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Human Chromosomal Assignments for 14 Argininosuccinate Synthetase Pseudogenes: Cloned DNAs as Reagents for Cytogenetic Analysis

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SUMMARY

There are multiple, processed, dispersed pseudogenes for human argininosuccinate synthetase. Chinese hamster × human somatic cell hybrids were used to map DNA fragment groups corresponding to the single expressed gene and 14 pseudogene loci. Each chromosomal assignment was confirmed using hybrids containing very few human chromosomes and/or by demonstrating monosomic or trisomic dosage in human cell lines with chromosomal abnormalities. Pseudogenes were mapped to chromosomes 2cen-p25, 3q12-qter, 4q21-qter, 5 (two loci), 6, 7, 9p13-q11, 9q11-q22, 11q, 12, Xp22-pter, Xq22-q26, and Ycen-q11. DNA fragments from the expressed gene were mapped to 9q34-qter in agreement with the previous assignment for enzyme activity. A high-frequency restriction fragment length polymorphism mapped to 9q11-q22. The analyses emphasized the feasibility of using chromosomally abnormal human cell lines for confirmation and regionalization of gene-mapping assignments made using somatic-cell hybrids. Conversely, cloned DNA probes, once mapped and characterized, can be very valuable for determining the chromosomal composition of interspecies hybrids and the dosage of loci in human cells. The argininosuccinate synthetase cDNA is a convenient reagent for dosage analysis of 15 human loci on 11 different chomosomes. Improved reagents could be designed that would simplify Southern blot patterns by eliminating overlapping DNA fragments and providing a single DNA fragment for each locus.

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INTRODUCTION

The human argininosuccinate synthetase locus is remarkable for the existence of an unusually large number of processed dispersed pseudogenes [1–3]. Previous work demonstrated that these pseudogenes were distributed to at least 10 different human chromosomes including 6, X, and Y [2, 4]. Enzyme activity for argininosuccinate synthetase was mapped to chromosome 9q34-qter [5]. Analysis of genomic DNA clones indicated that the pseudogenes were intron-free in seven instances examined, and three pseudogenes were fully or partially sequenced [3]. Processed pseudogenes consistently have been dispersed to chromosomal sites away from the expressed locus, and we have discussed the mechanistic and evolutionary significance of this pseudogene family elsewhere [3]. We now report assignments to 11 different chromosomes for what we believe to be all 14 of the highly homologous pseudogenes for argininosuccinate synthetase.

MATERIALS AND METHODS

Twenty-six cell lines were obtained from the Human Genetic Mutant Cell Repository (Camden, N.J.) and are specified in the presentation of data. The chromosomal aberrations are published in a catalog provided by the Repository [6]. Cell lines trisomic for chromosomes 13, 18, and 21 were obtained locally. Somatic-cell hybrids were prepared by hybridization of human lymphoblasts or leukocytes with a Chinese hamster cell line deleted for hypoxanthine-guanine phosphoribosyltransferase (HPRT) followed by selection in HAT medium as described [7]. Hybrids containing human X;9 translocation chromosomes were described previously [8]. The hybrid panel analyzed in table 1 included 10 hybrids containing an average of 10 human chromosomes (range 5–13) with an intact X in all cases. All human chromosomes were represented at least three times except that chromosome 17 was present in only one hybrid. Additional hybrids were analyzed including those shown in figure 2.

DNA was isolated from cultured cells as described by Wigler et al. [9]. The [^{32}P] nick-translated probe was synthesized as described [1] from pAS4/1, which contained all available cDNA sequence [10]. Blotting analysis of DNA was performed according to Southern [11] with modifications [1]. Densitometry was performed on a Gilford 2600 densitometer with a Radio Shack TRS-80 for data processing.

At the time of DNA preparation for the somatic-cell hybrids, a small aliquot of cells was plated in T-25 flasks for cytogenetic analysis. Care was taken to perform chromosome analysis on an aliquot of the cells at the time of harvesting for DNA. Trypsin G-banding analysis [12] was performed on 20 cells from each hybrid. G-11 staining [13] was performed on selected hybrids. A chromosome was scored as absent if observed $\leqslant$ three times in 20 cells and scored as present if observed $\geqslant$ four times.

