GENOMICS 17, 299–305 (1993)

Mapping of Human γ-Glutamyl Transpeptidase Genes on Chromosome 22 and Other Human Autosomes

DENISE A. FIGLEWICZ,*,[1] OLIVIER DELATTRE,† GEORGES GUELLAEN,‡ ALDIS KRIZUS,* GILLES THOMAS,† JESSICA ZUCMAN,† AND GUY A. ROULEAU*

*Department of Neurology, Montreal General Hospital, and Center for Research in Neuroscience, McGill University, Montreal, Canada; †Genetique des Tumeurs, Institut Curie, Paris Cedex 05, France; and ‡Unite INSERM 99, Hospital Henri Mondor, Creteil, France

Received April 1, 1992; revised November 7, 1992

γ-Glutamyl transpeptidase (GGT; EC 2.3.2.2) is a membrane-associated enzyme that plays a role in the metabolism of glutathione and in the transpeptidation of amino acids; changes in GGT activity may reflect preneoplastic or toxic conditions in the liver or kidney. In contrast to the rat, in which GGT is represented by a single gene, at least four GGT genomic sequences have been identified in man and two of these have been localized to two distinct regions of chromosome 22. To characterize this gene/pseudogene family further, we have used somatic cell hybrids to map GGT by hybridization with probes from a human kidney GGT cDNA clone and by amplification of 3′ GGT sequence by PCR. We clearly map three GGT loci to chromosome 22: two loci between the centromere and the breakpoint cluster region (BCR) gene and one locus telomeric to the BCR gene. In addition, we have been able to identify GGT-related sequences on chromosomes 18, 19, and 20.

INTRODUCTION

γ-Glutamyl transpeptidase (GGT; EC 2.3.2.2) is an enzyme with important metabolic and transport roles in mammals. It catalyzes reactions in which the γ-glutamyl group of glutathione (or related compounds) is transferred to an appropriate receptor, such as certain amino acids, dipeptides, or glutathione itself; alternatively, glutathione can be hydrolyzed to yield the dipeptide Cys–Gly and free glutamate (Tate and Meister, 1981; Meister and Anderson, 1983). A second important role is the transpeptidation of free amino acids to form γ-glutamyl amino acids providing an alternative system for cellular amino acid uptake. The localization of GGT (Tate and Meister, 1981; Curthoys, 1983; Nakamura *et al.*, 1985; Lebargy *et al.*, 1990) as a membranous enzyme that is concentrated in cells of secretory or absorptive functions is consistent with these roles. In mammals, GGT activity can be detected in a number of tissues, with the highest levels in kidney and gastrointestinal tract. However, some significant species differences, particularly between rodent and human, have been reported (Tate *et al.*, 1981, 1988).

Mammalian GGT is dimeric, containing two glycosylated subunits of unequal size that are synthesized simultaneously from the same mRNA (Barouki *et al.*, 1984; Finidori *et al.*, 1984; Nash and Tate, 1984). The glycosylated peptide of approximately 78 kDa is subsequently cleaved, resulting in a large subunit which is anchored at the external surface of the cell membrane by its N-terminal and a small subunit which exhibits catalytic activity. GGT became a subject of particular interest when it was discovered that measurable activity changes occur under preneoplastic or toxic conditions in liver or kidney (Barouki *et al.*, 1982; Ohmori *et al.*, 1982; Barouki *et al.*, 1983; Sawabu *et al.*, 1983; Hanigan and Pitot, 1985; Tsuda *et al.*, 1985). GGT activity in choroid plexus or capillary endothelium has been investigated in light of a putative role in amino acid transport across the blood–brain barrier (Ghandour *et al.*, 1980; Shine and Haber, 1981; Stastny and Lisy, 1981; Papandrikopoulou *et al.*, 1989). Isozyme variants of GGT have been recognized as the product of variable sialylation.

The cloning of rat kidney GGT cDNA (Laperche *et al.*, 1986; Coloma and Pitot, 1986) has allowed extensive characterization of its expression in development, in various tissues, and in experimental pathology (Goodspeed *et al.*, 1989; Chobert *et al.*, 1990). Several rodent GGT mRNAs (Griffiths *et al.*, 1989; Chobert *et al.*, 1990), all of which are the product of a single GGT gene, have been isolated. Cloning of human GGT has revealed a much more complex picture (Goodspeed *et al.*, 1989; Rajpert-De Meyts *et al.*, 1988; Sakamuro *et al.*, 1988). Four distinct genomic clones have been identified (Pawlak *et al.*, 1988). GGT-related sequences have been mapped to human chromosome 22 (Bulle *et al.*, 1987), to at least two different loci, one proximal and one distal to the breakpoint cluster region (BCR; Heisterkamp and Groffen, 1988). In addition, separate genomic clones

[1] To whom correspondence should be addressed at Department of Neurology, Livingston Hall, Room 7-220, Montreal General Hospital, 1650 Cedar Avenue, Montreal, P.Q. H3G 1A4, Canada. Telephone: (514) 937-6011, ext. 2524 or 4567.

0888-7543/93 $5.00

have been identified from relatively liver- or kidney-specific GGT cDNAs (Pawlak *et al.*, 1989). It is postulated that at least some of the GGT loci may represent pseudogenes and some of the variant GGT mRNAs that have been isolated may not result in the synthesis of a functional enzyme (Pawlak *et al.*, 1990).

