Hum Genet (2008) 123:321–332
DOI 10.1007/s00439-008-0487-7

REVIEW

The human gamma-glutamyltransferase gene family

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Received: 9 November 2007 / Accepted: 6 March 2008 / Published online: 21 March 2008
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Abstract Assays for gamma-glutamyl transferase (GGT1, EC 2.3.2.2) activity in blood are widely used in a clinical setting to measure tissue damage. The well-characterized GGT1 is an extracellular enzyme that is anchored to the plasma membrane of cells. There, it hydrolyzes and transfers γ-glutamyl moieties from glutathione and other γ-glutamyl compounds to acceptors. As such, it has a critical function in the metabolism of glutathione and in the conversion of the leukotriene LTC4 to LTD4. GGT deficiency in man is rare and for the few patients reported to date, mutations in *GGT1* have not been described. These patients do secrete glutathione in urine and fail to metabolize LTC4. Earlier pre-genome investigations had indicated that besides *GGT1*, the human genome contains additional related genes or sequences. These sequences were given multiple different names, leading to inconsistencies and confusion. Here we systematically evaluated all human sequences related to *GGT1* using genomic and cDNA database searches and identified thirteen genes belonging to the extended *GGT* family, of which at least six appear to be active. In collaboration with the HUGO Gene Nomenclature Committee (HGNC) we have designated possible active genes with nucleotide or amino acid sequence similarity to *GGT1*, as *GGT5* (formerly *GGL*, *GGTLA1/GGT-rel*), *GGT6* (formerly rat *ggt6* homologue) and *GGT7* (formerly *GGTL3*, *GGT4*). Two loci have the potential to encode only the light chain portion of GGT and have now been designated *GGTLC1* (formerly *GGTL6, GGTLA4*) and *GGTLC2*. Of the five full-length genes, three lack of significant nucleotide sequence homology but have significant (*GGT5*, *GGT7*) or very limited (*GGT6*) amino acid similarity to *GGT1* and belong to separate families. *GGT6* and *GGT7* have not yet been described, raising the possibility that leukotriene synthesis, glutathione metabolism or γ-glutamyl transfer is regulated by their, as of yet uncharacterized, enzymatic activities. In view of the widespread clinical use of assays that measure γ-glutamyl transfer activity, this would appear to be of significant interest.

Electronic supplementary material The online version of this article (doi:10.1007/s00439-008-0487-7) contains supplementary material, which is available to authorized users.

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Introduction

Mature gamma-glutamyltransferase (GGT; EC 2.3.2.2) is anchored to the cell surface through a small N-terminal transmembrane domain. It is one of the most widely used clinical indicators of tissue damage and GGT assays form part of the routine screening procedures on blood or plasma. GGT is an important enzyme in the metabolism of extracellular glutathione. GGT is able to cleave the

γ-glutamyl peptide bond in glutathione and other proteins and transfer the γ-glutamyl moiety to acceptors. GGT is also key to glutathione homeostasis because it provides the substrates for glutathione synthesis. GGT and glutathione are important to several organ systems such as the fetal liver, the kidney and the intestines (reviewed in Ikeda and Taniguchi 2005). GGT plays a critical role in lung antioxidant defense, both at normoxia and at hyperoxia (Jean et al. 2002; Barrios et al. 2001). Mice lacking *ggt1* have decreased lung fibrosis in a bleomycin injury model but are more sensitive to oxygen-induced lung injury (Pardo et al. 2003; Barrios et al. 2001; Jean et al. 2002). In addition, GGTs regulate the catabolism of LTC4, a potent vasoconstrictor (Han et al. 2002; Potdar et al. 1997; Zaitsu et al. 2003) in the lung and vasculature. Moreover, mice deficient in *ggt5* (formerly *ggl*, *GGT-rel/GGT-LA1*, see below for revised nomenclature) have significantly more airway hyper-reactivity in a model of experimental asthma (Han et al. 2002) and rat *ggt5* was shown to be highly expressed in airway epithelial cells and whole lung (Potdar et al. 1997). Thus, the GGT gene family plays an important role in maintaining lung homeostasis but the exact composition of this family was not clear. We have therefore systematically analyzed the human genome and identified all family members. Our analysis points to the existence of GGT family members other than the well characterized *GGT1* and *GGT5*, that may be involved in glutathione metabolism and leukotriene catabolism.

Data source and analysis

Database searching and sequence retrieving

We performed BLASTN nucleotide–nucleotide database searches (human build 35.1 and 36 RNA alternate and reference assemblies) at NCBI (http://www.ncbi.nlm.nih.gov) using *GGT1* cDNA (J04131) sequence to identify all human sequences with significant nucleotide identity with *GGT1*. We used MapViewer at NCBI to investigate localization of each human GGT member using human genome build 35.1.

Construction of protein tree

Protein RefSeqs (Human GGT1, NP_005256; Mouse Ggt1, NP_032142; Rat Ggt1, NP_446292; *S. pombe* GGT1, NP_593457; Human GGT2, XP_001129377; *S. pombe* GGT2, NP_593273; Human GGT5, NP_004112; Mouse Ggt5, NP_035950; Rat Ggt5, NP_062108; Human GGT6 NP_699169, Mouse Ggt6 NP_082095, Rat Ggt6 NP_001002820; Human GGT7, NP_821158; Mouse Ggt7, NP_659035; Rat Ggt7, NP_569107; Human GGTLC1, NP_842563; Human GGTLC2, NP_954578; Human GGTLC3, XP_001128296; *E. coli* GGT, NP_417904; Human TGM1, NP_000350; Mouse Tgm1, NP_064368; Rat Tgm1, NP_113847; Yeast (*S. cerevisiae*) ECM38, NP_013402; were aligned using BioEdit (Hall 1999) and phylogenetic analysis conducted using MEGA version 3.1 (Kumar et al. 2004). A neighbor-joining tree was generated using Poisson correction with pair wise deletion and bootstrap 500 replicates using the transglutaminase 1 (TGM1/Tgm1) sequences as an out group.

