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Assignment of the Structural Gene Encoding Human Aspartylglucosaminidase to the Long Arm of Chromosome 4 (4q21→4qter)

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SUMMARY

The structural gene for the human lysosomal enzyme aspartylglucosaminidase (AGA) has been assigned to chromosome 4 using somatic cell hybridization techniques. The human monomeric enzyme was detected in Chinese hamster-human cell hybrids by a thermal denaturation assay that selectively inactivated the Chinese hamster isozyme, while the thermostable human enzyme retained activity. Twenty informative hybrid clones, derived from seven independent fusions, were analyzed for the presence of human AGA activity and their human chromosomal constitutions. Without exception, the presence of human AGA in these hybrids was correlated with the presence of human chromosome 4. All other human chromosomes were excluded by discordant segregation of the human enzyme and other chromosomes. Two hybrid clones, with interspecific Chinese hamster-human chromosome translocations involving the long arm of human chromosome 4, permitted the assignment of human AGA to the region 4q21→4qter.

INTRODUCTION

The lysosomal enzyme aspartylglucosaminidase (1-aspartamido-β-*N*-acetylglucosamine amidohydrolase, E.C.3.5.1.26; AGA), cleaves the *N*-acetylglucosamine-asparagine linkage of oligosaccharide chains in a variety of glycoproteins and glycopeptides [1, 2]. The deficient activity of AGA results in the lysosomal

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storage disorder, aspartylglucosaminuria (AGU) [3]. This disorder is characterized by the progressive accumulation of aspartylglucosamines in tissues and fluids, and affected individuals have severe mental retardation, characteristic facial features, and mild dysostosis multiplex [4–6]. The disease is inherited as an autosomal recessive trait and is particularly prevalent in individuals of Finnish ancestry [7].

Our recent studies on the physicokinetic properties of the human AGA demonstrated that the enzymatic activity was remarkably thermostable [8]. Homogeneous enzyme purified from human liver had a half-life of about 3 hrs at 50°C; partially purified enzyme preparations were even more thermostable. This finding, and the fact that AGA activity in cultured mouse, rat, or Chinese hamster cell lines was thermolabile (our unpublished results, 1984), led to the development of a thermal denaturation assay that discriminated the human and rodent AGA isozymes. Using this assay and a panel of Chinese hamster-human somatic cell hybrids, we assigned the human gene encoding AGA to the long arm of chromosome 4.

MATERIALS AND METHODS

Chinese Hamster-Human Cell Hybrids

Clones derived from seven independent fusion experiments between human fibroblasts or leukocytes (series XII and XIII) and Chinese hamster cell lines were used. The rodent parental cell lines were either a hypoxanthine phosphoribosyltransferase negative (V 79/380-6, a continuous Chinese hamster fibroblast line in series XII, XIII, XV, XVII, and XVIII) or a thymidine-kinase negative cell line (Don/a23 in series XXI and XXV). Details of the derivation and characterization of the hybrid lines have been reported [9–12]. The chromosome constitution of each hybrid line and derivative subclones were determined using trypsin G-banding [13] and G-11-staining techniques [14]. Each hybrid line was rekaryotyped during these experiments, despite the relative stability of their chromosomal constitutions. A derivative of the parental Chinese hamster cell line, E-36, and normal human fibroblast cultures were used as sources of normal Chinese hamster and human AGA, respectively. All cell cultures were grown under standard tissue-culture conditions using RPMI 1640 (Gibco, Grand Island, N.Y.) supplemented with 10% fetal calf serum and antibiotics.

AGA Assay

Cells from three to five 75-mm^2 culture flasks were pooled, and the cell pellet was suspended in 0.6 ml of 0.15 M sodium chloride containing 0.2% Triton X-100. Cell supernatants were prepared by sonicating (3 × 20-second bursts) the cell pellet using a Branson sonicator model 200. The sonicates were centrifuged at 14,000 *g* for 30 min, and the supernatants were removed for assay. AGA activity was determined in the cell supernatants as described [8]. The reaction mixture contained 50 μl of cell supernatant, 25 μl of 15 mM 2-acetamido-1-β-(L-aspartamido)-1,2,-dideoxy-β-D-glucose (Sigma, St. Louis, Mo.), and 75 μl of 50 mM potassium phosphate buffer, pH 6.1. The reaction mixture was incubated at 37°C for 6 hrs, and the liberated *N*-acetylglucosamine was measured spectrophotometrically at 585 nm according to the Morgan-Elson reaction as described [8, 15]. One unit (U) of enzyme activity equals that amount of enzyme that liberates 1 nmol of *N*-acetylglucosamine per hr. All assays were carried out in duplicate or triplicate, and the results represent mean values.

