SHORT COMMUNICATION

Localization of the Gene for ATP Citrate Lyase (ACLY) Distal to Gastrin (GAS) and Proximal to D17S856 on Chromosome 17q12–q21

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The gene encoding ATP-citrate lyase, designated ACLY, was mapped to human chromosome 17q12–q21 by PCR on a panel of human/rodent somatic cell hybrids and localized to 17q21.1 by PCR on a panel of radiation hybrids. The radiation hybrid panel indicates that the most likely position of ACLY on 17q21.1 is between gastrin (GAS) and D17S856 at a distance of 170–290 kb from the GAS locus. © 1994 Academic Press, Inc.

ATP–citrate lyase (ACLY) is the primary enzyme responsible for the synthesis of cytosolic acetyl–CoA in many tissues. Studies have suggested that the enzyme functions as a regulatory point in glucose utilization, lipogenesis, and cholesterogenesis and therefore has particular relevance to the study of dietetics, nutrition, and diabetes. The human enzyme is 1105 amino acids in length, with a calculated molecular mass of 121,419 Da (6). The full-length cDNA is 4.3 kb. Human, mouse, and rat ATP–citrate lyase cDNAs have been isolated (5, 6, 13), and comparison of the human and rat ACLY amino acid sequences reveals 96.3% identity.

ACLY was previously mapped by genetic linkage studies to rat chromosome 10 between markers for the genes for the cellular homologue of the viral oncogene erbB2 (ERBB2) and pancreatic polypeptide (PPY), a region that exhibits linkage conservation with a segment of human chromosome 17 (11). Both ERBB2 and PPY map to human chromosome 17q12–q21 (2), suggesting that the ACLY gene is located in this interval. As a first step to accurately map the human ACLY gene, we regionally localized the gene using a panel of human/rodent somatic cell hybrids. This panel consists of (1) the human/rat hybrid 7AE4 (9) containing chromosome 17 as its only human chromosome; (2) the human/mouse hybrid NF13 (10) containing chromosome 17q11.2–qter with the breakpoint at the neurofibromatosis 1 locus (NF1); (3) P12.3B, a human/mouse hybrid containing chromosome 17q12–pter with a breakpoint at the retinoic acid receptor α subunit locus (RARA) (4, 14); and (4) the human/hamster UMHG-17/1 hybrid (8) containing chromosome 17q22–qter; see Fig. 1. Polymerase chain reaction (PCR) primers specific for the 3′ untranslated region of the human ACLY cDNA were designed and used to PCR amplify the human/rodent somatic cell hybrid DNA. Analysis placed the ACLY gene distal to the P12.3B hybrid breakpoint in RARA and proximal to the UMHG-17/1 hybrid breakpoint at 17q22, as shown in Fig. 1. This result is consistent with the position of ACLY by the rat linkage analysis. A combination of the two mapping techniques suggests that ACLY can be placed between RARA and PPY.

To refine the localization of ACLY within this interval further, a recently published radiation reduced hybrid (RH) panel (1) was utilized. The panel of radiation hybrids consisted of 76 hybrids generated by radiation reduction of the 7AE4 rat hepatoma microcell hybrid cell line. The hybrids had previously been characterized with 22 markers from chromosome 17q12–q21. Statistical analysis of marker retention provided a map of the region consistent with both meiotic mapping and fluorescence *in situ* hybridization (FISH). All 76 hybrids were typed with the ACLY sequence tagged site (STS). The PCR results for each of the 76 hybrids are shown in Table 1. Multipoint analysis of the RH data was performed. The location of greatest likelihood of ACLY on the 1000:1 framework map of 17q12–q21 was between ERBB2 and D17S855 (248YG9). This result, in combination with the somatic cell hybrid data, suggested that ACLY was located between RARA and D17S855. Analysis of the comprehensive radiation hybrid map by multipoint maximum likelihood analysis (3) of the marker retention data placed ACLY with greatest likelihood between gastrin (GAS) and D17S856 (OF2), which is consistent with the framework map analysis. The placement of ACLY between GAS and topoisomerase II α subunit (TOP2A) had max-

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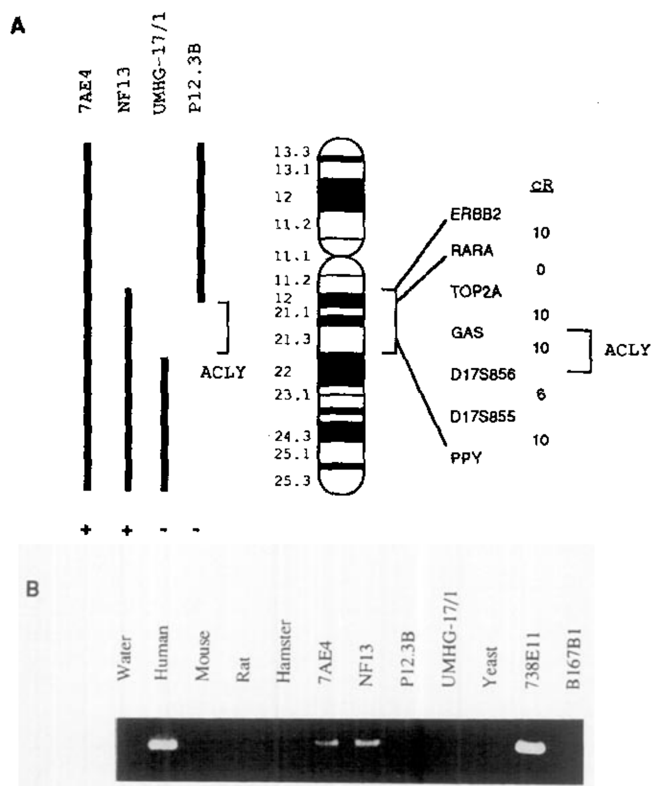