Analysis and discussion

Review of preexisting literature on human *GGT* genes

GGT is synthesized as a single polypeptide that is catalytically inactive and is post-translationally processed to form a heavy and a light chain (reviewed in Ikeda and Taniguchi 2005). After the isolation of cDNA clones for rat and mouse γ-glutamyltransferase, these were shown to be the product of a single gene, *Ggt1* (Coloma and Pitot 1986; Laperche et al. 1986; Pawlak et al. 1988; Goodspeed et al. 1989; Chobert et al. 1990; Rajagopalan et al. 1990, 1993; Shi et al. 1995). In human, cDNA clones encoding the most ubiquitously expressed human *GGT* gene, *GGT1*, were also sequenced (Rajpert-De Meyts et al. 1988; Sakamuro et al. 1988; Goodspeed et al. 1989). The transcriptional regulation of this *GGT1* gene is complex (reviewed by Ikeda and Taniguchi 2005; also Visvikis et al. 2001; Diederich et al. 1993). An alternatively spliced transcript from the *GGT1* locus was reported that contains intron sequences 5′ to the beginning of exon 7. These extra sequences cause a frameshift and premature termination of the polypeptide (Pawlak et al. 1990). There is also a shorter mRNA that is initiated from a promoter in the intron separating coding exons 7 and 8 and is found specifically in the lung (Leh et al. 1998).

In addition to these *GGT1* "variant transcripts", a second distinct gene, *GGT2* (formerly *GGT type II*), was first described by Pawlak et al. (1989) who cloned a partial 0.8-kb cDNA from a human kidney cDNA library. Although closely related to *GGT1*, this gene has the potential to encode a polypeptide with several amino acid residues distinct from those in GGT1. Also, Wetmore et al. (1993) identified a third species of *GGT* mRNA by cloning a cDNA from a lung cDNA library, that could encode another distinct polypeptide, but which would include only the light chain region. This gene has now been designated γ-glutamyltransferase light chain-1 (*GGTLC1*, formerly *GGTL6, GGTLA4*).

We previously identified a human cDNA that encodes a protein lacking significant nucleotide identity but clearly homologous to GGT1 at the amino acid level (Heisterkamp

et al. 1991). This gene, *GGT5* (previously named γ-glutamyl leukotrienase {*GGL*}, *GGT-rel/GGTLA1*), was subsequently also characterized in mouse and rat (Shi et al. 1995, 2001; Potdar et al. 1997; Carter et al. 1998; Han et al. 2002).

In situ hybridization (Morris et al. 1993; Figlewicz et al. 1993) and Southern blot analysis had suggested the presence of additional loci with nucleotide homology to *GGT1*, and in two studies cosmid or phage clones containing such segments were isolated and characterized (Pawlak et al. 1988; Courtay et al. 1994). Collins et al. (1997) also analyzed and numbered *GGT* sequences on chromosome 22q11 using YACs. As a result, there was agreement on the existence of multiple GGT-related sequences, but their location and nomenclature remained confusing. Therefore, the presence and location of all human sequences related to GGT have been systematically analyzed here and in collaboration with the HGNC, we propose a consistent nomenclature for all these loci.

Biochemical significance

GGT is an enzyme of major medical diagnostic significance. Biochemical assays for GGT activity in blood are one of the most widely used tests to measure, for example, liver damage such as that found in hepatocellular carcinoma or alcoholic liver, since elevated GGT is found in the circulation when liver injury occurs (reviewed by Whitfield 2001).

In general, GGT hydrolyzes γ-glutamyl peptides but can also catalyze the transfer of γ-glutamyl groups between different compounds. However, not all possible substrates have been systematically evaluated and only two, namely glutathione and LTC4, have been characterized in detail. One major function of GGT1 is as one of the six enzymes that constitute the γ-glutamyl cycle responsible for the metabolism of the tripeptide glutathione. A second important biochemical activity identified for GGT is the synthesis and metabolism of leukotrienes. These are biologically active lipid derivatives synthesized in hematopoietic cells (mast cells, macrophages, eosinophils and basophils), which are involved in acute hypersensitivity and inflammation. One of the metabolites in the leukotriene pathway is LTC4. GGT cleaves and removes the γ-glutamyl moiety from LTC4, yielding LTD4. Other compounds, which GGT uses as substrates include γ-glutamyl-β-cyanoalanine, γ-glutamyl-β-aminopropiononitrile, γ-glutamyl-Se-methylselenocysteine (active component of garlic) and the dipeptide γ-glutamyl-taurine (γ-GT). The latter compound is found naturally in parathyroid and brain. Interestingly, the administration of γ-GT has multiple effects on the central nervous system (reviewed by Bittner et al. 2005) suggesting that GGT may affect CNS function through this pathway.

Null mutant mice for *ggt1* and *ggt5*

The generation of null mutant mice for *ggt1* and *ggt5* (formerly *GGTLA1/GGT-rel* or *GGL*) has provided insight but also controversy into the consequences of ablation of these two major GGT family members. *Ggt1* null mutants have relatively normal LTD4 synthesis in some cell types (Carter et al. 1997; Shi et al. 2001) whereas *ggt1 x ggt5* double knockouts lack synthesis of LTD4 (Han et al. 2002; Shi et al. 2001). These studies showed that gg5 is the main enzyme converting LTC4 in (the lymphocytes present in) spleen, in the liver and in the uterus and plays an important role in LTC4 metabolism performed by endothelial cells (Han et al. 2002). However, ggt1 also contributes to LTC4 conversion in murine lung (Carter et al. 1998; Shi et al. 2001). The two enzymes appear to differ in their role in glutathione metabolism. Carter et al. (1998) reported that murine *ggt5* (formerly *ggl*, *ggtla1/ggt-rel*) transfected into NIH3T3 cells did not cleave glutathione or L-γ-glutamyl-*p*-nitronilide, an artificial substrate commonly used to assay Ggt1 activity. Also, there was no activity on glutathione in spleen, small intestine or kidney homogenates from mice lacking ggt1, suggesting that ggt5 does not compensate for loss of ggt1 in these tissues (Carter et al. 1997). This result contrasts with an earlier report (Heisterkamp et al. 1991) in which human *GGT5* (formerly *GGTLA/GGT-rel*, *GGL*) was first identified and found to have substantial metabolizing activity on glutathione in transfected NIH3T3 cells.