RESULTS

A panel of 10 Chinese hamster $\times$ human hybrids provided a base of data for extending the mapping of the argininosuccinate synthetase sequences. As described [2], restriction fragments were assigned to groups when they consistently were present or absent together in DNA from hybrids when analyzed by Southern blotting after restriction digestion with *Eco*RI, *Pst*I, and *Hind*III. The data are summarized in table 1, and for convenience, each chromosomal site is designated according to its final assignment, which is substantiated by data presented below. The restriction fragments for each chromosomal locus are listed in table 2. In all but four cases, there were no discordances for the presence or absence of a DNA fragment group as correlated with the final chromosome assignment (table 1).

TABLE 1

DISCORDANCE SUMMARY FOR HYBRID PANEL

CHROMOSOME	ARGININOSUCCINATE SYNTHETASE LOCI										
	2	3	4	5*	6	7	9*	11	12	X*	Y
1	5	5	6	4	4	4	2	6	5	7	3
2	1	5	6	5	6	3	3	4	5	5	3
3	4	0	1	5	3	5	7	3	4	2	6
4	6	2	1	3	3	7	7	5	4	4	4
5	7	5	4	0	2	6	4	6	5	7	3
6	5	3	2	2	0	6	6	4	3	5	3
7	2	6	7	7	7	1	3	5	4	6	6
8	5	5	4	4	4	4	4	6	3	7	3
9	5	7	8	4	6	2	0	6	7	7	5
10	2	4	5	7	5	3	5	5	2	6	4
11	3	3	4	6	4	4	6	0	5	1	7
12	5	5	4	4	4	6	6	6	1	7	3
13	3	7	8	8	6	4	4	4	5	5	5
14	4	6	5	5	5	5	5	5	2	6	4
15	5	7	6	4	6	4	4	6	3	7	5
16	5	3	4	4	6	6	6	6	5	5	5
17	7	7	6	2	4	6	4	8	3	9	3
18	6	6	7	3	5	7	5	5	6	6	4
19	5	5	4	2	2	6	4	6	5	7	1
20	4	4	3	5	3	3	5	5	2	6	4
21	6	2	3	3	3	7	5	5	6	4	4
22	5	5	6	6	4	6	6	4	3	5	5
X	4	2	3	7	5	5	7	1	6	0	8
Y	4	6	5	3	3	7	5	7	4	8	0

NOTE: Each no. in the table is the no. discordances observed when the presence or absence of a set of DNA fragments was correlated with the chromosomal composition of 10 hybrids. One hybrid contained the short arm of chromosome 2 translocated to a Chinese hamster chromosome, and this hybrid was scored as concordant for chromosome 2 in all cases. Underlined numbers represent final assignments.

* Multiple loci on chromosomes 5, 9, and X showed identical patterns of presence and absence.

For assignments to chromosomes 2, 4, 7, and 12, there was one discordance each. In all cases, these discordances represented presence of the DNA fragment group, with absence of the chromosome on cytogenetic analysis. This type of discordance could occur if fragments of human chromosomes or translocation chromosomes were present in those hybrids. There were no instances of presence of a human chromosome and failure to observe the assigned DNA fragment group. The latter type of discordance would be of greater importance, since it could not be easily explained. All chromosome assignments were confirmed by additional analyses as described below. The DNA fragments are shown for reference in figure 1.

As one method of confirmation for assignments, we analyzed hybrids containing the human X plus one or a few human chromosomes as shown in figure 2. Restriction fragments that had been assigned to particular chromosomes based on the hybrid panel are listed in table 2. For example, figure 2 demonstrates *Eco*RI digestion of DNA from particular hybrids. The hybrid with human chromosomes X and 12 contained the Chinese hamster DNA fragments and the following human DNA fragments: 19, 3.0, and 1.2 kilobases (kb) assigned to X; and 6.9 kb assigned to 12. The hybrid with human chromosomes 6, 8, 11, and X contained the following

DNA fragments: 19, 3.0, and 1.2 kb assigned to X; 10 kb assigned to 6; and 4.0 and 1.6 kb assigned to 11. Using this approach, assignments to chromosomes 3, 4, 6, 11, 12, Xq, and Xp were substantiated as shown in figure 2. Similarly, analysis of a hybrid containing human chromosomes 7, 20, and X supported the assignment to chromosome 7 (data not shown). The fragments predicted to be present from analysis of the hybrid panel were found without any discrepancies or unexpected results. Two loci are present on the X chromosome. All hybrids were selected for expression of human HPRT present on Xq, and all hybrids retained the 19-kb *Eco*RI fragment assigned to Xq. However, one hybrid in figure 2 did not demonstrate the 3.0 or 1.2 kb *Eco*RI fragments assigned to Xp. The absence of the Xp fragments is explained by the presence in this hybrid of an X;11 translocation chromosome in which all of Xp and all of 11p were deleted.