In this paper, we more precisely localize three GGT sequences and the previously reported GGT *Pvu*II polymorphism (Rouleau *et al.*, 1988), on chromosome 22 using many defined breakpoints from somatic cell hybrids. In addition, we demonstrate the presence of the GGT sequence on three other human autosomes: 18, 19, and 20.[2]

MATERIALS AND METHODS

Hybrid cell lines. Chromosome 22 somatic cell hybrid EyeF3A6 was characterized by Van Keuren *et al.* (1987). Ajo-9, Alu-2, Cerch-S, Cerch-H, Raj 5, and Asv 3.2 were described by Delattre *et al.* (1991). NF13-2 was obtained from D. Ledbetter (Menon *et al.*, 1989) and subcloned to eliminate the intact copy of chromosome 22. Further information concerning chromosome 22 loci in this hybrid was provided by M. Budarf and B. Emmanuel (Penn., pers. comm., 1991). Cell lines A3EW and ICB were obtained from Geurts van Kessel (Geurts van Kessel *et al.*, 1985; Dumanski *et al.*, 1990). H83 was described by Aubry *et al.* (1991, submitted for publication).

Monochromosomal cell lines GM10478, GM10449, GM07299, GM06318B, GM10479, GM10868, GM10611, GM10790, GM10629, GM10114, GM10567 (CY18), GM10253 (314-1b), and GM08854 (WA17) were obtained from the Coriell Institute (Camden, NJ). Cell lines 342-A2, L17A14, and K015 were received from G. A. Rouleau (unpublished data), R. E. K. Fournier (Leach *et al.*, 1989), and L.-C. Tsui (Arfin *et al.*, 1983), respectively.

The NIGMS human/rodent somatic cell hybrid mapping panel (No. 1) was obtained from the Coriell Institute (Camden, NJ). The characterization of human chromosomal content of each cell hybrid is available from the Coriell Institute.

Probes. 14HS and 14HL, 1.3- and 1.6-kb (respectively) *Eco*RI fragments of a human kidney GGT cDNA clone, were previously described (Rouleau *et al.*, 1988).

Hybridizations. Total genomic DNA was prepared from human, mouse, and hamster cells as well as from EyeF3A6 and the monochromosomal hybrid cell lines. Southern blots were prepared from DNA digested with *Hin*dIII or *Pvu*II. Filters were hybridized at high stringency with probes labeled by the random priming method. These methods have been described previously (Rouleau *et al.*, 1987, 1989, 1990a,b).

Polymerase chain reaction. Primers for polymerase chain reaction (PCR) were chosen from the 3′ region of human kidney GGT sequence: A-T-C-G-C-G-T-C-C-A-C-C-T-T-C-A-T-C-G and C-C-C-A-A-A-G-T-C-C-T-C-T-T-C-C-T-C-A, 5′ to 3′. PCR was performed using standard concentrations of template DNA, dNTP (Pharmacia), and primers; final $MgCl_2$ concentration was 1.5 m*M*. Samples were first denatured at 97°C for 10 min, *Taq* polymerase (BioCan) was added, and 32 cycles of amplification were carried out at 94°C for 1.0 min, 55°C for 1.5 min, and 72°C for 1.5 min. PCR-amplified sequences were separated by electrophoresis on 7% acrylamide gels and visualized using ethidium bromide.

[2] The HGMW-approved symbols for the genes discussed in this article are as follows: GGT1, γ-glutamyltransferase 1 (probes 14HS-5, 14HS-8, 14HL-2, 14HL-6); GGT2, γ-glutamyltransferase 2 (probes 14HS-4, 14HL-2, 14HL-3); GGT3, γ-glutamyltransferase 3; GGTL1, γ-glutamyltransferase-like 1 on chromosome 18; GGTL2, γ-glutamyltransferase-like 2 on chromosome 19; and GGTL3, γ-glutamyltransferase-like 3, chromosome 20.

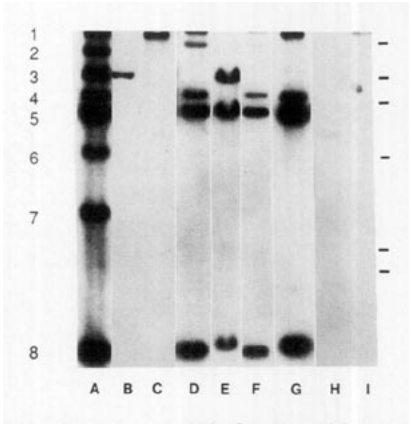

FIG. 1. Hybridization of 14HS. Southern blots probed with ^{32}P-labeled 14HS containing *Hin*dIII-digested DNA samples. Lanes (from left to right): **A**, human genomic DNA; **B**, mouse genomic DNA; **C**, hamster genomic DNA; **D**, EyeF3A6; **E**, NF13-2; **F**, Ajo-9; **G**, Asv 3.2; **H**, Alu-2; **I**, A3EW. Lambda molecular weight standards are indicated by the dashes to the right of lane I.

RESULTS

Mapping on Chromosome 22

14HL, a 1.6-kb *Eco*RI fragment from the 5′ end of a human kidney cDNA for GGT, and 14HS, a 1.3-kb fragment from the 3′ end of the same clone, were used to probe Southern blots containing *Hin*dIII-digested genomic DNA from human, mouse, and hamster, as well as from human/rodent somatic cell hybrids containing defined fragments of chromosome 22.