Phenotypically, *ggt1* knockouts have growth retardation, skeletal abnormalities and cataracts (Harding et al. 1997; Levasseur et al. 2003; Lieberman et al. 1996), whereas *ggt5* null mutants are normal (Shi et al. 2001). Mutants that lack both proteins have a very severe phenotype and die at an early age (Shi et al. 2001).

Human GGT deficiency

In general, inherited disorders of the six enzymes that form the glutathione cycle are very rare and all disorders are recessive (reviewed by Ristoff and Larsson 2007). Gamma-glutamyl-transferase deficiency (OMIM 231950) has been reported in only 7–8 patients total worldwide. Interestingly, a genotype–phenotype relationship has not been established for any of these and oddly, no mutations at the gene level (i.e., in *GGT1*) have been reported.

The earliest reports of GGT deficiency in man predate the identification of the *GGT1* gene and the *GGT* gene family. These were an abstract from O'Daley in (1968), Goodman et al. (1971), Schulman et al. (1975) and Wright et al. (1980). More recently Hammond et al. (1995) described two sisters and Iida et al. (2005) two brothers as GGT deficient. All patients had glutathionuria. Other biochemical findings reported included increased plasma glutathione levels, low GGT activity, and the presence of

Table 1 List of all GGT-homologous sequences, new approved gene symbols and chromosomal location

Approved gene symbol	Approved gene name	Previous symbols and aliases	Chromosomal location (in Mb)[c]	Functional protein possible?[d]	Accession number mRNA or predicted (pred) transcript	Remarks	NCBI entrez gene ID number
(Potential) Full length proteins							
GGT1	Gamma-glutamyltransferase 1	[b]Gene 6 GGT type I GeneCard: GGT1	Chr 22: 23.3	Functional protein	NM_001032364 NM_013430 NM_005265 NM_001032365 J05235	Variant mRNAs 1-4 J05235 mRNA with 22 bp insert	2678
GGT2	Gamma-glutamyltransferase 2 (putative)	[a]Clone F15 [b]Gene 3 (L10396) GGT type II GeneCard: GGT2	Chr 22: 19.89	Possible. Highly homologous to GGT1	XM_001129425 (pred) XM_001129412 (pred) XM_001129377 (pred)	Variant mRNAs 1-3	728441
GGT3P	Gamma-glutamyltransferase 3 pseudogene	[a]Clone F11 GeneCard: GGT3	Chr 22: 17.15	No	NR_003267	See Suppl. Fig 2	2679
GGT4P	Gamma-glutamyltransferase 4 pseudogene	[b]Gene 12 (L10398) [a]Clone F30	Chr 13: unplaced	No	XR_016938 (pred)	See Suppl. Fig. 2	643171
GGT5	Gamma-glutamyltransferase 5	GGL, gamma-glutamyl leukotrienase GGTLA1/GGT-rel GGT5 precursor GeneCard: GGTLA1	Chr 22: 22.95	Functional protein	NM_004121	See Fig. 2	2687
GGT6	Gamma-glutamyltransferase 6 (putative)	Rat ggt6 homolog	Chr 17: 4.4	Possible	NM_153338 AK074646	See Fig. 3	124975
GGT7	Gamma-glutamyltransferase 7 (putative)	GGTL3, GGT4, GGTL5 GeneCard: GC20M032896	Chr 20: 32.9	Protein	NM_178026	See Figs. 1, 2	2686
GGT8P	Gamma-glutamyltransferase 8 pseudogene		Chr 2: 91.3	No	NR_003503	Homology to GGT1 coding exons 1, 4 and 5	645367
Light chain only genes							
GGTLC1	Gamma-glutamyltransferase light chain 1	GGTL6 GGTLA4; GeneCard: GGTLA4	Chr 20: 23.92	Possible. Light chain only.	NM_178311 BC040904 NM_178312 NM_080920 L20492	Homology only to GGT light chain. NM_080920 mRNA variants A, B, C. A and B differ in their 5' ends; see Suppl Fig. 1	92086
GGTLC2	Gamma-glutamyltransferase light chain 2	[b]Gene 1 (L10394). GGTL4; GeneCard: GGTL4	Chr 22: 21.31	Possible. Light chain only.	NM_080839 NM_199127	Homology only to GGT light chain. Variant mRNAs 1, 2; see Suppl. Fig. 1	91227

Table 1 continued

Approved gene symbol	Approved gene name	Previous symbols and aliases	Chromosomal location (in Mb)[c]	Functional protein possible?[d]	Accession number mRNA or predicted (pred) transcript	Remarks	NCBI entrez gene ID number
GGTLC3	Gamma-glutamyltransferase light chain 3; GeneCard LOC728226	[b]Gene 11 (L10397)	Chr 22: 18.75	Possible. Light chain only.	XM_001128310 (pred) XM_001128302 (pred) XM_001128296 (pred)	Homology only to GGT light chain. See Suppl. Fig. 1	728226
GGTLC4P	Gamma-glutamyltransferase light chain 4 pseudogene	[b]Gene 2 (L10395);	Chr 22: 22.97	No	XR_015549 (pred) XR_015675 (pred)	Homology only to GGT light chain.	729838
GGTLC5P	Gamma-glutamyltransferase light chain 5 pseudogene	[b]Gene 13 (L10399)	Chr 22: 18.95	No	XR_016962 (pred)	Homology only to GGT light chain.	653590