Thermal Discrimination Assay

For the standard thermal denaturation assay, the AGA activities in parental or hybrid cell supernatants were diluted to 50 U/ml with 50 mM potassium phosphate buffer, pH

6.1. A 50-μl aliquot of the cell supernatant (2.5 U) was mixed with 75 μl of the same buffer and incubated at 65°C. After 20 min, the mixture was immediately cooled to 4°C in an ice bath. The thermostable AGA activity was measured as described above. The percent of thermostable AGA activity in each cell supernatant was determined by comparison to that in aliquots that were not subjected to thermal denaturation. The results represent the mean values of duplicate or triplicate determinations for at least two separate experiments for each hybrid cell line.

RESULTS

Development of the Thermal Discrimination Assay for AGA Isozymes

Figure 1 compares the stability of the AGA activities (50 U/ml) in supernatants of cultured human fibroblasts and Chinese hamster cells (line E-36) after incubation for 20 min at 60°C–75°C. Under these conditions, human AGA was heat-stable at 60°C and 65°C; at 70°C, approximately 50% of the initial human activity was retained. Interestingly, a 10%–20% increase in AGA activity was consistently observed following heat treatment at 60°C or 65°C. In contrast, the AGA activity in Chinese hamster cell supernatants lost about 85% of the initial activity after 20 min at 60°C; treatment at 65°C destroyed almost 100% of the Chinese hamster AGA activity. Based on these findings, a standard thermal denaturation assay (20 min at 65°C) was chosen for the selective inactivation of the Chinese hamster AGA in the hybrid cell lines. Under these conditions, Chinese hamster cell supernatants (line E-36) always had levels of activity that were 5% or less than that in untreated supernatants.

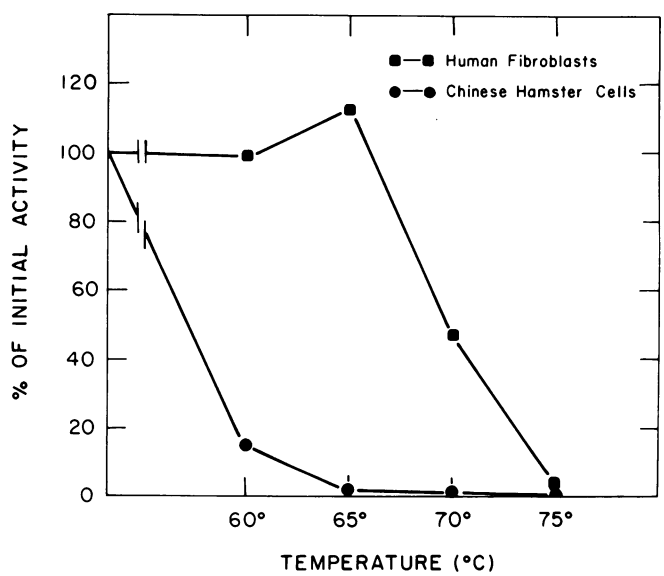

FIG. 1.—Effect of thermal denaturation on the AGA activity in parental Chinese hamster cell (line E-36) and human fibroblast supernatants. Aliquots of each supernatant (containing an initial activity of 50 U/ml) were incubated for 20 min at the indicated temperatures and then were assayed for AGA activity. The results are expressed as percent of initial (untreated) activity. See MATERIALS AND METHODS for details.

The accuracy of the thermal discrimination assay was further supported by the results of mixing experiments. As shown in figure 2, when mixtures of cell supernatants from human fibroblast and Chinese hamster cell line, E-36, were subjected to the standard thermal denaturation assay, the remaining heat-stable enzyme activity was directly proportional to the amount of human AGA activity in the mixture, from 0.25 U to 2.5 U.