For confirmation of some chromosomal assignments, for further evidence against any assignment to certain chromosomes, and for regional assignments, we analyzed various human cell lines containing chromosomal aberrations. Only examples of the data are shown in figures 3 and 4, but about 30 abnormal cell lines and multiple control cells were examined. Each cell line was analyzed using more than one restriction enzyme digestion. Figure 3 demonstrates: (1) expected dosage effects for X and Y chromosomal sites, (2) no evidence of hybridizing sequences on chromosomes 16 or 21, (3) increased intensity of a band assigned to chromosome 2 in a trisomy 2 cell line, and (4) increased and decreased dosage for Xq and Xp as expected for a cell line with an X isochromosome causing trisomy for Xq and monosomy for Xp. Two of the lanes in panel A of figure 3 are represented in a

TABLE 2

CHROMOSOMAL ASSIGNMENTS OF RESTRICTION DNA FRAGMENTS DETECTED BY ARGININOSUCCINATE SYNTHETASE cDNA

CHROMOSOMAL LOCUS	RESTRICTION FRAGMENTS IN KILOBASES		
	*Eco*RI	*Pst*I	*Hin*dIII
2cen-p25	15	9.5, 7.2†	32
3q12-qter	7.7a†	2.1†	20†
4q21-qter	9.0	5.7	8.2†
5 (two)	11, 9.5	8.5, 7.3†	1.9†
6	10	1.5†, 0.75	27
7	4.0, 3.4	0.96	2.5, 2.3
9q34-qter(e)‡	31, 6.2, 5.6 3.7, 3.0a*†	5.6* 4.2, 3.5, 2.9*†, 1.4*†	19*†, 16, 10.5*, 8.3*†, 8.1*†, 6.6a*†
9q11-q22 (ψa)	3.0b†	6.5	3.9/2.7
9p13-q11 (ψb)	7.7b†	2.9†	1.4
11q	4.0, 1.6	2.2†	7.3† or 7.2†
12	6.9	1.7†	2.1†
Xq22-q26	19	1.8†	6.6b†
Xp22-pter	3.0c†, 1.2	4.7	6.1
Ycen-q11	4.3	?	?
Uncertain	...	...	11, 9.5, 7.3† or 7.2†, 1.0

NOTE: Underlined fragments are more easily scored. * = faint fragments not usually seen in routine blot, but known to be present by structural gene analysis. † = doublet or triplet band. (e)‡, (ψa), and (ψb) after the loci on chromosome 9 refer to the expressed gene and two pseudogenes, respectively.

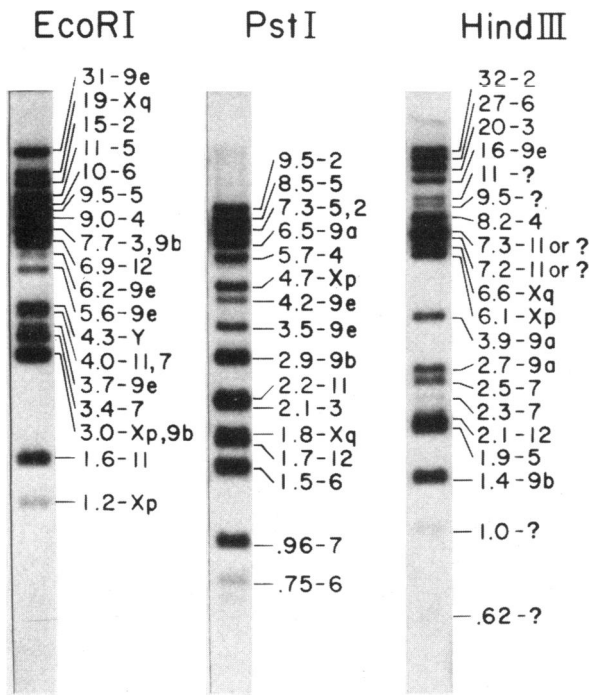

FIG. 1.—Southern blot pattern for human DNA using the argininosuccinate synthetase cDNA probe. Approximate fragment sizes in kb are listed followed by the chromosome assignments. Faint fragments indicated by an asterisk in table 2 are not listed in this figure. Loci on chromosome 9 are designated *e* for the expressed gene and *a* or *b* for pseudogenes.