[a] Pawlak et al. (1988) cloned segments of *GGT3* (called F11) and *GGT2* (called F15)

[b] Courtay et al. (1994) cloned genomic DNA segments with nucleotide sequence homology to *GGT1* exons 9, 10 and 11 from 7 different loci and examined expression using oligonucleotide probes and human mRNA dot blots

[c] The numbers after the chromosome number refer to the distance in Mb from the top of the chromosome in the human genome reference assembly. We used human genome build 35.1 to define the chromosomal locations in this table

[d] Based on experimental data or amino acid identity to GGT1. Also see "Supplementary information"

γ-glutamylcysteine and cysteine in urine. Moreover, Mayatepek et al. (2004) was able to measure leukotriene levels in monocytes, plasma and urine of three of these patients (the ones reported by Hammond et al. and Wright et al.) and found that they were completely deficient in LTD4 synthesis.

Phenotypically, patients varied in their symptoms. Five of seven patients listed in OMIM had central nervous system symptoms. One of the two patients described by Iida et al. had Marfan syndrome, and both brothers had mild mental retardation. The two sisters (Hammond et al. 1995) both bruised easily and one experienced seizures early in life. She was diagnosed with Prader–Willi syndrome. Both Marfan (OMIM #154700) and Prader-Willi (OMIM #176270) syndromes have been mapped to chromosome 15q (PW an interstitial deletion 15q11–q13, MS a mutation in the fibrillin-1) a therefore correlation with the GGT deficiency is not likely.

The combined biochemical data on mouse and human GGT mutants form a puzzle. Three human patients had glutathionuria and also failed to convert LTC4, suggesting that both biochemical defects were caused by homozygous mutation of the two *GGT1* alleles. Yet studies using *ggt1* and *ggt5* null mutants (Carter et al. 1997, 1998) show that ggt1 is the main enzyme involved in glutathione metabolism whereas ggt5 mainly is responsible for LTC4 metabolism. Thus, further studies including biochemical and genetic analysis of the different *GGT* genes will be needed to explain this.

Identification of *GGT* genes using database searches

We performed a database search by BLAST at http://www.ncbi.nlm.nih.gov on the human build 35.1 and 36 genome database (all assemblies) using a human *GGT1* cDNA (J04131) to identify all sequences with significant nucleotide or amino acid identity with *GGT1*. Table 1 shows the location of the thirteen loci identified. Chromosome 22 contains the majority (8 of 13) of these. We then evaluated the amino acid encoding potentials of these genes (Table 1). A select number of mRNAs or predicted mRNAs representing each locus were analyzed for a reading frame consistent with the GGT1 polypeptide. Based on this criterion, only a limited number of the *GGT* related genes can be considered potentially functional, if a functional protein is defined as resembling the primary amino acid sequence of GGT1 across the entire length of heavy + light chain, or light chain only.

We could distinguish different categories of human GGT-related sequences (see Supplementary Data Table 1). Functional genes based on the definition above include highly related *GGT1*, *GGTLC1* and *GGTLC2*, as well as the less highly related *GGT5*, *GGT6* and *GGT7*. The other