14HS displayed a single band in rodent DNA and eight bands from total human DNA (see Fig. 1 and Table 1). The sizes of fragments 1 through 8, respectively, are 25, 16, 10, 7.3, 6.3, 4.6, 3.0, and 1.0 kb. EyeF3A6, a somatic cell hybrid containing the entire human chromosome 22, displays three *Hin*dIII fragments (identified as bands 4, 5, and 8) seen also in total human DNA, in addition to the hamster band (band 1). (The second band displayed by EyeF3A6 does not correspond in size to any human band.) These three 14HS fragments can be assigned to two loci on chromosome 22. Band 4 maps to the region of chromosome 22 defined by the centromeric breakpoint of Raj 5 and the telomeric breakpoint of the centromeric fragment of Asv 3.2. This corresponds to a small region on 22q11.2–q12.1 (group 5 of Delattre *et al.*, 1991; see Fig. 2). The two other fragments, bands 5 and 8, were always present together and map to a region more centromeric on 22q defined by the centromeric breakpoint of Ajo-9 and the telomeric breakpoint of NF13-2. This region cannot be distinguished from group 2 of Delattre *et al.* (1991).

14HL displayed six *Hin*dIII fragments in total human DNA (Table 1). The sizes of fragments 1 through 6, respectively, are 19.5, 10.8, 7.3, 6.1, 4.2, and 2.0 kb. Again, only one single band is seen in rodent DNA. Four of the six 14HL fragments could be identified in the hybrid EyeF3A6. Their assignment to chromosome 22 is confirmed by an identical hybridization pattern in the Wegroth-D2 cell line, which contains a normal chromosome

TABLE 1

Mapping of 14HS and 14HL on Chromosome 22

Somatic cell hybrid	Bands	
	14HS	14HL
EyeF3A6	4, 5, and 8	2, 3, 5, and 6
NF13-2	5 and 8	2 and 6
Ajo-9	4, 5, and 8	2, 3, 5, and 6
Asv 3.2	4, 5, and 8	2, 3, 5, and 6
Alu-2	— — — — — — — — — — —	— — — — — — — — — — —
A3EW	— — — — — — — — — — —	— — — — — — — — — — —
Cerch-S	4	2 and 3
Cerch-H	5 and 8	2, 5, and 6
Raj 5	4	2 and 3
H83	4, 5, and 8	2, 3, 5, and 6

Note. Mapping of GGT cDNA clones 14HS and 14HL to chromosome 22 somatic cell hybrids. DNA from these hybrids as well as human, mouse, and hamster genomic DNA was digested with *Hin*dIII and used to prepare Southern blots. Total human DNA gave eight bands when hybridized with 14HS and six when hybridized with 14HL. Under the same conditions, hamster and mouse DNA displayed only one band each.

22 as the unique human chromosome (Kaplan and Emanuel, 1991). These four fragments can be assigned to three distinct loci on chromosome 22 (see Fig. 2).

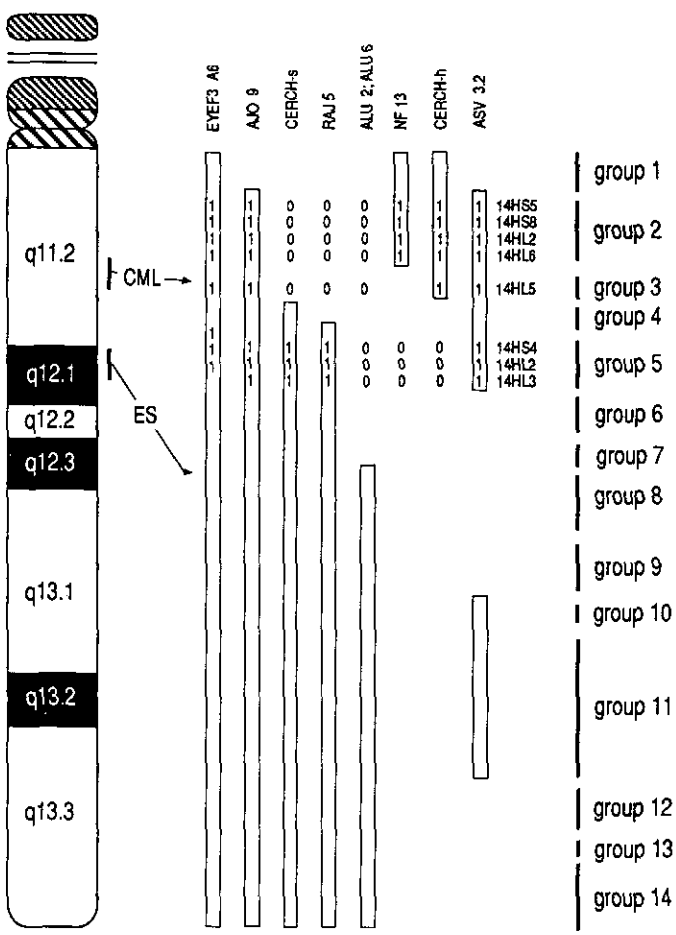

FIG. 2. Diagram of chromosome 22 indicating the mapping of human GGT fragments to three distinct loci, which correspond to groups 2, 3, and 5 of Delattre *et al.* (1991). The listing of 14HS and/or 14HL fragments in each region is not intended to indicate any ordering of the fragments in relation to each other. For more details see Delattre *et al.* (1991).

14HL band 6 colocalizes with 14HS bands 5 and 8 (group 2 of Delattre *et al.*, 1991). 14HL band 5 must lie between the telomeric breakpoint of NF13-2 and the telomeric breakpoint of Cerch-H (group 3 of Delattre *et al.*, 1991).

14HL band 2 gave a very strong hybridization signal with both of the mutually exclusive hybrids, Cerch-H and Cerch-S, demonstrating the presence of GGT fragments of this size both proximal and distal to the Cerch breakpoint. The dosage of hybridization signal using total human DNA or the hybrid EyeF3A6 (containing human chromosome 22) was consistent with multiple copies of 14HL fragment 2 compared to other 14HL fragments. One locus for 14HL fragment 2 must map to the same region as, and so probably be associated with, 14HL fragment 6. A second locus for fragment 2 is the region defined by the centromeric portion of Raj 5 (group 5 of Delattre *et al.*, 1991), where it is probably associated with 14HL fragment 3. Whether a third locus for 14HL fragment 2 exists, in association with 14HL fragment 5, cannot be determined from the hybridizations.

