Expression of multiple γ-glutamyltransferase genes in man

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In clinical and pharmacological laboratories, the assay for γ-glutamyltransferase (GGT) activity is an important diagnostic test, but one with high biological variability. Although the human genome contains multiple GGT genomic sequences, the diagnostic tests generally assume that only a single GGT gene is active. In the current study, segments encompassing parts of seven different potential human GGT genes have been molecularly cloned. Based on sequence determination of exons within these distinct genomic clones, oligonucleotide primers were designed which would prime and PCR-amplify putative mRNA of all seven potential GGT genes, if expressed. Gene-specific oligonucleotide probes were then utilized to assay the transcriptional status of the seven possible GGT genes in a wide variety of human RNAs. Our results show that a single GGT gene exhibits ubiquitous expression in all RNAs tested, including those from fetal and adult liver. A surprisingly large number of four additional GGT genes is expressed in man. Interestingly, these novel GGT genes are expressed in a tissue-restricted manner, which suggests that their corresponding gene products exhibit distinct functions in these specific tissues.

INTRODUCTION

γ-Glutamyltransferase [GGT; (5-glutamyl)-peptide: amino acid 5-glutamyltransferase, EC 2.3.2.2.] is an important enzyme participating in the metabolism of glutathione (Meister, 1988). GGT is located on the external surface of cells and is predominantly active in epithelial cells which have secretory and absorptive functions (Tate and Meister, 1982; Curthoys, 1983). The heavy, membrane-associated subunit and the light subunit, which contains the catalytic site of the molecule, are encoded on a single mRNA (Hughey et al., 1979; Tsuji et al., 1980; Tate and Khadse, 1986).

GGT activity is high in adult kidney but low in adult liver. In fetal and newborn rats, however, liver activity is high and kidney activity relatively low (Meister, 1988). The liver enzyme can be induced in adult animals and in man by glucocorticoids (Billon et al., 1980), and by carcinogens, drugs and alcohol (Fiala et al., 1972; Rosalki and Rau, 1972; Edwards and Lucas, 1985; Hanigan and Pitot, 1985; Power et al., 1987). The mammary gland has an increased level of GGT during lactation (Puente et al., 1979). GGT is also elevated early in the development of some types of neoplasia in human and in experimental chemically induced tumours (Solt, 1982; Peraino et al., 1983; Taniguchi et al., 1985; Dempo et al., 1988). In human plasma, GGT has high intra- and inter-individual variability. GGT levels are increased in plasma after alcohol consumption and in liver cancer.

Differential expression of GGT is found in a variety of different tissues, in different physiological states, during development and it is also inducible. The question arises, whether different GGTs detected by enzymic assays are products of a single gene or are encoded by multiple separate genes: four or more distinct GGT genomic clones have been isolated previously from human (Heisterkamp and Groffen, 1988; Pawlak et al., 1988). However, it is at present unknown how many of these possible human GGT genes are transcribed into mRNA. The coding sequences of human cDNAs from placenta, fetal liver, pancreas and HepG2 cells are identical, indicating they are probably transcripts from a single gene (Rajpert-DeMeyts et al., 1988; Sakamuro et al., 1988; Goodspeed et al., 1989; Courtay et al., 1992). However, a kidney cDNA, clearly distinct from other cDNAs isolated to date, had a 30 bp deletion and 22 point mutations in the coding region (Pawlak et al., 1989). This suggested that perhaps one additional active GGT gene existed. In contrast, only one GGT gene has been demonstrated to exist in rat. At least four types of mRNAs are transcribed from this single gene. These mRNAs differ in their 5′ untranslated sequences while the coding sequences are the same (Darbouy et al., 1991).

In the current study, we have addressed the question of multiple GGT genes by constructing a primary human genomic library to clone segments of as many different potential GGT genes as is feasible. We have isolated seven distinct types of GGT genomic clones, and have examined whether these are expressed in a large number of human RNAs isolated from different tissues. Surprisingly, our results indicate that of the seven potential GGT genes, at least five are expressed, with some exhibiting a restricted, tissue-specific expression.