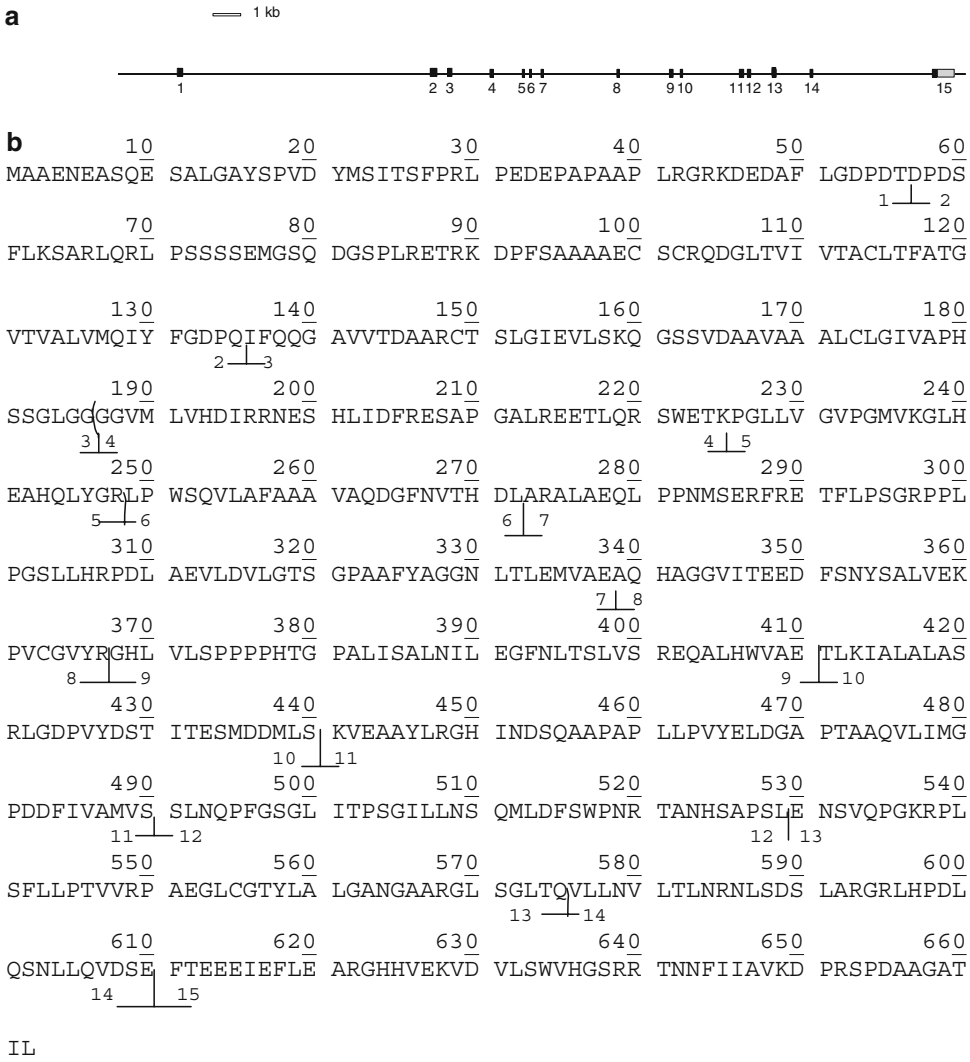

Fig. 1 *GGT7* gene organization. **a** Schematic representation of the *GGT7* gene on chromosome 20q. The *gray box* represents the 3′ untranslated region. **b** Deduced amino acid sequence (http://www.xpasy.org/uniprot/Q9UJ14) of the GGT7 protein encoded by the 15 exons. The distribution of the coding sequence over exons is as indicated

genes would encode polypeptides with only segments of amino acid identity to GGT1. It should be noted however, that only for *GGT1* (i.e., Accaoui et al. 2000; Karp et al. 2001; Thioudellet et al. 1994) and *GGT5* (formerly *GGTLA1/GGT-rel*, *GGL*; Heisterkamp et al. 1991; Enoiu et al. 2000; Carter et al. 1997) has it been experimentally demonstrated that a protein is made with enzymatic activity. The existence of an actual protein has not been demonstrated for the other genes with the exception of *GGT7* (formerly *GGTL3*, *GGT4*), of which a partial cDNA clone was isolated in a yeast two hybrid screen of a human placental cDNA library. He et al. (2002) reported that their cDNA encoded a novel isoform of GGTL3, which included 252 amino acid residues and "contained the active site of GGT".

Light chain only genes

Interestingly, the light chain of GGT1 contains the catalytic activity of the protein (Tate and Khadse 1986; Hughey et al. 1979). An alignment of the deduced amino acid sequence of the *GGTLC1*, *GGTLC2* and *GGTLC3* genes shows that their amino acid sequence is very similar (Supplementary Fig. 1). Leh et al. (1998) showed that the *GGT1* gene also has the capacity to encode only a light chain, because of the presence of a cryptic promoter present in the intron between coding exons 7 and 8. Interestingly, this promoter drives the expression of a shorter mRNA that is found in human lung (Wetmore et al. 1993). When a database search by BLAST at http://www.ncbi.nlm.nih.gov on the human build 35.1 genome data base (all assemblies) was performed with the sequence of this promoter (Y09833) seven sequences with a high degree of identity were detected on chromosome 22. These correspond to the seven highly related *GGT* genes on this chromosome. Because mRNAs for *GGTLC1* and *GGTLC2* have been isolated, it is highly likely that these promoters are active and are responsible for the transcripts produced.

GGT1 in human and *E. coli* is synthesized as a single polypeptide that undergoes autocatalytic cleavage to form a