FIG. 1. Mapping of ACLY to chromosome 17q12–q22. (**A**) Regional localization of ACLY on chromosome 17q by somatic cell hybrid analysis. Vertical bars to the left of the chromosome indicate the region of chromosome 17 retained in the somatic cell hybrids. The bracket indicates the region to which ACLY is assigned. Localization of ACLY by radiation hybrid analysis is illustrated to the right of chromosome 17. Previously mapped genes from the 17q12–q21 region with intervening distances in centirays, cR(8000), are shown. The bracket indicates the most likely localization of ACLY. (**B**) Ethidium-bromide-stained 1.5% agarose gel analysis of ACLY STS PCR amplified human/rodent hybrid and YAC DNA. Lane 1 contains results of PCR amplification from water. Lanes **2–5** contain PCR results from human, mouse, rat, and hamster genomic DNA. Lanes **6–9** contain PCR amplified human/rodent clones 7AE4, NF13, P12.3B and UMHG-17/1, respectively. Lanes **10, 11,** and **12** contain PCR results from AB1380 yeast genomic DNA, YAC 738E11, and YAC B167B1. ACLY STS primer sequences were 5′-GACCAACATCCACAGGCTAACACC-3′ and 5′-CCTACAATGAGGAAGACCCCATCC-3′. PCR was carried out in 25-μl reactions containing 10 m*M* Tris–HCl, pH 8.3, 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 200 μ*M* dNTPs, 1.0 μ*M* primers, 1 unit *Taq* DNA polymerase (BMB), and 125 ng hybrid DNA. PCR reactions were performed on a PTC-100 Programmable Thermal Controller (MJ Research Inc.) and consisted of 4 min at 94°C; 32 cycles of 1 min at 94°C, 1 min at 63°C, 1 min at 72°C; and 7 min at 72°C. The expected product size for human ACLY is 174 bp.

imum likelihood 240-fold less. RH mapping placed ACLY at an estimated distance of 3.2 cR(8000) from GAS, as calculated by multipoint analysis of the comprehensive RH map. Centirays (cR) refers to distances on the radiation hybrid map, where 100 cR(8000) corresponds to one expected break per hybrid after exposure to 8000 rads. Using the relationship of 50–90 kb/cR(8000) (1), ACLY is estimated to be 170–290 kb from GAS in a 500–900 kb (10 cR) interval between GAS and D17S856; see Fig. 1.

RH statistical analysis predicted close physical linkage between GAS and ACLY. This prediction was confirmed by colocalization studies of both markers on two yeast artificial chromosomes (YACs) from chromosome 17q12–q21. The YACs B167B1 from the Washington University library and 738E11 from the Genethon library, which were isolated using the GAS STS, were tested for the presence of the ACLY STS by PCR, as shown in Fig. 1. The B167B1 YAC, which is nonchimeric but only 90 kb in size, did not contain the ACLY STS, while the 1.5 Mb, chimeric, 738E11 YAC did contain the STS. Neither YAC contained the nearest proximal (TOP2A) and distal (D17S856) markers. YAC chimerism studies were performed by FISH (7). The amount of chromosome 17q21.1 DNA in the 738E11 YAC is presently unknown. This result indicates that ACLY must be within 1.5 Mb of the GAS STS.

In summary, a combination of techniques has been used to accurately map the ACLY gene between TOP2A and D17S856 on chromosome 17q12–q21, most probably 170–290 kb distal to the GAS locus. This places ACLY in the candidate interval for the familial early onset breast cancer gene (BRCA1) (distal to RARA, proximal to PPY) (12). This STS should prove useful in physical mapping of this intensively studied region.

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TABLE 1

Retention Scores for the ACLY STS on the Chromosome 17 Radiation Hybrid Panel

RH	ACLY	RH	ACLY	RH	ACLY	RH	ACLY
1	+	18b	+	31	−	66	+
3	−	18c	−	32b	+	67	+
4	−	19	−	32c	+	68	+
5	+	20a	+	37	−	69	+
6	−	20b	−	42a	−	70	+
7	−	20c	+	42b	−	71	+
8a	+	21a	+	43	−	72b	+
8b	+	21b	+	44a	+	72c	+
8c	+	22a	−	44b	+	73	+
9	−	22b	+	45	−	74b	+
10a	−	22d	+	49b	+	74c	+
10b	−	23	+	49d	+	75	+
11	+	24	−	50	+	76a	+
12	−	25a	+	54	−	76b	+
13	−	25b	+	55	+	76c	+
15	−	26	+	56	+	76d	+
16a	+	27a	−	57	+		
16b	−	27c	−	61	+		
17	−	28	−	64a	−		
18a	+	29	−	64b	−		

Note. A hybrid scored (+) indicates the presence of the ACLY STS in that hybrid. A hybrid scored (−) indicates the absence of the STS. Hybrid scores can be matched to previously published retention scores for other markers in the 17q12–q21 region (1).

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