EXPERIMENTAL

Isolation of human GGT genomic clones

GM 7387A (obtained from the National Institute of General Medical Sciences Human Genetic Mutant cell repository, Camden, NJ, U.S.A.) is a lymphoblast cell line derived from the mother of a proband with cystic fibrosis. GM 7387A high-molecular-mass DNA partially digested with *Mbo*I was size-selected in the 20–10 kb range on a low-melting-point agarose gel. DNA was ligated into EMBL3 digested with *Bam*HI, packaged *in vitro*, and the resulting library of 530000 recombinants was screened with a 0.4 kb *Bgl*II/*Pst*I GGT placental cDNA probe (nucleotides 1858–2240, Rajpert-DeMeyts et al., 1988). After post-hybridization stringency washing in 0.03 × SSC (1 × SSC; 0.15 M NaCl/0.015 M sodium citrate) at 65 °C, 12

Abbreviations used: GGT, γ-glutamyltransferase; RT, reverse transcriptase; SSC, 0.15 M NaCl/0.015 M sodium citrate.

The nucleotide sequence data reported will appear in the EMBL, Genbank and DDBJ Nucleotide sequence Databases under the following accession numbers: GGT1, L10394; GGT2, L10395; GGT3, L10396; GGT11, L10397; GGT12, L10398; GGT13, L10399.

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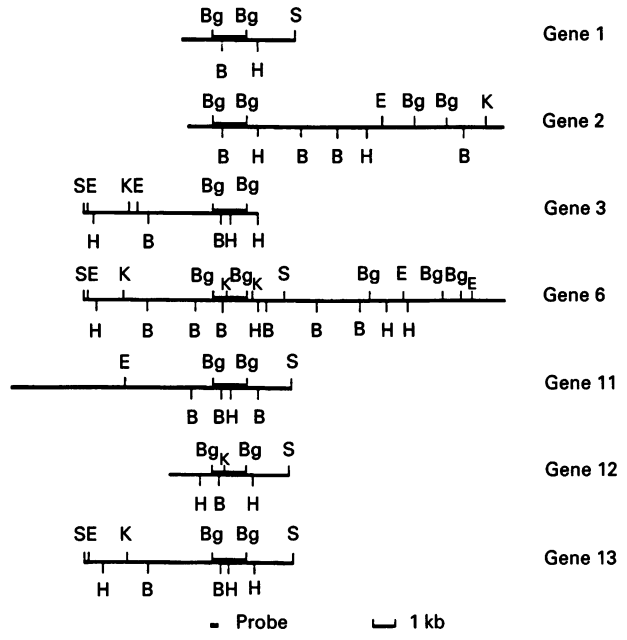

Figure 1 Restriction-enzyme maps of segments of seven different GGT genomic clones.

Restriction enzymes used include B, *Bam*HI; Bg, *Bgl*I; E, *Eco*RI; H, *Hind*III; K, *Kpn*I; S, *Sal*I. The location of the 1.5 kb *Bgl*I fragments containing 3′ exons is indicated by thick solid lines in the maps. The location of the 0.3 kb *Bgl*I/*Bam*HI probe is shown below the map of gene 13.

positive clones were obtained, which were mapped with restriction enzymes. Genes 1, 2 and 12 were isolated as single positive clones, genes 3, 11 and 13 each in two overlapping phage clones, and gene 6 in three overlapping phage clones. *Sal*I restriction-enzyme fragments hybridizing to the GGT cDNA probe were subcloned from the phage DNA; in many cases insert DNA contained one internal *Sal*I site, resulting in the generation of two *Sal*I fragments. The non-hybridizing fragment represented sequences 3′ to the GGT gene (not shown). We have previously also isolated different cosmids which overlap with genes 1, 3, 6 and 13.