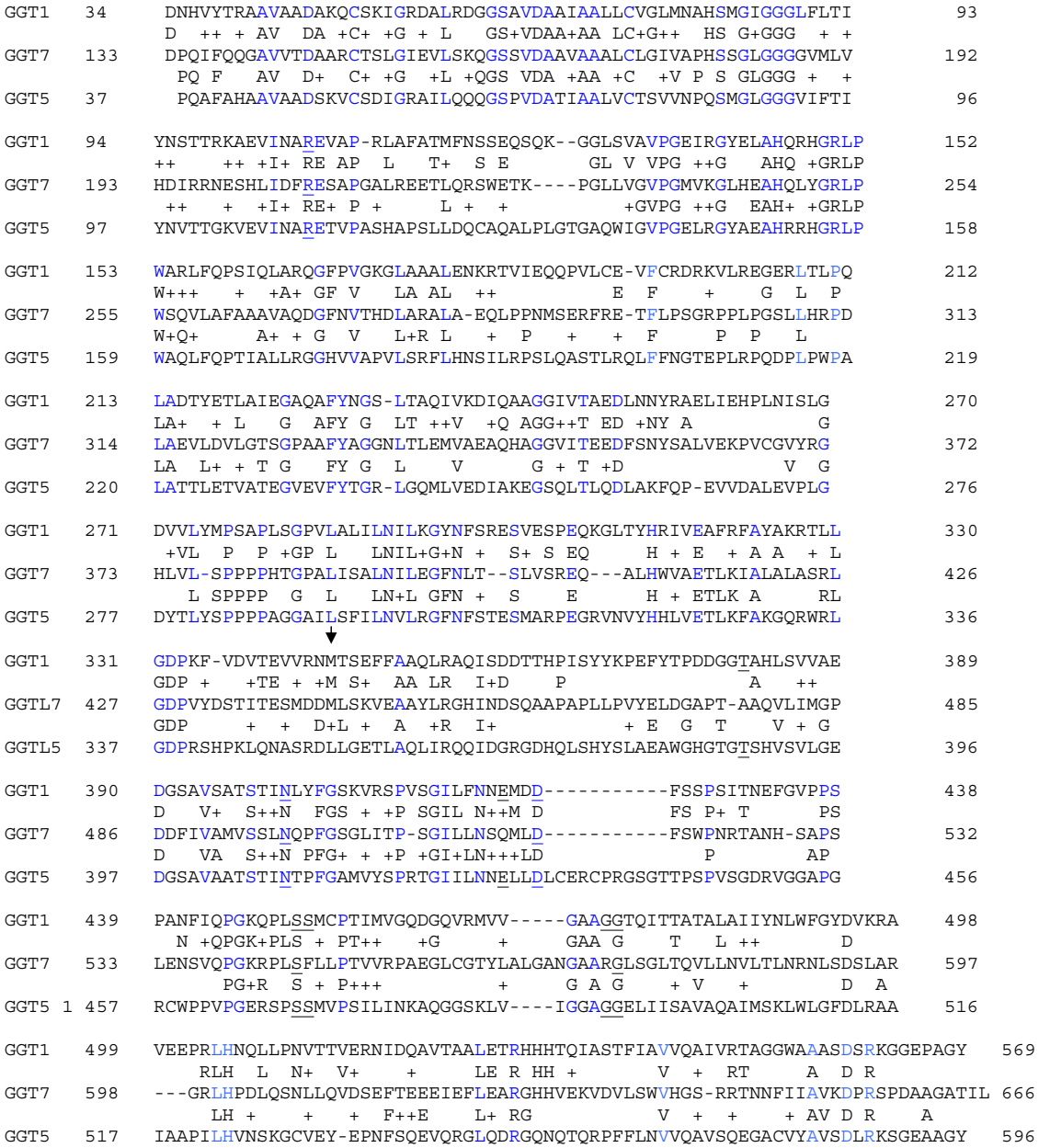

Fig. 2 Alignment *GGT1*, *GGT5* and *GGT7* deduced amino acid sequences. We used NP_005256, NP_004112 and NP_821158 for the alignment of the GGT1, GGT5 and GGT7 amino acid sequences, respectively using BlastP and manual alignment. Amino acid residues common between the sequences are indicated in blue. The "M" in GGT1 that is the first methionine acid residue of the light chain is indicated with an arrow. The number of amino acid residues of the largest sequences is indicated at the end of the sequence. Residues proposed to interact with glutathione (Okada et al. 2006; Han et al. 2007) in GGT1 are *underlined*

heavy and a light chain that associate with each other. Hashimoto et al. (1995) compared the catalytic activity of the *E. coli* light chain with that of the holoenzyme generated by the natural autocatalytic cleavage. They found that the small subunit had little activity on its own. In human GGT1, the glutamate-binding site includes eight residues encoded by its light chain and R107 encoded by the heavy chain (Han et al. 2007). Han et al. (2007) proposed which active site residues of human GGT1 would interact with glutathione, based on analysis of the crystal structure of *E. coli* GGT (Okada et al. 2006). Interestingly, only GGTLC1 and GGTLC2 would include all of these present in the light chain. GGTLC3 lacks D423 (numbered as in GGT1) and S451 (Supplementary Fig. 1). Residue R107 encoded by the heavy chain would also be missing in the products encoded by the light chain-only genes and because of this the glutamate binding site of these putative polypeptides may not be functional.

Table 2 mRNA expression of transcribed *GGT* genes

Approved gene symbol	UniGene	Expression (EST counts)-normal[a]	Expression (EST counts)-cancer[b]	Total numbers of GEO Profile records[c]	Disease/state[d]
GGT1	Hs.645535	Many, i.e., Blood Mammary gland Lymph prostate	Many, i.e., Breast carcinoma Leukemia Uterine Lung	3694	Increased in many, including liver disease, oxidative stress[e], metabolic syndrome[f]
GGT5 (formerly GGTLA1)	Hs. 437156	Wide-spread, i.e., adrenal gland, spleen, vascular thymus bladder	Many, i.e., PNET Kidney Glioma Esophageal	713 (GGT5) 1702 (GGTLA1)	Many, i.e., GDS1252 idiopathic pulmonary fibrosis GDS1857 and 2126 reduced in synovial arthritis tissues GDS1725; GDS961; GDS1021; GDS2429; GDS330; GDS2096
GGT6	Hs. 130749	Limited, i.e., Esophagus, trachea Kidney, bladder Adrenal gland intestine	Limited: Adrenal Colorectal Breast carcinoma	263	GDS1891 renal mesangial cells GDS1009, GDS1361; GDS1832 Meibomian gland expression
GGT7 (formerly GGTL3, GGTL5)	Hs. 433738	Wide-spread, i.e., Larynx Lung Pancreas prostate	Many, i.e., Bladder carcinoma Glioma Head and neck Lung, PNET	961 (GGTL5) 412 (GGTL3)	GDS2052 Endometrium throughout menstrual cycle GDS824, GDS1348 Bronchial epithelium exposed to cig. smoke GDS2615 airway epithelial cell differentiation
GGTLC1 (formerly GGTLA4)	Hs. 355394	Restricted Blood Lung testis	Restricted leukemia	363 (GGTLA4)	GDS2695, GDS2696, GDS2697 teratozoospermia GDS1329, GDS505 Higher in apocrine type br ca, lower renal clear cell ca GDS1376 platelets, GD1973 prostate luminal secretory
GGTLC2 (formerly GGTL4)	Hs. 632765	Very limited testis	Very limited Germ cell tumor	47 (GGTL4)	GDS1696 reduced in Jurkatt cells after nanosecond pulsed electric fields

[a,b] Abundance of an mRNA is deduced by the frequency of its cDNAs in EST databases

[c] A search with the (former) gene symbol retrieved the indicated number of records in the GEO Profiles database (version as per 3/4/08) and is also a measure for the ubiquitousness of expression

[d] For some genes, there are large numbers of GEO records and only a few interesting patterns were selected here. A caveat is, that individual records frequently show data of a condition or treatment that is only represented once in this database

[e,f] Reviewed in for example Lee et al. (2004, 2007)