densitometer tracing in panel B. The densitometry was found to be very reliable when the loading of DNA and general intensity of the autoradiographic signal were well matched, as for the two lanes scanned in panel B. Proceeding from left to right across the scan, there is good matching for a fragment from the expressed locus on chromosome 9, XX vs. XY dosage for the locus on Xq, the expected dosage effect for trisomy 2, a group of matched peaks, presence of a Y fragment in the male cell line, and dosage for the Xp site.

Two additional instances where chromosomally abnormal cell lines were used to confirm assignments and obtain regional assignments are shown in figure 4. In panel A, the DNA fragment assigned to chromosome 3 showed increased dosage on densitometric analysis of a cell line that is trisomic for 3q12-qter. A previously described *Hin*d III polymorphism [14] also is demonstrated in peaks labeled 9ψa. The data in figure 4*A* demonstrate that some inconsistencies in the intensity of autoradiographic bands may arise when imperfectly matched lanes are subjected to densitometry. In addition to the marked variation for the fragment on chromosome 3, there are other more modest deviations, most prominently for fragments mapped to Xp and Xq. Theoretically, the tracing for the two analyses in figure 4*A* should be superimposable except for the fragment from chromosome 3 and for polymorphic fragments. In our experience, these differences typically

arise when lanes that are imperfectly matched for DNA loading are analyzed. In figure 4*B*, DNA fragments are confirmed to map on chromosome 4 and given regional assignment, since there is increased dosage for these fragments in a cell line trisomic for 4q21-qter. We also analyzed cell lines trisomic for chromosomes 8 (mosaic, GM 496), 13, 15 (GM 3184), 18, and 22 (GM 3371). No DNA fragments showed increased dosage with these cell lines.

All assignments can be reviewed as follows. Assignment for chromosome 2 is in agreement with data from the hybrid panel and is supported strongly by the analysis of a trisomic cell line (fig. 3). Failure to detect monosomy in cell line GM 501 as shown in figure 4*B* indicated that the pseudogene is not at distal 2p and lies within 2p25-qter. In one somatic cell hybrid containing DNA fragments ultimately assigned to chromosome 2, G-banding and G-11 analysis showed that the entire short arm of chromosome 2 was translocated to a Chinese hamster chromosome; therefore, this pseudogene maps within 2cen-p25. The assignments for chromosomes 3 and 4 are detailed above.

Our assignment for chromosome 5 is based solely on data from the hybrid panel, but no discordances were observed, and these assignments have been confirmed by J. Wasmuth (personal communication, 1983) using a Chinese hamster × human cell hybrid that retains chromosome 5 exclusively. Our data indicate two sets of fragments that hybridize with both the 5′ end and the 3′ end of the

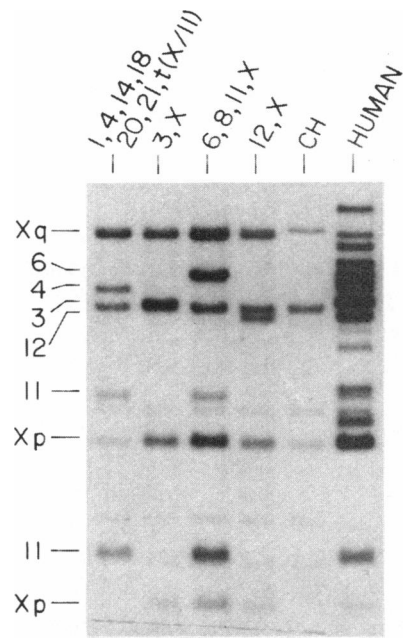

FIG. 2.—Analysis of Chinese hamster × human hybrid cells containing few human chromosomes. The human chromosome composition of the hybrids is *indicated at the top of each lane* except where Chinese hamster (CH) and human DNA were analyzed alone. The X;11 translocation chromosome indicated for *the left lane* contains Xq and 11q. Restriction digestion is with *Eco*RI and the DNA fragments *designated at the left* are as follows: chromosome, DNA in kb; Xq, 19; 6, 10; 4, 9.0; 3, 7.7; 12, 6.9; 11, 4.0; Xp, 3.0; 11, 1.6; Xp, 1.2.