Sequencing

The 1.5 kb *Bgl*II fragments, which contained the most 3′ five exons of the genes, were subcloned into pSK (Stratagene) and the sequence was obtained from both ends of the insert to determine which GGT exons were included within the 1.5 kb *Bgl*II fragment using Stratagene primers (KS and T3). Additional oligonucleotide primers (derived from the GGT cDNA sequence; Rajpert-DeMeyts et al., 1988 and from the sequence data obtained from the ends of the 1.5 kb *Bgl*II inserts) were prepared in order to sequence both strands of all clones of the region (shown in Figure 2) encompassing a *Bam*HI and *Kpn*I site (gene 6; see Figure 2). This region was chosen because it was likely to contain multiple nucleotide differences between the different clones: some GGT genes contained a *Hin*dIII and/or a *Kpn*I site, while others lacked one or both restriction sites (Figure 1). Sequence primers included: sequence primer 1 (nucleotides 2209–2225, 5′-ATGTTTCTCTCCACTGT-3′; this primer is located 3′ of Amp2 in Figure 2), sequence primer 2 (Figure 2, nucleotides 1989–2005, 5′-CTGGCTGGATGAAATTG-3′), sequence primer 3 (Figure 2, nucleotides 2051–2071, 5′-CCATCCGG-ACCTGGCCGTCCT-3′), Ampl (see section on reverse transcriptase and PCR) and probe gene 11+13 (see section on hybridization and washing). Sequencing was performed according to a double-stranded DNA sequencing protocol using Sequenase II (United States Biochemical, Cleveland, OH, U.S.A.).

Reverse transcriptase (RT) and PCR

As controls for the specificity of the oligonucleotide hybridizations, all genomic GGT clones (1 μg of DNA) were amplified separately and approx. 200 ng of the reaction products were co-electrophoresed on the same gel together with the RT-PCR products of RNA. RNAs were isolated using guanidine isothiocyanate as described (Chomczynski and Sacchi, 1987). RNAs include those isolated from human thyroid, liver, uterus, gall bladder, lung, muscle, colon, sigmoid, small intestine, normal-term placenta, normal lymphocytes, lymphocytes from a diabetic patient, fetal brain, fetal liver, fetal pancreas, fetal kidney and from the KYN2 hepatoma cell line. The lung tissue was from a lung carcinoma; other adult, apparently healthy, tissues were from surgical procedures. Fetal brain was from an 18-week-old male, spontaneously aborted (due to infection), foetus. Fetal pancreas, kidney and liver were from an 11-week-old foetus with cystic fibrosis. RT reactions were performed essentially as described for total RNA (Heisterkamp et al., 1991) using the sequence 5′-AGCCGAACCAGAGGTTGTAGA-3′ (Amp2). PCR primers included the aforementioned oligonucleotide as well as a 5′ amplimer: 5′-AATGGACGACTTCAGCTCTCC-3′ (Ampl).

Hybridization and washing

Part (5–20%; approx. 200 ng of DNA) of each PCR reaction mixture was electrophoresed on a 1.2% (w/v) agarose gel and blotted on to Magna NT (Micron Separations, Westboro, MA, U.S.A.) as described. Blots were prehybridized for 2 h at 65 °C in 3 × SSC/10 × Denharts/50 μg/ml salmon sperm DNA/0.1% SDS and for 2 h at 65 °C in 5 × SSC/10 × Denharts/50 μg/ml salmon sperm DNA/10% (w/v) dextran sulphate/7% (w/v) SDS/50 mM sodium pyrophosphate (oligonucleotide hybridization mix); hybridization was overnight at 53 °C in oligonucleotide hybridization mix with oligonucleotide probes which were end-labelled with [γ-^{32}P]dATP.

Some of the oligonucleotide probes used in initial experiments were designed to hybridize to two GGT genes. If both genes were found to be expressed, an additional oligonucleotide probe was designed to discriminate between the expression of the two genes. Oligonucleotide probes used to distinguish the different genes include: gene 1+2, 5′-CCAACGAGTTTGGGGTGCCC-3′; gene 6, 5′-CCAACGAGTTTGGGGTACCC-3′; gene 12, 5′-CCAATGAGTTTGGGGTACCC-3′; gene 12+13, 5′-GCATC-ACCAATGAGTTTGGG-3′ (these probes all correspond to probe region A in Figure 2); gene 1, 5′-CTCTCGTCAATGT-GCCCGAC-3′; gene 3, 5′-CTCTTGTCCATGTGCCTGAC-3′; gene 11+13, 5′-CTCTTGTCCATGTGCCCGAC-3′ (these three probes correspond to probe region B in Figure 2); gene 2, 5′-TCCGGATGGTTGTGGGAGCT-3′ (Figure 2, probe gene 2).

Post-hybridization washings were in 3 × SSC at 65 °C for gene 6 and gene 12 oligonucleotides; in 3 × SSC, 67 °C for gene 12+13, gene 3, gene 11+13, gene 1 and gene 2 oligonucleotides and in 3 × SSC at 71 °C for the gene 1+2 oligonucleotide.