An uncharacterized gene related to *GGT-GGT7*

He et al. (2002) reported that CT120, a plasma membrane-associated protein with unknown function, interacts with what in the databases was formerly named GGTL3, which they isolated in a yeast two-hybrid screen using CT120 as bait. An examination of this sequence revealed that it represents a previously uncharacterized gene, spanning at least 28 kb with 15 coding exons, on chromosome 20q (Fig. 1a, b), that has substantial amino acid similarity but no nucleotide similarity to *GGT1* and *GGT5* (Fig. 2). Interestingly, it is located within 60 kb of the glutathione synthase gene which catalyses the last step in the synthesis of glutathione (Ristoff and Larsson 2007). The largest mRNA would encode a protein of 662 amino acid residues with a calculated molecular mass of around 70 kDa. Alignment of *GGT1* and *GGT5* (formerly *GGL*, *GGTLA1/GGT-rel*) with this new gene, now designated *GGT7*, shows that they are equally distant: GGT7 shares 29% identity (47% positives) with GGT5 and 34% identity (52% positives) with GGT1 in amino acid sequence. The conceptual organization of GGT7 is similar to that of GGT1 and GGT5 with a putative N-terminal encoded heavy and C-terminal light chain. The mouse genome also contains a *GGT7* gene. Translation of mouse NM_144786 and alignment with human NP_821158 shows 637/662 identities (648/662 positives), indicating a high degree of evolutionary conservation.

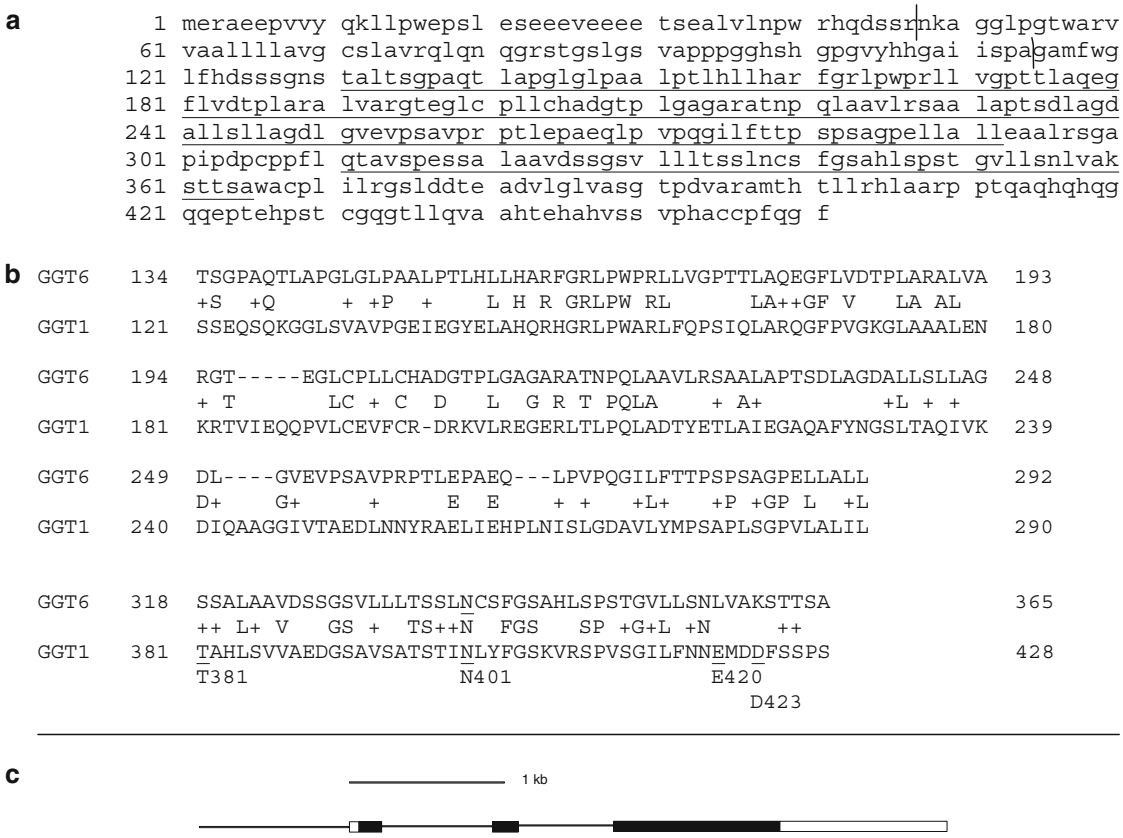

Fig. 3 GGT6. **a** GGT6 amino acid sequence (NP_005256). The regions with amino acid similarity to GGT are *underlined* and the distribution of the amino acids over exons is indicated with a vertical line in the sequence. **b** Alignment of GGT1 and GGT6 amino acid sequence homology. **c** schematic organization of the human *GGT6* locus on chromosome 17p13.2. Black boxes indicate the coding regions, open boxes the 5′ and 3′ untranslated regions and lines introns and genomic DNA outside the gene

As mentioned above, based on inhibitors and active site residues of *E. coli* GGT observed in the X-ray crystal structure (Sakai et al. 1996; Okada et al. 2006), Han et al. (2007) proposed residues in human GGT1 that would interact with glutathione in the active site. Interestingly, of these, residues R107, N401, D423, G474 and S451 are common between all three species, whereas residues T381, E420, G473 and S452 are only present in GGT1 and GGT5 (Fig. 2).

The database lists many possible splice variants deduced from the genomic sequence for this gene (i.e., isoforms 2–6 described under Q9UJ14) and their deduced amino acid sequences. The significance of these is currently unclear in the absence of evidence for their existence as actual mRNA species.

This gene shows widespread expression in normal tissues and is also detected in human tumor specimen (Table 2). Its expression may be dynamically regulated in some conditions (see Table 2 GEO Profile records). Apart from its function in the metabolism of glutathione and LTC4, GGT1 appears to have other unexpected activities such as induction of bone resorption (Hiramatsu et al. 2007). GGT5 (formerly GGL, GGTLA1/GGT-rel) has a role in inflammation by converting LTC4 to LTD4 (Carter et al. 1997). Based on these data and the amino acid residue differences in the GGT7 light chain that could affect binding to substrates, it should be of interest to examine the substrate specificity and normal cellular function of GGT7.