Finally, it is to be expected that one *Hin*dIII fragment could be recognized by both 14HS and 14HL. Based on molecular weight, fragment 3 (identified by 14HL) is identical to fragment 4 (identified by 14HS).

Figure 2 summarizes the results of mapping on chromosome 22.

Mapping on Other Human Chromosomes

Several of the human *Hin*dIII fragments displayed by 14HS and 14HL hybridization do not map to chromosome 22. Therefore, similar hybridization experiments were carried out using hybrid cell lines that are (largely) monochromosomal. The results of this experiment can be seen in Table 2. A very weak hybridization signal was obtained for 14HL with the chromosome 19-containing hybrid 10449. Using either 14HL or 14HS, hybrid 10478 (which contains chromosome 20) gave a very strong hybridization signal for the band corresponding to hamster

TABLE 2

Mapping of GGT Sequence on Other Human Chromosomes

Cell line	Composition	Bands		180 bp	245 bp
		14HS	14HL		
10449	19		2 and 4	+	
10478	20 (80%); 4 (8%)	5 and 8	2, 3, and 6	+	
EyeF3A6	22	4, 5, and 8	2, 3, 5, and 6	+	
Total human	Genomic	1–8	1–6	+	+

Note. DNA from (largely) monochromosomal cell lines was digested with *Hin*dIII and used to prepare Southern blots. Blots were hybridized with GGT probe 14HL or 14HS. Bands listed above correspond to one or more of the bands obtained by hybridization to similarly treated human genomic DNA. Alternatively, DNA from hybrid cell lines served as template for PCR amplification of GGT-related sequences (180-bp and/or 245-bp fragments). The positive results are listed. Neither hybridization signal nor PCR product was obtained from the majority of cell lines tested. These cell lines—7299, 314-16b, 10114, 10629, 10790, 10611, 342-A2, 10868, 10479, CY18, L17A14, WAV17, and 6318B—contain human chromosomes 1 and X, 3, 5, 6, 7, 9, 10, 12, 14, 16, 17, 21, and X, respectively.

DNA and a much weaker signal for bands corresponding to human GGT fragments already localized to chromosome 22 (14HS bands 5 and 8; 14HL bands 2, 3, and 6). It could be hypothesized that this hybrid line includes a small proportion of chromosome 22-containing cells. However, this is unlikely because only some, not all, of the chromosome 22 fragments are displayed using this hybrid. Moreover, dosage of hybridization signal for 14HS bands 5 and 8 compared to those of other bands is consistent with the existence of multiple copies of this locus in the genome.

As some of the hybridization results were difficult to interpret, the same monochromosomal cell lines were screened for GGT-specific sequence using PCR. Primers were chosen from the 3′ region of the human kidney GGT sequence. Based on this sequence, the predicted PCR product size was 180 bp. Using these primers, two products are synthesized from human genomic DNA—one as expected at 180 bp and another at approximately 245 bp. EyeF3A6, the chromosome 22 somatic cell hybrid, contains only the 180-bp sequence. Two of the hybrid lines (10478 and 10449) that gave some hybridization signal also contained the 180-bp GGT-related sequence (see Table 2).

Several chromosomes (2, 4, 11, 13, 15, and 18) could not be tested for GGT-related sequence by these approaches, as monochromosomal lines were not available. Moreover, several of the human genomic *Hin*dIII 14HS or 14HL fragments had not been localized to the chromosomes that had been tested. The NIGMS human/rodent somatic cell hybrid panel, which allows for chromosomal mapping, was also screened for GGT-specific sequences using the PCR primers described above. Mapping of the 180-bp fragment (predicted from the gene sequence) and the larger GGT-related 245-bp fragment is shown in Table 3. Cell lines from the mouse or hamster background cells did not yield PCR products of either 180 or 245 bp. In some instances, a 180-bp fragment was synthesized in very high yield (see Fig. 3). Such a product (marked ++) represents GGT-like sequence that could be mapped to chromosome 22 (Table 3). A ++180-bp fragment was synthesized in 7 of 7 somatic cell hybrid lines containing chromosome 22 and in 0 of 9 somatic cell hybrid lines that did not contain chromosome 22, giving 100% concordance and 0% discordance, respectively. Hybridization of a Southern blot containing the NIGMS somatic cell hybrid mapping panel confirmed these results. In other instances, a 180-

TABLE 3

Mapping of PCR-Generated GGT Sequences Using NIGMS Human/Rodent Somatic Cell Hybrid Mapping Panel

Hybrid DNA	++180-bp product	+180-bp product	245-bp product
NA09925 a, b, c	++		+
NA09927 a, b		+	+
NA09928 c	++		—
NA09929 b		+	—
NA09930A a, b, c	++		+
NA09931 b		+	—
NA09932	—	—	—
NA09933 a, b, c	++		+
NA09934 a, b		+	+
NA09935A a, c	++		+
NA09936 b, c	++		—
NA09937 a	—	—	+
NA09938 b, c	++		—
NA10324	—	—	—
NA10567	—	—	—
NA10611	—	—	—

Note. The NIGMS somatic cell mapping panel was screened for GGT sequence by PCR. Identity of individual hybrids is listed in column 1. Cell hybrids containing human chromosome 18 are labeled "a." Those containing human chromosome 20 are labeled "b." Those containing human chromosome 22 are labeled "c." The predicted PCR product of 180 bp was obtained in high yield (++) (column 2), low yield (+) (column 3), or no yield (—) (columns 2 and 3). A ++ 180-bp PCR product was obtained in all cell lines containing human chromosome 22. A +180-bp PCR product was obtained in all (nonchromosome 22 containing) cell lines containing human chromosome 20. A 180-bp PCR product was never obtained in a cell line that contained neither human chromosome 20 nor 22. Likewise, amplification of a 245-bp GGT-related fragment is shown in column 4. A 245-BP PCR product was obtained in all cell lines containing human chromosome 18 and never obtained in a cell line that did not contain chromosome 18.