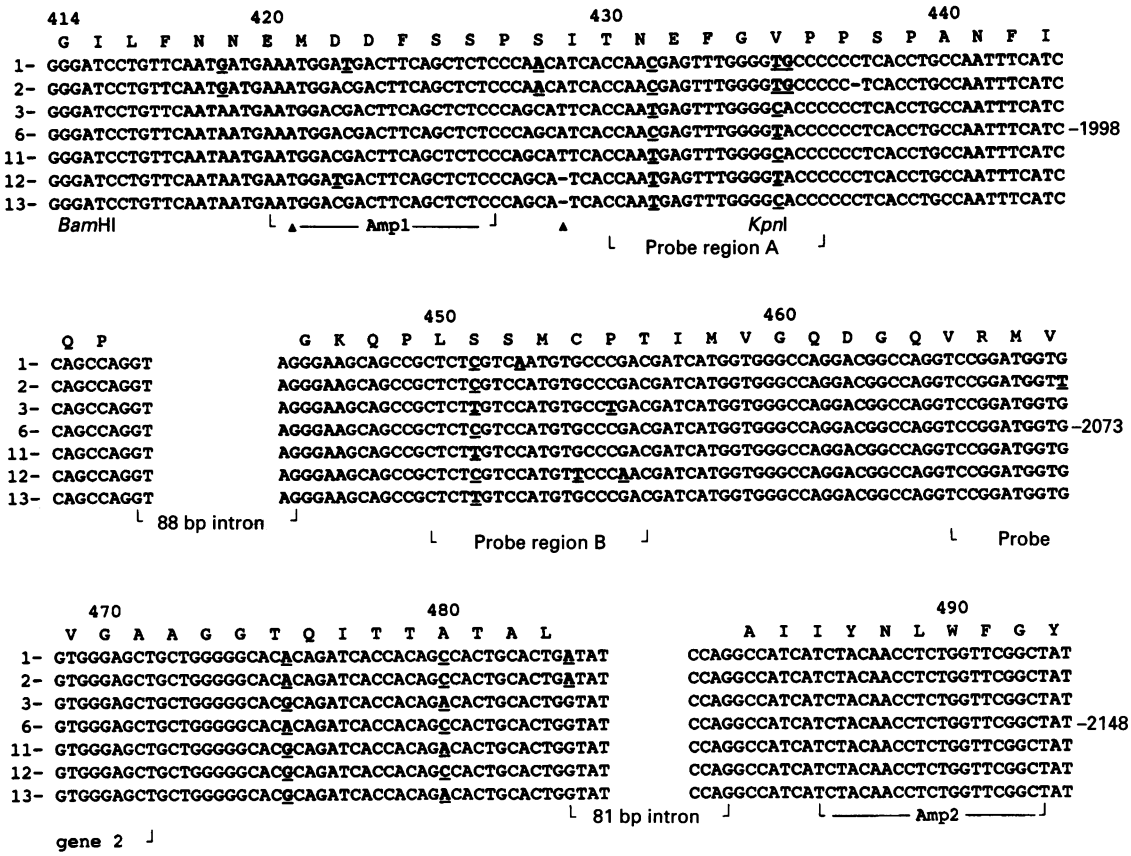

Figure 2 Nucleotide sequence used to distinguish the seven GGT genes

The number of the genes is indicated to the left (genes 1–13). The position of amplimers Amp1 and Amp2 used for cDNA synthesis and PCR is as indicated below the sequences. The location of the regions from which specific probes were derived is indicated below the sequence. Nucleotide differences with the human placental cDNA (gene 6) are indicated in bold and are underlined; the deduced amino acid sequence and number (also for the shown nucleotide sequence) is from the corresponding region of the placental cDNA (Rajpert-DeMeyts et al., 1988) and is shown above the sequence (for the nucleotide sequence on the right-hand side) for purpose of orientation. Arrowheads beneath the sequence highlight a region containing a frame shift in genes 3, 11, 12 and 13. The location of two introns within this region and of *Bam* HI and *Kpn*I sites are also shown.