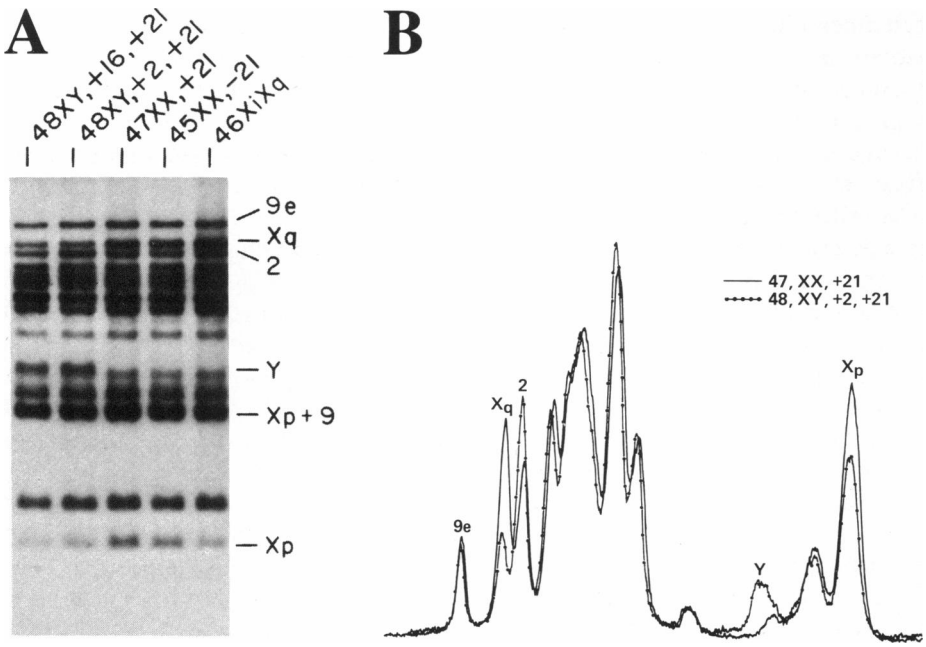

FIG. 3.—Analysis of chromosomally abnormal human cell lines with argininosuccinate synthetase cDNA probe. In *panel A*, the chromosome composition of the cell lines is indicated and identification is as follows *from left to right:* GM 4435, GM 3576, local patient, GM 230B, and GM 88. Restriction digestion is with *Eco*RI. DNA fragments are identified as follows: chromosome, DNA in kb; 9e, 31; Xq, 19; 2, 15; Y, 4.3; Xp + 9, 3.0; and Xp, 1.2. *Panel B* demonstrates densitometry of *lanes designated 48XY, +2, +21 and 47XX, +21 in panel A*. Densitometry ends after the 3.0-kb fragment.

cDNA probe, indicating the presence of two pseudogenes on chromosome 5. This also is confirmed because these two loci are separable in deleted chromosomes 5 (J. Wasmuth, personal communication, 1983). Assignment of one pseudogene to chromosome 6 was documented previously by multiple lines of evidence [2], and the data presented here are in agreement. Assignment to chromosome 7 is based on the data in table 1 and on analysis of a hybrid containing human chromosomes 7, 20, and X.

Assignments to chromosome 9 are complicated. Initial evidence that multiple widely separated loci existed on chromosome 9 arose from analysis of hybrids containing X;9 reciprocal translocation chromosomes with breakpoints at Xq13 and 9q34. These results indicated sequences on 9q34-qter, and these fragments agreed with ongoing structural analysis of the expressed gene in agreement with the assignment for enzyme activity [5]. However, the data also indicated hybridizing sequences on 9q34-pter. Analysis of hybrids selected for expression of human argininosuccinate synthetase by growth in tissue-culture medium with citrulline substituted for arginine also indicated multiple loci because some hybrids retained DNA fragments from the expressed gene but lost other DNA fragments mapped to chromosome 9. Analysis of these hybrids indicated the presence of two pseudogenes on chromosome 9 in addition to the expressed gene. Analysis of seven