Segregation Analysis of Human AGA in Hybrid Cell Lines

The percent of thermostable AGA activity and the human chromosomal constitution in 20 informative hybrid clones from seven independent fusions are shown in table 1. A hybrid cell line was considered positive for human AGA if more than 10% of the total enzymatic activity was thermostable. Of the 20 hybrid clones studied, 10 were scored positive, having had thermostable AGA activities ranging from 11.8% to 45% of initial activity. Hybrid cell lines scored as negative had thermostable activities that were 2.1%–5.3% of initial activities.

The segregation data for human AGA and the human chromosomes are summarized in table 2. There was complete concordance for human AGA and chromosome 4 in all 18 informative hybrids studied. Every hybrid line positive for the human enzyme, as determined by the thermal discrimination assay, had retained human chromosome 4 or a chromosomal rearrangement involving chromosome 4 in at least 80% of metaphases studied, whereas hybrid lines negative for the enzyme did not contain this chromosome. For all other human chromosomes, at least three hybrids were discordant for human AGA.

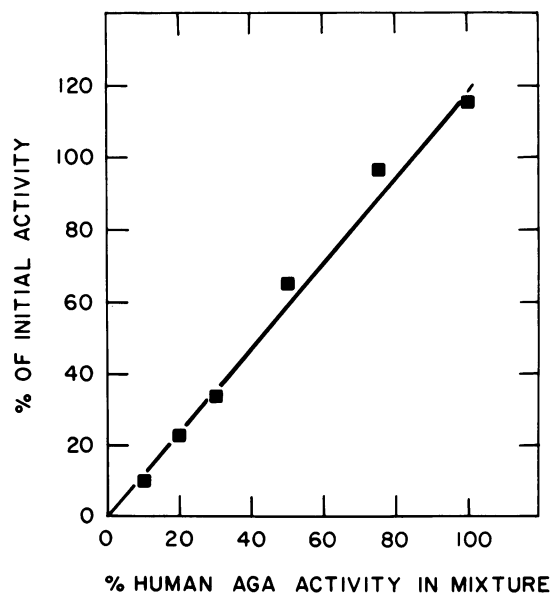

FIG. 2.—Detection of human AGA in mixtures of parental Chinese hamster (line E-36) and human fibroblast supernatants by the standard thermal denaturation assay. Total amount of AGA activity (2.5 U) in the assay was the same for all mixtures, which contained the indicated percentage of human fibroblast activity. See MATERIALS AND METHODS for details.

Regional Assignment

Two Chinese hamster-human hybrid cell lines were known to carry interspecific rearrangement chromosomes involving the long arm of human chromosome 4. The other derivative chromosomes, as well as intact chromosome 4s, were not present in these hybrid clones. Hybrid XV-N contained a derivative rearrangement chromosome due to a de novo interspecific translocation between the short arm of Chinese hamster chromosome 2 and the region 4q21→qter of human chromosome 4 as illustrated [12]. The other derivative chromosome with 4pter→q21, as well as a normal chromosome 4, were not present in this hybrid, which, consequently, lacked the short arm and proximal long arm of human chromosome 4. As shown previously [12], this hybrid did not express human peptidase S nor phosphoglucomutase 2, which are encoded by genes localized to 4p12→q12 and 4p14→q12, respectively [16]. The second informative hybrid clone, XVII-E, carried a derivative chromosome containing the entire long arm of human chromosome 4 (4cen→qter) translocated to the euchromatic arm of the Chinese hamster X chromosome. This hybrid did not contain the short arm of human chromosome 4 or a normal human chromosome 4. Both interspecies translocations were confirmed by Giemsa 11 staining as documented elsewhere [17]. Both of these hybrid clones were positive for the expression of the human AGA. On the basis of these findings, the structural gene encoding human AGA was assigned to the region 4q21→qter.