RESULTS

Cloning of seven discrete GGT genomic clones

To isolate DNA fragments representative of all possible GGT genes in the human genome, a primary unamplified genomic library was constructed from human DNA partially digested with *Mbo*I. The library was screened with a 0.4 kb *Bgl*II/*Pst*I probe containing GGT light-chain-encoding sequences, isolated from a human placental cDNA (Rajpert-DeMeyts et al., 1988). A total of 12 new clones were obtained, in addition to seven clones previously isolated from cosmid libraries (unpublished results and Heisterkamp and Groffen, 1988). Based on restriction enzyme mapping, the clones were divided into seven groups, each group representing a unique GGT genomic clone with a characteristic pattern of restriction enzyme sites (see Figure 1); the primary genomic library was found to contain one or more clones from each of the seven groups. Clones were arbitrarily numbered.

To correlate the cloning of the seven different GGT clones with hybridization patterns seen in total human genomic DNA, a repeat-free 0.3 kb *Bgl*II/*Bam*HI fragment, which was present in all seven clones, was prepared (see Figure 1). Different enzymes yielded various numbers of hybridizing bands in human DNA, (Figure 3, lanes 1–5), indicating, not unexpectedly, that fragments from distinct members of the GGT gene family co-migrate in some digests. For example, all genomic clones have a *Bgl*II fragment of approx. 1.5 kb (see Figure 1) which contains approx. 75 bp of heavy-chain-encoding sequence and the entire light-chain-encoding region, including the 3′ end and polyadenylation signal (Pawlak et al., 1989). In agreement with the presence of multiple similar-sized *Bgl*II fragments in the cloned DNAs, only one very strongly hybridizing band was visible in total human DNA (Figure 3, lane 3). A *Hin*dIII digest of total human DNA yielded five bands (lane 2); one of 25 kb, three bands in the size range of 6–7 kb and of 2.8 kb. The 25 kb fragment belongs to gene 1, the 2.8 kb fragment to gene 12 and the three 6–7 kb fragments originate from genes 3, 6 and 13. The *Hin*dIII-fragment sizes of genes 2 and 11 are unknown. Four *Bam*HI fragments of 4.0, 3.4 , 2.8 and 1.1 kb were seen (Figure 3, lane 4) of which the 3.4 kb fragment hybridized most strongly. Genes 3, 12 and 13 all contain a 3.4 kb *Bam*HI fragment, gene 1 a 2.8 kb fragment, gene 6 a 1.1 kb fragment and, most likely, gene 2 a 4.0 kb fragment. Gene 11 contains a 1.1 kb *Bam*HI fragment which appears to be polymorphic: although this fragment is present in a number of DNAs analysed including the DNA from the cell line GM 7387A, from which gene 11 was cloned, it was absent in some genomic DNAs, including the DNA shown in Figure 3. The size of the *Bam*HI fragment in this genomic DNA is probably 2.8 kb, as the hybridization signal of the 2.8 kb fragment is higher compared with DNAs which contain the 1.1 kb fragment. This

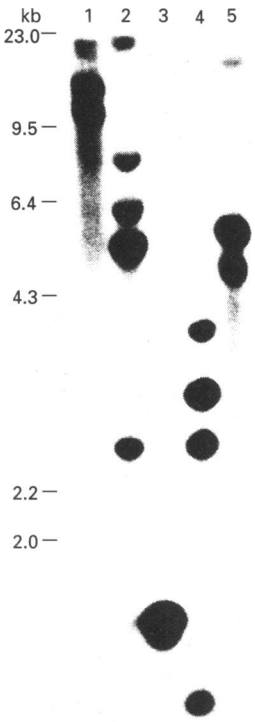

Figure 3 GGT Southern blot analysis

Human DNA digested with *Eco*RI (lane 1), *Hin*dIII (lane 2), *Bgl*II (lane 3), *Bam*HI (lane 4) or *Sst*I (lane 5) was probed with an 0.3 kb *Bgl*II/*Bam*HI probe (see Figure 1).

complicated the question of whether all members of the GGT gene family had been cloned. However, the genomic blot analysis, in combination with the isolation of a total of 19 independent GGT clones strongly indicates that seven different members of a family of GGT genes exist in the human genome which contain, at minimum, a partial GGT sequence.