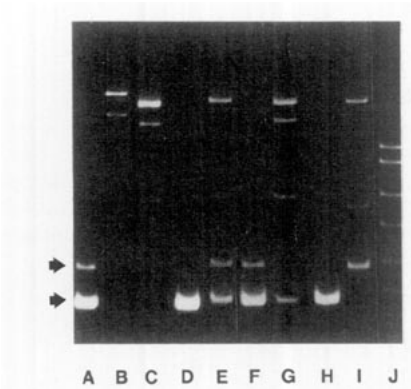

FIG. 3. Amplified GGT sequences. GGT-related sequences were amplified by PCR using the following sources of template DNA. Lanes (from left to right): **A**, human genomic DNA; **B**, hamster genomic DNA; **C**, mouse genomic DNA; **D**, EyeF3A6; **E**, GM09927 (+180 bp, 245 bp); **F**, GM09935 (++180 bp, 245 bp); **G**, GM09931 (+180 bp only); **H**, GM09928 (++180 bp only); **I**, GM09937 (245 bp only); **J**, MW standards—*Msp*-digested Puc 19. Arrows at the left indicate the 245-bp (upper arrow) and the 180-bp (lower arrow) products of PCR amplification.

bp fragment was synthesized, but in considerably lower quantities. This GGT-like sequence (marked +) maps to chromosome 20 (Table 3). A +180-bp product was obtained in 4 of 4 nonchromosome-22-containing somatic cell hybrid lines that did contain chromosome 20 and in 0 of 5 somatic cell hybrid lines that did not contain chromosome 20. Repeated testing of both low- and high-yield cell lines gave consistent results. The 245-bp GGT-like sequence maps to chromosome 18 (Table 3). This product was obtained in 7 of 7 somatic cell hybrid lines containing chromosome 18 and in 0 of 9 somatic cell hybrid lines not containing chromosome 18.

Mapping of 14HS PvuII Polymorphism

A previously identified *Pvu*II polymorphism displayed by 14HS, with fragment sizes of 3.5 and 3.8 kb, was mapped using the chromosome 22 somatic cell hybrids (see Fig. 4). In addition to the results shown in Fig. 4, the polymorphic fragment could be identified in the hybrids H83, ICB, and Cerch-H but not in hybrid A3EW or Cerch-S. The polymorphic *Pvu*II fragment maps between the centromeric breakpoint of Ajo-9 and the telomeric breakpoint of NF13-2. This is the same locus as that of the 14HS *Hin*dIII fragments, bands 5 and 8.

DISCUSSION

GGT was previously mapped to human chromosome 22 (q11.1/q11.2 interface) by *in situ* hybridization using a rat kidney cDNA clone (Bulle *et al.*, 1987). However, subsequent cloning of human GGT demonstrated at least four different genomic sequences, each with intron/exon structure (Pawlak *et al.*, 1988). It is clear that in contrast to rodents, which have a single GGT gene, humans have a complex gene/pseudogene family of GGT sequences. Heisterkamp identified human GGT sequence in two separate loci while searching for copies of chromosome 22 breakpoint cluster region (BCR) gene sequences (Heisterkamp and Groffen, 1988). While BCR and GGT cDNAs have no sequence in common, this surprising finding allowed the mapping of GGT-related sequences to two different regions of chromosome 22, proximal and distal to the BCR gene itself. We have used somatic cell hybrids to map chromosome 22 GGT sequences more precisely and to identify nonchromosome 22 sequences.

Hybridization experiments were performed using 14HL and 14HS representing the 5′ and 3′ fragments of a human kidney GGT cDNA. 14HL should contain the coding sequence of the large GGT subunit, and 14HS of the small subunit. The results obtained are consistent with previous reports of a single rodent gene and multiple gene copies in humans. Southern analysis of the chromosome 22-containing somatic cell hybrid (EyeF3A6) allowed us to assign 3 of 8 human *Hin*dIII fragments that hybridize with 14HS and 4 of 6 *Hin*dIII fragments that are displayed by 14HL to three distinct loci on chromosome 22. Figure 2 depicts the three separate GGT loci on chromosome 22, two of which contain both 5′ and 3′ sequences, and one which contains only the 5′ sequence.