DISCUSSION

The remarkable thermostability of human AGA activity permitted the development of a sensitive thermal denaturation assay for the selective inactivation of Chinese hamster AGA activity. This assay reliably detected the expression of the thermostable human enzyme in Chinese hamster-human somatic cell hybrids. Similarly, the difference in thermostability between mouse and human isozymes of acid α-glucosidase was used to map the human structural gene to chromosome 17 [18]. Prior efforts to separate the human AGA monomeric enzyme [8] from the Chinese hamster, rat, or mouse isozymes by starch gel or cellulose acetate electrophoresis in our laboratory were unsuccessful since the human activity comigrated with the rodent isozymes in a variety of buffer systems. In addition, the human and rodent isozymes could not be separated by Western blotting techniques (K. H. Astrin, unpublished observations, 1984).

Using the thermal denaturation assay and a panel of 20 Chinese hamster-human hybrid cell clones, the gene encoding human AGA was assigned to chromosome 4. All clones containing human chromosome 4 were positive for human AGA (table 1). Every other human chromosome was ruled out by a considerable number of discordant hybrids (table 2). Furthermore, two hybrids containing only portions of human chromosome 4 permitted the regional assignment of human AGA to 4q21→qter.

Human AGA joins the 26 other genes assigned to chromosome 4, including 21 structural genes and five cloned DNA fragments [16–18]. Among these gene loci, several have been mapped to the long arm of chromosome 4: MN and Ss (q28→q31), the fibronectin gene family (q21→q31), albumin (q11→q22), α-

TABLE 1

HUMAN AGA ACTIVITY AND HUMAN CHROMOSOMES IN 20 CHINESE HAMSTER-HUMAN SOMATIC CELL HYBRID CLONES

HYBRID SERIES	CLONE CODE	HUMAN AGA*	HUMAN CHROMOSOME†										
			1	2	3	4	5	6	7	8	9	10	11
XII	W	+(17.8)	+	−	+	+	−	+	−	+	−	−	−
XIII	C	− (4.2)	−	−	−	−	−	−	−	−	−	−	−
XIII	U	− (2.1)	−	−	−	−	+	−	+	+	−	−	−
XV	A	+(30.3)	−	+	P	+	+	−	−	+	−	−	+
XV	B	− (3.9)	P	+	+	−	+	+	−	+	−	−	−
XV	D	+(26.1)	P	+	+	+	+	P	−	+	+	−	+
XV	N	+(21.7)	+	+	+	P	+	P	−	+	+	−	P
XV	R	+(45.3)	+	+	+	+	+	+	+	−	+	+	+
XV	T	+(21.5)	+	+	+	+	−	+	+	−	−	+	+
XVII	E	+(35.5)	−	−	+	P	−	+	+	−	−	+	+
XVII	J	− (4.9)	−	+	+	−	−	+	−	−	−	−	+
XVII	S	− (3.0)	−	+	+	−	−	+	P	+	−	−	+
XVIII	O	− (5.3)	−	−	L	−	−	−	−	−	−	−	+
XXI	F	− (3.8)	−	−	+	−	+	−	−	+	−	−	P
XXI	G	+(24.3)	+	+	+	+	+	+	+	+	−	+	+
XXI	H	+(45.4)	−	−	+	+	+	+	+	+	−	+	+
XXI	K	− (3.1)	+	−	−	−	−	−	−	+	−	−	P
XXI	L	+(18.9)	−	−	+	+	+	−	+	+	+	+	+
XXI	P	− (2.4)	−	−	+	−	+	+	−	+	−	+	+
XXV	I	− (4.0)	−	−	−	−	−	−	−	−	−	+	−