Sequence determination of segments of all seven GGT genomic clones

To obtain sequence information for the design of PCR amplimers and for hybridization probes, exons localized in the 1.5 kb *Bgl*II fragments of all clones were subcloned into plasmid pSK and the nucleotide sequence of three exons shown in Figure 2 was obtained for both strands. The two introns were also sequenced, largely but not completely, for both strands. This suggested an intron size of 88 bp and 81 bp (Figure 2; only splice donor and acceptor sequences are shown). Segments (coding sequences) with minimal differences between all clones were then identified for use as PCR amplimers (Figure 2, Amp1 and Amp2). Two regions in the coding sequence appeared to have a substantial amount of nucleotide differences between the seven types of genomic clones and these were used to design gene specific hybridization probes (see the Experimental section and Figure 2, probe regions A and B). In the region sequenced here some of the nucleotide changes would result in the substitution of amino acids.

Expression of GGT genes in human RNAs

The common amplimers Amp1 and Amp2 were used to amplify genomic DNA from the seven types of GGT genomic clones as controls. All DNAs yielded a PCR product of 387 bp, demonstrating that this set of oligonucleotides was suitable for amplifying all possible seven types of GGT genes (not shown). The genomic DNA differs from possible RNA transcripts in the presence of two introns of 88 and 81 bp (see Figure 2). The expected size of a cDNA segment amplified with the same primers would be 218 bp, and should be clearly distinguishable from genomic DNA amplification products, if present.

Subsequently, RT-PCR was performed on a number of different human RNAs. On an ethidium-bromide-stained gel with the RT-PCR products, all RNAs gave rise to bands of approx. equal intensity (not shown). Blots were then successively probed with different oligonucleotide probes and washed to high stringency (see the Experimental section). All oligonucleotide probes specifically hybridized with the gene(s) they were designed to hybridize to, with the exception of the oligonucleotide probe for genes 12+13, which also showed substantial hybridization with gene 2.

A gene 1-specific oligonucleotide probe detected expression in placental and sigmoidal tissue (Figure 4a, lanes 8 and 10); a signal was also seen in lung and thyroid upon longer exposure (not visible in Figure 4a). Interestingly, the amplified cDNA product was slightly larger than that found with other probes (also see below), but was smaller than the amplification product of genomic DNA. As gene 1 contains a mutation at the splice donor of the 81 bp intron (see Figure 2), we suspect that this larger product includes the 81 bp intron. Gene 2 also contains this splice donor mutation (Figure 2). Other nucleotide differences between the intron sequences of the different genes were tentatively identified (results not shown; intron sequence data was not available for both strands of all clones), but no additional changes were found in the splice consensus sequences. An oligonucleotide probe unique for gene 2 detected expression in sigmoid (Figure 4b, lane 8) and the PCR product was similar in size to that of gene 1. Other tissues were essentially negative for gene 2 expression.

We also found gene 3 to be expressed, in thyroid, sigmoid and placenta (Figure 4c, lanes 1, 8 and 10). Expression was also found in lung (not visible in Figure 4c). The widest expression range could be attributed to gene 6, with a ubiquitous expression in all adult and fetal tissues tested, including fetal liver and kidney (Figure 4d).

In contrast, gene 12 lacked clear expression (Figure 4e, compare exposure time of 210 min with that of gene 3 in Figure 4c, 50 min). An oligonucleotide probe hybridizing to transcripts of gene 12 and gene 13 showed a signal in lanes containing cDNA of thyroid, sigmoid and fetal brain (Figure 4g, lanes 1, 8 and 15). This indicates that gene 13 is transcribed. An oligonucleotide probe for gene 13 and gene 11 (Figure 4f) showed essentially the same pattern and intensity as that for gene 13, suggesting that gene 11 is not transcribed.

DISCUSSION

In the present study we have identified seven distinct types of human GGT genomic clones by molecular cloning and have investigated whether they represent active genes. Previous studies by ourselves and others had demonstrated the presence of more than one GGT genomic sequence in man (Heisterkamp and Groffen, 1988; Pawlak et al., 1988). The present data describing the molecular cloning of seven different types of clones confirms that the human genome contains a GGT gene family. The

