different profiles, indicating no cross-hybridization between the two genes.

It has been reported that the degree of homology between human ALDH1 and horse ALDH1 was 91% at the protein level [18]. Homology between the human ALDH1 and rodent ALDH1, and that between human ALDH2 and rodent ALDH2, are probably high, as demonstrated by cross-hybridization with the cDNA probes (figs. 1, 2, and 3). Some of these cross-hybridization bands either coincided with or were located close to the hybridization bands of human DNA. However, when the DNA samples were hybridized with the fragment of ALDH1 cDNA and the fragments of ALDH2 cDNA as probes, the human bands were clearly distinguishable from the rodent bands (figs. 2 and 3). Thus, segregation analysis could be performed without ambiguities. We found that the noncoding 3′-fragment of ALDH2 cDNA hybridized only with human DNA. Since the noncoding 3′-region is expected to hybridize with only a functional *ALDH2* locus, but not with a pseudogene for human ALDH2 or rodent ALDH2, the noncoding region would be the most specific probe for detection of the functional gene. Thus, the results presented here assign the functional gene for ALDH2 to chromosome 12.

The gene for mouse cytosolic ALDH isozyme (corresponding to human ALDH1) was assigned on mouse chromosome 19 [19], and the gene for mouse mitochondrial ALDH isozyme, which functionally differs from the human ALDH2, was assigned to chromosome 4 [20]. The present results indicate that the *ALDH1* locus is located on the long arm of chromosome 9 and the *ALDH2* locus is on chromosome 12 in man.

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