cell lines (GM numbers 870, 1387, 1414, 1667, 1893, 3226, and 3563) with monosomy and trisomy for various portions of chromosome 9 documented the presence of two pseudogene loci at 9q11-q22 and 9p13-q11. The limits for the regional assignment of the pseudogene at 9q11-q22 are based on the monosomic dosage in cell line GM 1893 which is deleted for this region. The limits for the regional assignment of the pseudogene at 9p13-q11 depend on trisomic dosage in a cell line triplicated for 9q11-pter (GM 3226) and disomic dosage in a cell line deleted for 9p13-pter (GM 870). Analysis of all cell lines was consistent for the three regional assignments on chromosome 9.

Assignments to chromosomes 11 and 12 rest on the panel analysis shown in table 1 and on the data in figure 2, with regional assignment to 11q as discussed above. The locus on chromosome 12 is within 12p13-qter since the fragments were not monosomic in cell line GM 4127.

Assignments to the X chromosome were first indicated to be multiple by analysis of the X;9 translocation chromosomes described above, since hybridizing sequences were present on both sides of the Xq13 breakpoint. Analysis of six additional cell lines (GM numbers 74, 88, 2470, 3624A, 3827, and 3923) mapped the X-chromosome loci to Xp22-pter and Xq22-q26. The regional limits for the pseudogene at Xq22-q26 were determined by locating the site between two deletions; GM 3624 is deleted for Xq26-qter, and GM 3923 is deleted for Xq13-q22. The regional limits for the pseudogene at Xp22-pter depend on the fact that the locus is monosomic in the cell line GM 314, which contains idic(X) (qter-p22::p22-qter). GM 314 is a mosaic cell line but the Xq pseudogene is clearly at greater

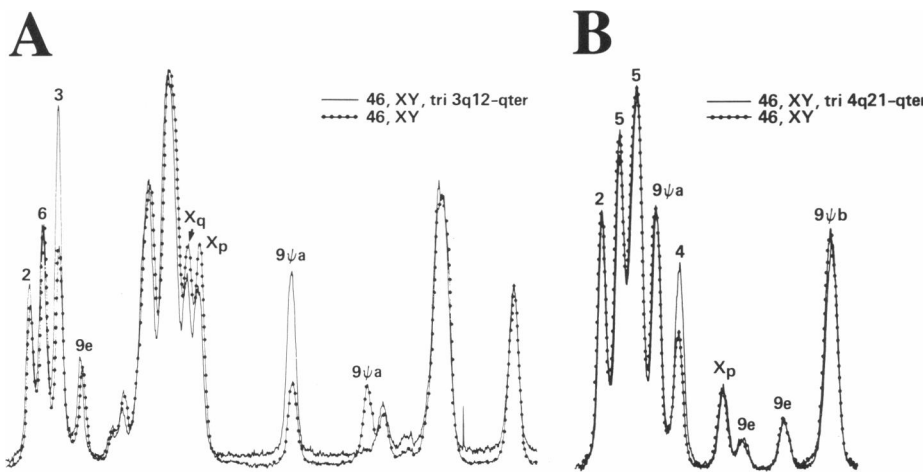

FIG. 4.—Densitometry for confirmation and regional assignment of pseudogenes on human chromosomes 3 and 4. In *panel A*, DNA was digested with *Hin*dIII, and fragments are identified as follows: chromosome, DNA in kb; 2, 32; 6, 27; 3, 20; 9e, 16; Xq, 6.6; Xp, 6.1; 9ψa, 3.9, and 2.7. Fragments labeled 9ψa represent a restriction fragment length polymorphism. The chromosomally abnormal cell line in *panel A* is GM 3563, which is monosomic for 9p24-pter as well as trisomic for 3q12-qter. In *panel B*, DNA was digested with *Pst*I and fragments are identified as follows: chrosomosome, DNA in kb; 2, 9.5; 5, 8.5, and 7.3; 9ψa, 6.5; 4, 5.7; Xp, 4.7; 9e, 4.2, and 3.5; 9ψb, 2.9. The chromosomally abnormal cell line in *panel B* is GM 501, which is monosomic for 2p25-pter as well as trisomic for 4q21-qter.

than disomic dosage and the Xp pseudogene is at monosomic dosage. Analysis of all cell lines was consistent with these regional assignments.