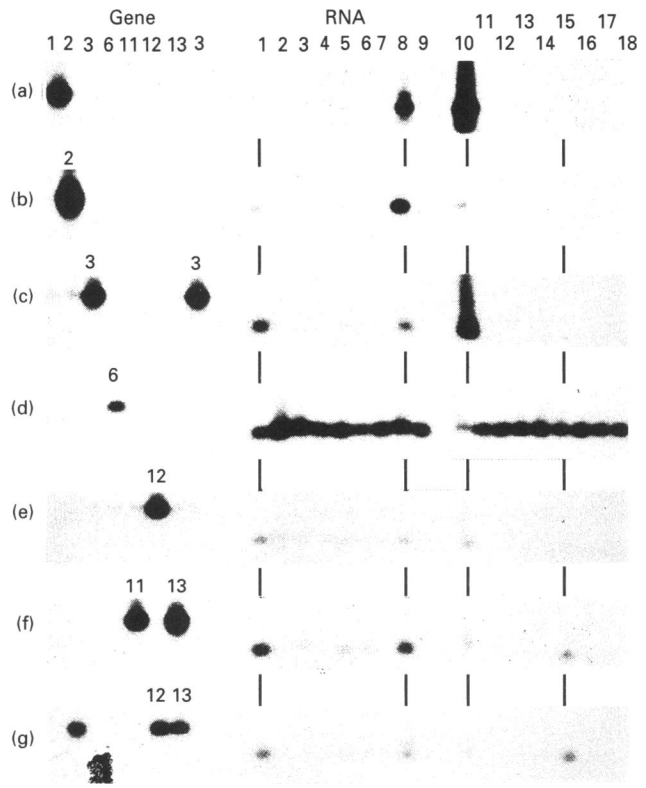

Figure 4 Expression of the seven possible GGT genes in different human tissues

PCR products from the GGT genomic clones serve as positive and negative controls and are indicated as genes 1–13. The 18 RNAs used in this study include thyroid (lane 1), liver (lane 2), uterus (lane 3), gall bladder (lane 4), lung (lane 5), muscle (lane 6), colon (lane 7), sigmoid (lane 8), small intestine (lane 9), placenta (lane 10), the hepatoma cell line Kyn-2 (lane 11), normal lymphocytes (lane 12), lymphocytes from a diabetic patient (lane 13), bone marrow (lane 14), fetal brain (lane 15), fetal liver (lane 16), fetal pancreas (lane 17), and fetal kidney (lane 18). **(a)** shows a 90 min exposure of the hybridization of a probe for gene 1; **(b)** a 150 min exposure for gene 2; **(c)** a 50 min exposure for gene 3; **(d)** a 30 min exposure for gene 6; **(e)** a 210 min exposure for gene 12; **(f)** 90 min exposed for genes 11 and 13 and **(g)** a 150 min for genes 12 and 13 (this probe also detects gene 2). Cloned DNA representing gene 3 was loaded twice.

conservation of restriction enzyme sites between the different genomic clones in both introns and exons is a measure of the degree of relatedness of the members of this gene family. On a restriction enzyme level, clones 3 and 13 are remarkably similar (only distinguishable by an *Eco*RI site), yet sequence analysis revealed that they are distinct and are unlikely to be (polymorphic) alleles of each other. Clones representing all of these seven possible genes were present in a human genomic library constructed with DNA from a karyotypically normal cell line GM 7387A. This excludes the possibility that these seven types of genomic clones are alleles of a single highly polymorphic gene. These sequences must have been derived from each other relatively recently in evolution.

Previously, segments of four different possible human GGT genes designated F30, F15, F19 and F11 were isolated by Pawlak et al. (1988). A comparison of their restriction enzyme maps with those presented here shows that our clones 3, 12 and 13 most probably correspond to their clones F15, F30 and F11. Interestingly, clone F30 was designated as the gene which encoded the common transcript (type-I mRNA) isolated as cDNAs from placental, fetal liver, pancreatic and HepG2 cDNA libraries. Their conclusion was based on the presence of a *Kpn*I restriction enzyme site which was present in one of their clones, F30, and is also found in the cDNAs (Pawlak et al., 1988, 1989). Of the seven clones isolated by us, two contain a coding *Kpn*I site (clones 6 and 12). Our sequence analysis unambiguously demonstrates that our gene 6 represents the gene encoding the ubiquitously expressed GGT. This demonstration confirms our previous observations that this gene is located on chromosome 22, distal to the BCR gene and is physically linked to a non-functional BCR gene segment (Heisterkamp and Groffen, 1988).