Hybrid series	Clone code	Human AGA*	Human chromosome†											
			12	13	14	15	16	17	18	19	20	21	22	X
XII	W	+(17.8)	+	−	P	+	+	L	+	+	−	+	+	P
XIII	C	− (4.2)	−	−	P	−	+	−	−	−	−	−	+	+
XIII	U	− (2.1)	−	+	−	+	−	−	+	−	+	−	+	−
XV	A	+(30.3)	+	−	+	+	+	+	−	+	+	+	+	+
XV	B	− (3.9)	−	+	+	−	+	−	+	+	+	+	+	−
XV	D	+(26.1)	+	+	+	+	+	−	−	+	+	+	+	−
XV	N	+(21.7)	+	−	+	+	+	+	+	−	+	+	+	+
XV	R	+(45.3)	+	+	+	+	+	−	+	+	+	+	+	+
XV	T	+(21.5)	+	+	+	+	−	−	+	+	+	+	+	+
XVII	E	+(35.5)	+	−	−	+	−	−	−	−	+	+	+	+
XVII	J	− (4.9)	+	+	+	P	−	−	+	+	+	+	P	P
XVII	S	− (3.0)	+	+	+	P	+	−	+	+	+	+	P	+
XVIII	O	− (5.3)	+	−	+	−	−	−	L	+	+	L	−	−
XXI	F	− (3.8)	−	+	+	+	+	+	−	L	L	+	+	+
XXI	G	+(24.3)	+	+	+	+	−	+	+	+	+	+	+	+
XXI	H	+(45.4)	+	+	+	−	−	−	+	+	+	+	+	+
XXI	K	− (3.1)	−	−	+	−	−	+	−	+	−	P	+	+
XXI	L	+(18.9)	−	−	+	+	+	+	+	+	+	−	+	−
XXI	P	− (2.4)	+	−	+	−	−	−	−	−	−	+	+	+
XXV	I	− (4.0)	+	−	+	−	−	+	−	−	−	−	−	−

* + or − indicates presence of human AGA activity based on % thermostable activity; % thermostable AGA activity indicated in parentheses.

† + indicates chromosome present in at least 10% of cells, L indicates low frequency (less than 0.1 copies/cell), P denoted the presence of only a defined part of the chromosome. P and L data were excluded from calculating the discordancy ratio in table 2.

TABLE 2

SEGREGATION OF HUMAN AGA AND HUMAN CHROMOSOMES IN CHINESE HAMSTER CELL-HUMAN SOMATIC CELL HYBRID CLONES

PRESENCE OF HUMAN AGA/ CHROMOSOME	HUMAN CHROMOSOME										
	1	2	3	4	5	6	7	8	9	10	11
+/+	5	6	9	8	7	6	6	7	4	6	8
−/−	8	7	4	10	6	6	8	4	10	8	4
+/−	4	4	0	0	3	2	4	3	6	4	1
−/+	1	3	5	0	4	4	1	6	0	2	4
Discordant hybrids/	5	7	5	0	7	6	5	9	6	6	5
Total informative hybrids	18	20	18	18	20	18	19	20	20	20	17

PRESENCE OF HUMAN AGA/ CHROMOSOME	HUMAN CHROMOSOME										
	12	13	14	15	16	17	18	19	20	21	22	X
+/+	9	5	8	9	6	4	7	8	9	9	10	7
−/−	5	5	1	6	6	7	5	4	4	3	2	4
+/−	1	5	1	1	4	5	3	2	1	1	0	2
−/+	5	5	8	2	4	3	4	5	5	5	6	5
Discordant hybrids/	6	10	9	3	8	8	7	7	6	6	6	7
Total informative hybrids	20	20	18	18	20	19	19	19	19	18	18	18

fetoprotein (q11→q22) [16] and epidermal growth factor (q21→qter). Also notable is the recent assignment of a cloned restriction length fragment polymorphism (D4S10), which is tightly linked to the Huntington disease locus, to chromosome 4 [19].

The assignment of the gene encoding human AGA provides additional insight into the organization of the genes encoding the lysosomal enzymes. To date, the locations of 20 structural genes for different lysosomal hydrolases (or their subunits) have been mapped to specific chromosomes or chromosomal regions (for review, see [20]). Previously, no lysosomal structural gene was assigned to chromosome 4, as the provisional assignment of α-fucosidase 2 by family studies presumably represents a gene responsible for the post-translational modifications of the plasma form of this enzyme [21]. With the inclusion of AGA, 20 lysosomal genes have been mapped to 12 autosomes and the X chromosome. Although several chromosomes contain two or more genes for lysosomal enzymes (chromosomes 1, 5, 10, 11, 19, 22, and X), none of the syntenic genes, with the exception of arylsulfatase B and β-hexosaminidase B on chromosome 5, encode enzymes involved in the same degradative pathway nor do they share similar catabolic functions (e.g., sulfatases). Thus, the structural genes encoding the lysosomal hydrolases are dispersed throughout the human genome.

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