Five *Hin*dIII fragments displayed by 14HS and two additional fragments displayed by 14HL do not map to chromosome 22. Monochromosomal somatic cell hybrids representing 16 other autosomes, as well as the X chromosome, were used to identify GGT-related sequence on chromosomes 19 and 20 (displayed both by hybridization and by PCR). In addition to the predicted 180-bp product, PCR using human genomic DNA as template also amplified an unexpected 245-bp fragment that maps to none of the chromosomes contained in the monochromosomal hybrids. PCR amplification was then carried out using the NIGMS human/rodent somatic cell hybrid mapping panel. Because at least two 3′ GGT loci map to chromosome 22 (Heisterkamp and Groffen, 1988; this paper), one might predict, and in fact

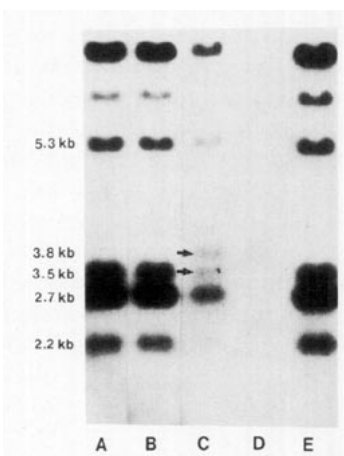

FIG. 4. 14HS *Pvu*II polymorphism. Southern blots probed with ^{32}P-labeled 14HS, containing *Pvu*II-digested DNA samples. Lanes (from left to right): **A**, human genomic DNA; **B**, EyeF3A6; **C**, NF13-2; **D**, Alu-2; **E**, Ajo-9. Arrows indicate the polymorphic fragment. Fragment sizes are indicated to the left.

we see, that PCR amplification yields noticeably more 180-bp product in chromosome 22-containing hybrids. Based on clearly quantitative yields (++ or +), GGT-like sequence could be mapped to chromosome 20 (+) and to chromosome 22 (++). Unfortunately, due to the composition of the various hybrids of the mapping panel (no cell lines containing chromosome 19 without also containing either chromosome 20 or 22), it was impossible to demonstrate a GGT-like sequence specific to chromosome 19, as was suggested by the monochromosomal data. The GGT-related 245-bp PCR product was mapped to chromosome 18, using the somatic cell mapping panel. We also determined that a previously reported *PvuII GGT polymorphism* (Rouleau *et al.*, 1988) maps to the locus containing 14HS fragments 5 and 8 and 14HL fragments 2 and 6.

What is the role of these GGT-related loci in the human genome? Pawlak *et al.* (1988) have proposed that some of these copies of GGT sequence may represent pseudogenes. If we assume that a functional gene must contain both 14HS and 14HL hybridizing fragments, the coding gene could be in either of two regions of chromosome 22 or on chromosome 20. However, definitive localization of the coding sequence awaits better characterization and mapping.

The discovery of a GGT multigene family in man, in contrast to a single locus in the rat, has led to the speculation that control of GGT activity in man may be related to the activation of different GGT genes, as opposed to the recognition of different promoters (Chobert *et al.*, 1990) and alternate splicing that is postulated for the rat GGT gene (Griffiths and Manson, 1989). However, recent evidence indicates that measurable changes in GGT activity are not always directly related to changes in GGT expression (Goodspeed *et al.*, 1989). Thus control of GGT activity at the post-translational stage must also be kept in mind. The identification of GGT-related sequences in cDNA fragments containing BCR-like sequence (Heisterkamp and Groffen, 1988) suggests an alternate explanation for the multiple copies of GGT-like sequence in the human genome. That is, its close proximity to a sequence of DNA that is clearly involved in multiple recombination and translocation events in the human genome may have resulted in the coincidental translocation of adjacent GGT-related sequence. Recently, the isolation of the first active human transposable element was reported (Dombroski *et al.*, 1991a,b). This locus, an L1 element, was mapped to chromosome 22q11.1–q11.2. We will now be able to examine the possible relationship between this transposable element and the chromosome 22 GGT sequences in GGT-containing cosmids.

ACKNOWLEDGMENTS

The authors thank Edward Cohen for use of the probes 14HL and 14HS. G.A.R. is supported by the Medical Research Council of Canada and the Fonds de Recherche en Sante du Quebec.

REFERENCES

Arfin, S. M., Cirullo, R. E., Arredondo-Vega, F. X., and Smith, M. (1983). Assignment of structural gene for asparagine synthetase to human chromosome 7. *Somatic Cell Genet.* **9:** 517–531.

Barouki, R., Chobert, M. N., Billon, M. C., Finidori, J., Tsapis, R., and Hanoune, J. (1982). Glucocorticoid hormones increase the activity of gamma-glutamyl-transferase in a highly differentiated hepatoma cell line. *Biochim. Biophys. Acta* **721:** 11–21.

Barouki, R., Chobert, M. N., Finidori, J., Aggerbeck, M., Nalpas, B., and Hanoune, J. (1983). Ethanol effects in a rat hepatoma cell line: Induction of gamma-glutamyl transferase. *Hepatology* **3:** 323–329.

Barouki, R., Finidori, J., Chobert, M.-N., Aggerbeck, M., Laperche, Y., and Hanoune, J. (1984). Biosynthesis and processing of gamma-glutamyl transpeptidase in hepatoma tissue culture cells. *J. Biol. Chem.* **259:** 7970–7974.

Bulle, F., Mattei, M. G., Siegrist, S., Pawlak, A., Passage, E., Chobert, M. N., Laperche, Y., and Guellaen, G. (1987). Assignment of the human gamma-glutamyl transferase gene to the long arm of chromosome 22. *Hum. Genet.* **76:** 283–286.

Bulle, F., Hillion, J., Grausz, D., Chikhi, N., Berger, R., Guellaen, G., and Bernheim, A. (1990). Co-amplification of γ-glutamyl transferase specific sequences with bcr and Igl genes in the K562 cell lines. Abstr. 1st Int. Workshop on the Mapping of Hum. Chrom. 22, Paris.

Chobert, M. N., Lahuna, O., Lebargy, F., Kurauchi, O., Darbouy, M., Bernaudin, J. F., Guellaen, G., Barouki, R., and Laperche, Y. (1990). Tissue-specific expression of two γ-glutamyl transpeptidase mRNAs with alternative 5′ ends encoded by a single copy gene in the rat. *J. Biol. Chem.* **265:** 2352–2357.