Previous assignment of a pseudogene to the Y chromosome [4] was confirmed in these studies, and regional assignment to Ycen-q11 was concluded since the locus was absent from a cell line containing Yq11-qter (GM 118) and was present in a cell line with iYq (GM 1709).

We have endeavored to confirm all chromosomal assignments by multiple means in order to establish this cDNA probe as a reliable reagent for analysis of human cells and rodent × human hybrids. The DNA fragments that can be used for this application are summarized in table 2, and the table can be used in conjunction with figure 1. It is important to emphasize that many bands represent doublets and can lead to confusion. We have underlined in table 2 those fragments that are reliable for analysis. A few of these fragments (e.g., *Hin*d III 20 kb and *Pst*I 2.9 kb) are quite reliable even though very faint fragments from the expressed gene are known to be present from other data. Fragments are designated by a difference of 0.1-kb unit if they are part of a doublet where the upper and lower portion are distinguishable. Two fragments are designated by an a or b if the two migrate indistinguishably in our analyses; for example, *Eco*RI 3.0a and 3.0b.

One distinct DNA polymorphism, mapped to the 9q11-q22 locus, involves the *Hin*d III 3.9- and 2.7-kb fragments, with gene frequencies of .6 and .4, respectively, reported for these alleles [14]. We are not aware of any other polymorphisms involving *Eco*RI, *Pst*I or *Hin*d III DNA fragments, but it is possible that DNA polymorphisms occur involving some doublet or triplet bands.

DISCUSSION

These results provide extensive chromosomal mapping data for argininosuccinate synthetase and 14 pseudogenes. The two pseudogenes on chromosome 9 are distant from the expressed gene and distant from each other, so we believe that there is no special significance to the fact that they occur on the same chromosome as the expressed gene. Extensive structural analysis of the pseudogenes indicates that each locus so far examined represents a single dispersed processed pseudogene [3]. Work is in progress to isolate unique flanking probes for each pseudogene and to relate each cloned pseudogene to a chromosomal site. Southern blotting analysis of DNA from chimpanzee [15] suggests that most or all of the pseudogenes characterized here will be present at analogous sites in higher primates. The high homology of the pseudogenes to the cDNA suggests that most of the pseudogenes are unlikely to be found at analogous chromosomal regions in the evolutionarily distant rodents. However, we have preliminary evidence that argininosuccinate synthetase sequences are found on multiple chromosomes in the mouse, and the DNA blot pattern is relatively complex [2]. It is possible that the pseudogenes characterized so far represent a more homologous or recently arisen subset from a continuum of pseudogene homology.

Well-characterized human cell lines with chromosomal aberrations can be very valuable for confirming gene mapping assignments and for obtaining regional data. The cell collection of the Human Genetic Mutant Cell Repository is extremely valuable for this purpose. For dosage analysis, a probe that provides control

fragments for comparison is essential. With proper attention to DNA loading and other variables, definitive differentiation of trisomic from disomic dosage (1.5 to 1 signal) as well as of monosomic from disomic dosage (2 to 1 signal) is possible as shown in figure 3.

Once DNA probes are well characterized, they become valuable for analyzing the karyotype of cells or somatic cell hybrids. The pAS1 probe is attractive for this application since one probe can demonstrate the presence, absence, and dosage for 15 different sites on 11 different chromosomes. Dosage analysis using cloned probes can have important medical applications. For example, dosage analysis will be particularly useful in detecting small deletions that occur in the Prader-Willi syndrome in humans [16]. It will also be helpful in characterizing de novo chromosomal aberrations where the identity of a region of the karyotype is unclear. Such analysis can delineate breakpoints in a chromosomal rearrangement relative to a DNA site.

Superior reagents could be generated by identifying a series of unique genomic DNA fragments of varying size using a single restriction enzyme. Pieces of these DNAs could be used to prepare mixed probes that would give a single DNA fragment for each chromosomal site. By judicious choice of restriction enzymes and DNA fragments, complex probes could be selected to avoid any doublet or triplet bands. Reagents such as those proposed would facilitate analysis of somatic-cell hybrids. Similar reagents could be designed for prenatal diagnosis of human chromosomal aneuploidy.

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