GGT6-limited amino acid sequence homology to GGT1

GGT6 represents a second uncharacterized gene that encompasses less than 5 kb on chromosome 17p13.2, consists of 3 exons and transcribes a 2.5 kb mRNA. *GGT6* is conserved in evolution. It was identified in the rat by Puente and Lopez-Otin (2004) as a threonine protease family member. There are no data published regarding the possible protein made by this gene (Fig. 3a). It has only very limited homology on an amino acid level to GGT1 and its organization on the genomic DNA is also very different from that of *GGT1*, *GGT5* or *GGT7*. As shown in Fig. 3b, the region of residues 134–292 shows some amino acid homology (27% identity; 41% positives) to residues 121–290 in the heavy chain of GGT1, and there is an additional stretch of homology with the light chain of GGT1. The polypeptide that could be encoded by the entire cDNA is

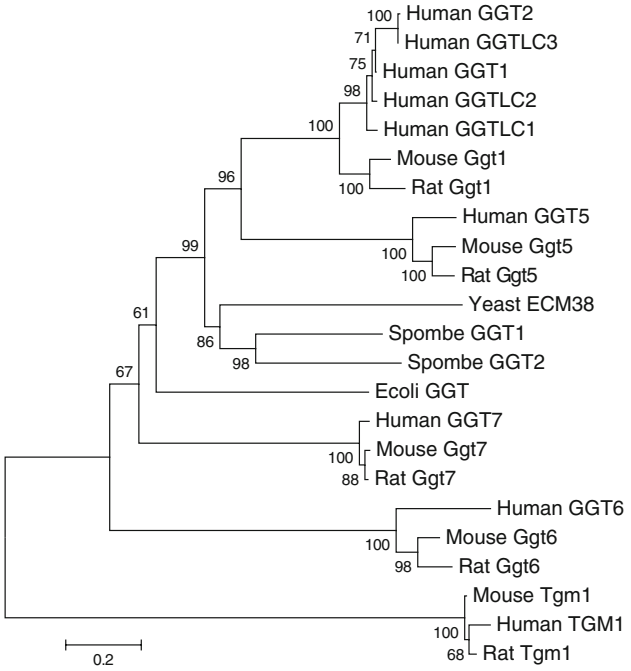

Fig. 4 Phylogeny of the human and rodent γ-glutamyltransferase proteins. Transglutaminase 1 (TGM1/Tgm1) is used as an outgroup; *scale bar* shows 0.2 changes per site

461 residues, and includes regions with low homology to domains in other human proteins (residues 380–452 with Hef1; residues 165–330 with chondroitin sulfate proteoglycan 3/neurocan). Whether this protein is likely to be involved in the activities ascribed to GGT1 and GGT5 is therefore difficult to assess. In comparison with *GGT1*, *GGT5* and *GGT7*, the *GGT6* gene appears to be relatively restricted in expression (Table 2).

GGT family of genes

Interestingly, rodents have orthologs of only four of the human GGT family members, including *GGT1* (MGI:95706; RGD:2683–*Ggt1*), *GGT5* (MGI:1340623; RGD:2684–*Ggt5*), *GGT6* (MGI:1918772; RGD:1303335–*Ggt6*) and *GGT7* (MGI:1913385; RGD:619870–*Ggt7*). Although human *GGT1* and *GGT5* have diverged in nucleotide sequence, they probably arose by tandem duplication (Fig. 4). In the mouse and rat, *Ggt1* and *Ggt5* lie in tandem, but in humans an inversion has separated these genes and their syntenic regions. This event may have also caused the duplication of the *GGT1* light chain only region. As shown in Fig. 4, this is reflected in the relatively high degree of relatedness of *GGT1* and *GGT5*, as well as the close homology of *GGT1* with *GGTLC1-3*. According to this analysis, *GGT7* is more distantly related to *GGT1*, and *GGT6* has the lowest degree of relatedness. Because *GGT1, GGT5, GGT6*, and *GGT7* each have orthologs in the mouse and rat, it is likely that the proteins encoded by them each have a distinct cellular function. Interestingly, the *GGTLC* genes appear to have arisen relatively late in evolution. A survey of the genome (build 1.1) of a more close relative to man, the rhesus macaque (*Macaca mulatta*) suggests that these are not present in this primate species.

Concluding remarks

One of the first reports measuring GGT activity dates from 1956 (Ball et al. 1956). Although this is more than 50 years ago, research on GGT is far from concluded. The finding that other genes exist with possible overlapping activity adds substantial interest as well as complexity to the subject. For example, it would be of interest to examine which gene(s) are exactly mutated in the seven patients that lack GGT activity, and also how the reported mental retardation or other CNS symptoms could be caused by GGT deficiency, for example by perturbation in the metabolism of the dipeptide γ-glutamyl-taurine. This could in fact also be measured in the *ggt1* and/or *ggt5* null mutant models. Also, it is of great interest to examine how ggt6 and ggt7 differ from ggt5 and ggt1 in subcellular location, cell type specificity or substrate preference, for example by generation of *ggt6* and *ggt7* null mutant mouse models. Finally, the possible existence of GGT light chain only proteins is intriguing, because these products miss the heavy chain that contains the membrane-anchoring N-terminal moiety. Thus GGT light chain products, if possessing enzymatic activity themselves, would not likely be membrane-associated and would act as soluble enzymes in the intracellular space. In view of the physiological importance of glutathione metabolism to the lung and other organs, it should be of significant interest to examine the enzymatic activities of each of these proteins, and to investigate if humans express light chain only polypeptides.

Acknowledgments The literature on GGT in human and other species is very extensive and we apologize to those authors of whom the work could not be cited. This work was supported by PHS grants HL071945 and HL060231, NHGRI grant P41 HG 003345, the UK Medical Research Council and the Wellcome Trust.

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