Coloma, J., and Pitot, H. C. (1986). Characterization and sequence of a cDNA clone of gamma-glutamyl transpeptidase. *Nucleic Acids Res.* **14:** 1393–1403.

Curthoys, N. P. (1983). Role of gamma-glutamyltranspeptidase in the renal metabolism of glutathione. *Min. Electrolyte Metab.* **9:** 236–245.

Delattre, O., Azambuja, C. J., Aurias, A., Zucman, J., Peter, M., Zhang, F., Hors-Cayla, M. C., Rouleau, G., and Thomas, G. (1991). Mapping of human chromosome 22 with a panel of somatic cell hybrids. *Genomics* **9:** 721–727.

Dombroski, B. A., Mathias, S. L., Nanthakumar, E. J., Scott, A. F., and Kazazian, H. H. (1991a). Isolation of the L1 gene responsible for a retrotransposition event in man. *Am. J. Hum. Genet.* **49S:** 403.

Dombroski, B. A., Mathias, S. L., Nanthakumar, E., Scott, A. F., and Kazazian, H. H. (1991b). Isolation of an active human transposable element. *Science* **254:** 1805–1808.

Dumanski, J. P., Geurts van Kessel, A. H. M., Ruttledge, M., Wladis, A., Sugawa, N., Collins, V. P., and Nordenskjold, M. (1990). Isolation of anonymous, polymorphic DNA fragments from human chromosome 22q12–qter. *Hum. Genet.* **84:** 219–222.

Finidori, J., Laperche, Y., Haguenauer-Tsapis, R., Barouki, R., Guellaen, G., and Hanoune, J. (1984). In vitro biosynthesis and membrane insertion of gamma-glutamyl transpeptidase. *J. Biol. Chem.* **259:** 4687–4690.

Geurts van Kessel, A. H. M., Turc-Carel, C., Klein, A., Grosveld, G., Lenoir, G., and Bootsma, D. (1985). Translocation of oncogene c-sis from chromosome 22 to chromosome 11 in an Ewing sarcoma-derived cell line. *Mol. Cell. Biol.* **5:** 427–429.

Ghandour, M. S., Langley, O. K., and Varga, V. (1980). Immunohistological localization of gamma-glutamyl transpeptidase in cerebellum at light and electron microscope levels. *Neurosci. Lett.* **20:** 125–129.

Goodspeed, D. C., Dunn, T. J., Miller, C. D., and Pitot, H. C. (1989). Human γ-glutamyl transpeptidase cDNA: Comparison of hepatoma and kidney mRNA in the human and rat. *Gene* **76:** 1–9.

Griffiths, S. A., and Manson, M. M. (1989). Rat liver gamma glutamyl

transpeptidase mRNA differs in the 5′ untranslated sequence from the corresponding kidney mRNA. *Cancer Lett.* **46:** 69–74.

Hanigan, M. H., and Pitot, H. C. (1985). Gamma-glutamyl transpeptidase—its role in hepatocarcinogenesis. *Carcinogenesis* **6:** 165–172.

Heisterkamp, N., and Groffen, J. (1988). Duplication of the bcr and gamma-glutamyl transpeptidase genes. *Nucleic Acids Res.* **16:** 8045–8056.

Johnson, G. D., Nogueira, C. D., and Aranjo, J. G. M. (1981). A simple method for reducing the fading of immunofluorescence during microscopy. *J. Immunol. Methods* **43:** 349–350.

Kaplan, J. C., and Emanuel, B. (1991). Chromosome 22 workshop proceedings. *Genomics* **9:** 214–218.

Laperche, Y., Bulle, F., Aissani, T., Chobert, M. N., Aggerbeck, M., Hanoune, J., and Guellaen, G. (1986). Molecular cloning and nucleotide sequence of rat kidney γ-glutamyl transpeptidase cDNA. *Proc. Natl. Acad. Sci. USA* **83:** 937–941.

Leach, R. J., Thayer, M. J., Schafer, A. J., and Fournier, R. E. K. (1989). Physical mapping of human chromosome 17 using fragment-containing microcell hybrids. *Genomics* **5:** 167–176.

Lebargy, F., Bulle, F., Siegrist, S., Guellaen, G., and Bernaudin, J. F. (1990). Localization by in situ hybridization of γ-glutamyl transpeptidase mRNA in the rat kidney using 35S-labeled RNA probes. *Lab Invest.* **62:** 731–735.

Meister, A., and Anderson, M. E. (1983). Glutathione. *Annu. Rev. Biochem.* **52:** 711–760.

Menon, A. G., Ledbetter, D., Rich, D. C., Seizinger, B. R., Rouleau, G. A., Michels, V. F., Schmidt, M. A., Dewald, G., Dellatorre, C. M., Haines, J. L., and Gusella, J. F. (1989). Characterization of a translocation within the vonRecklinghausen neurofibromatosis region of chromosome 17. *Genomics* **5:** 245–249.

Nakamura, Y., Azuma, T., Fukuyama, H., Suzuki, F., and Nagata, Y. (1985). Active site of intestinal gamma-glutamyltransferase faces luminal side of brush border membrane. *J. Nutr. Sci. Vitaminol.* **31:** 179–187.

Nash, B., and Tate, S. S. (1984). In vitro translation and processing of rat γ-glutamyl transpeptidase. *J. Biol. Chem.* **259:** 678–685.

Ohmori, T., Hiasa, Y., Murata, Y., and Williams, G. M. (1982). Gamma glutamyl transpeptidase activity in carcinogen-induced epithelial lesions of rat kidney. *Jpn. J. Cancer Res.* **73:** 543–548.