Based on restriction enzyme data, they also concluded that the cDNA (type-II mRNA) isolated from a human kidney cDNA library was encoded by the F15 genomic clone, corresponding to our clone 3 (Pawlak et al., 1989). A comparison of the partial sequence data of both clones establishes that these sequences are identical. This finding further confirms that our clone 3 and clone F15 are derived from the same gene. The cDNA transcribed from F15 contains a deletion (Pawlak et al., 1989) but there is no indication in the genomic sequence which we obtained why this deletion occurred. Our clones 3 and 13 are very similar. Interestingly, sequencing of genomic clone 13 revealed a splice acceptor mutation at the 3′ end of a 216 bp intron located 5′ to the *Bam*HI site (not shown). The sequence intron ... ag/CTT ... exon (clone 3) was mutated to the sequence intron ... ag/ATT ... exon in clone 13. This nucleotide substitution, at the interface of intron/exon sequences, could prevent splicing. To date, it has been generally assumed that only one human GGT gene was expressed. Our findings demonstrate that of the seven possible GGT genes, a surprisingly large number of five GGT genes were found to be expressed (genes 1, 2, 3, 6 and 13). Genes 11 and 12 are most probably non-expressed, exhibit an extremely low level of expression, or are restricted in their expression to rare specialized cells not included among the specimens examined in this study. There seems to be no obvious reason for the lack of expression of gene 12; hybridizations with GGT cDNA probes has shown that this genomic clone contains additional, 5′ exons (Pawlak et al., 1988). Clone 11 contains approx. 9 kb of sequences 5′ to the 1.5 kb *Bgl*II fragment (see Figure 1), but there is no indication for the presence of additional 5′ exons in this region, as we did not observe hybridization of this region with the entire GGT cDNA (Rajpert-DeMeyts et al., 1988). In genes 3, 6 and 13 a comparable region, 5′ to the *Bgl*II fragment, contains additional exons as determined by hybridization of the genomic clones with the entire GGT cDNA. Therefore, the lack of expression of gene 11 could be explained if the gene is truncated and lacks exons more 5′ to the region analysed in this study.

The genomic organization of the GGT gene in rat appears to be considerably different and much less complex than in human. The cloning of multiple identical genomic rat clones and Southern-blot analysis of rat genomic DNA established that the rat genome contains a single GGT gene, from which at least four different types of mRNAs are transcribed, which seem to differ only in their 5′ untranslated regions (Darbouy et al., 1991; Habib et al., 1992).

In the course of evolution the GGT gene was amplified and the level of complexity of regulation apparently increased. There is a ubiquitously expressed GGT mRNA species in man, which is encoded by a single gene, gene 6. It transcribes the only GGT mRNA found both in adult and in fetal liver as well as in a hepatoma cell line. We have found no evidence for the existence of fetal-specific GGT mRNAs, with the exception of the gene 13 transcript in fetal brain. Our results establish that placental, fetal kidney, pancreatic and hepatoma mRNAs isolated to date are all transcripts of a single gene, gene 6. It will be of obvious interest

to examine whether GGT genes other than gene 6 are induced in the liver under influence of stimuli such as alcohol and phenobarbital (Schiele et al., 1977; Siest et al., 1988) which are known to increase GGT enzymic activity *in vivo*. In this context it is interesting to note that our preliminary data suggest that in addition to gene 6, two additional GGT genes are expressed in normal adult liver (data not shown).

In man, four genes are expressed in a restricted, tissue-specific manner. Some tissues express one (always gene 6) or two (another gene in addition to gene 6) different GGT mRNAs; for example, fetal brain expresses gene 6 and gene 13. One gene, gene 2, is mainly expressed in one tissue, sigmoid. Other tissues exhibit expression of several genes simultaneously: placenta (genes 1, 3, 6 and 13), sigmoid (genes 1, 2, 3, 6 and 13) and thyroid (genes 3, 6 and 13).

In this study we have demonstrated the expression of five of seven existing GGT genes. Mutations in the regions we have sequenced indicate that the proteins encoded by these genes, although very similar, are likely to contain amino acid substitutions and may have different enzymic activities. Analysis of the primary structures and activities of the protein products may help explain why some tissues have expression of so many distinct GGT genes. But a definitive answer regarding protein activity and structure will have to await the molecular cloning of complete cDNAs for each RNA species, followed by overexpression in cells.

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