Papandrikopoulou, A., Frey, A., and Gassen, H. G. (1989). Cloning and expression of γ-glutamyl transpeptidase from isolated porcine brain capillaries. *Eur. J. Biochem.* **183:** 693–698.

Pawlak, A., Lahuna, O., Bulle, F., Suzuki, A., Ferry, N., Siegrist, S., Chikhi, N., Chobert, M. N., Guellaen, G., and Laperche, Y. (1988). γ-Glutamyl transpeptidase: A single copy gene in the rat and a multigene family in the human genome. *J. Biol. Chem.* **263:** 9913–9916.

Pawlak, A., Wu, S. J., Bulle, F., Suzuki, A., Chikhi, N., Ferry, N., Baik, J. H., Siegrist, S., and Guellaen, G. (1989). Different gamma-glutamyl transpeptidase mRNAs are expressed in human liver and kidney. *Biochem. Biophys. Res. Commun.* **164:** 912–918.

Pawlak, A., Cohen, E. H., Octave, J. N., Schweickhardt, R., Wu, S. J., Bulle, F., Chikhi, N., Baik, J. H., Siegrist, S., and Guellaen, G. (1990). An alternatively processed mRNA specific for gamma-glutamyl transpeptidase in human tissues. *J. Biol. Chem.* **265:** 3256–3262.

Rajpert-De Meyts, E., Heisterkamp, N., and Groffen, J. (1988). Cloning and nucleotide sequence of human γ-glutamyl transpeptidase. *Proc. Natl. Acad. Sci. USA* **85:** 8840–8844.

Rouleau, G. A., Wertelecki, W., Haines, J. L., Hobbs, W. J., Trofatter, J. A., Seizinger, B. R., Martuza, R. L., Superneau, D. W., Conneally, P. M., and Gusella, J. F. (1987). Genetic linkage of bilateral acoustic neurofibromatosis to a DNA marker on chromosome 22. *Nature* **329:** 246–248.

Rouleau, G. A., Bazanowski, A., Cohen, E. H., Guellaen, G., and Gusella, J. F. (1988). Gamma-glutamyl transferase locus (ggt) displays a PvuII polymorphism. *Nucleic Acids Res.* **16:** 1848.

Rouleau, G. A., Haines, J. L., Bazanowski, A., Colella-Crowley, A., Trofatter, J. A., Wexler, N. S., Conneally, P. M., and Gusella, J. F. (1989). A genetic linkage map of the long arm of human chromosome 22. *Genomics* **4:** 1–6.

Rouleau, G. A., Bazanowski, A., Gusella, J. F., and Haines, J. L. (1990a). A genetic map of chromosome 1: Comparison of different data sets and linkage programs. *Genomics* **7:** 313–318.

Rouleau, G. A., Seizinger, B. R., Wertelecki, W., Haines, J. L., Superneau, D. W., Martuza, R. L., and Gusella, J. F. (1990b). Flanking markers bracket the neurofibromatosis type 2 (NF-2) gene. *Am. J. Hum. Genet.* **46:** 323–328.

Sakamuro, D., Yamazoe, M., Matsuda, Y., Kangawa, K., Taniguchi, N., Matsuo, H., Yoshikawa, H., and Ogasawara, N. (1988). The primary structure of human gamma-glutamyl transpeptidase. *Gene* **73:** 1–9.

Sawabu, N., Nakagen, M., Ozaki, K., Wakabayashi, T., Toya, D., Hattori, N., and Ishii, M. (1983). Clinical evaluation of specific gamma-GTP isoenzyme in patients with hepatocellular carcinoma. *Cancer* **51:** 327–331.

Shine, H. D., and Haber, B. (1981). Immunocytochemical localization of gamma-glutamyl transpeptidase in the rat CNS. *Brain Res.* **217:** 339–349.

Stastny, F., and Lisy, V. (1981). Cortisol regulations of gamma-glutamyl transpeptidase in liver, choroid plexus, blood plasma and cerebrospinal fluid of developing chick embryo. *Dev. Neurosci.* **4:** 408–425.

Tate, S. S., and Meister, A. (1981). γ-Glutamyl transpeptidase: Catalytic, structural and functional aspects. *Mol. Cell. Biochem.* **39:** 357–368.

Tate, S. S., Khadse, V., and Wellner, D. (1988). Renal gamma-glutamyl transpeptidases: Structural and immunological studies. *Arch. Biochem. Biophys.* **262:** 397–408.

Tsuda, H., Moore, M. A., Asamoto, M., Satoh, K., Tsuchida, S., Sato, K., Ichihara, A., and Ito, N. (1985). Comparison of the various forms of glutathione-S-transferase with glucose-6-phosphate dehydrogenase and gamma-glutamyl transpeptidase as markers of preneoplastic and neoplastic lesions in rat kidney induced by N-ethyl-N-hydroxyethyl nitrosamine. *Jpn. J. Cancer Res.* **76:** 919–929.

Van Keuren, M. L., Hart, I. M., Kao, F. T., Neve, R. L., Bruns, G. A. P., Kurnit, D. M., and Patterson, D. (1987). A somatic cell hybrid with a single human chromosome 22 corrects the defect in the CHO mutant (Ade-I) lacking adenylosuccinate activity. *Cytogenet. Cell Genet.* **44:** 142–147.

Yunis, J. J., Sawyer, J. R., and Ball, D. W. (1978). Characterization of banding patterns of metaphase G-banded chromosomes and their use in gene mapping. *Cytogenet. Cell Genet.* **22:** 679–683.