Quantitative Analysis of Interindividual Variation of Glutathione S-Transferase Expression in Human Pancreas and the Ambiguity of Correlating Genotype with Phenotype

Brian F. Coles, Kristin E. Anderson, Daniel R. Doerge, Mona I. Churchwell, Nicholas P. Lang, and Fred F. Kadlubar

ABSTRACT

Analysis of glutathione S-transferases (GSTs) of the alpha, mu, and pi classes by reverse-phase high-performance liquid chromatography and electrospray-ionization mass spectrometry in 43 samples of normal human pancreas demonstrated a wide variation in expression of subunits P1, A1, A2, A4, M1, M2, and M3 and the presence of a novel form designated GSTA5. GSTA2 consisted of three forms that were differentially expressed between individuals in a manner consistent with allelic polymorphism at the hGSTA2 locus. Expression, in terms of μg GST subunit/mg cytosolic protein, varied by 6–15-fold for subunits P1, A2, and M3 and 17–30-fold in the case of GSTs A1 and M2. Less consistently expressed were GSTs M1a, M1b, A4, and A5. Among these, GSTM1 expression (excluding M1-null samples) varied 12-fold between samples, whereas GST A4 and A5 expression varied ~50–100-fold between samples, well beyond the range of other subunits, suggesting that their expression is highly inducible. Linear correlations (P < 0.001–0.003) existed between levels of the most consistently expressed GST, GSTP1, and total GSTs, GSTA2 and M3, and in GSTM1-positive samples, between GSTM1, M3, and P1. The correlation between GST subunits P1 and M3 was bimodal according to M1 genotype, reflecting the presence of the regulatory element in hGSTM3*B that is linked with the hGSTM1*A genotype. It is concluded that although a degree of regulation of expression of GSTs occurs in human pancreas, the variability of phenotype is high and might obscure the effects of genetic polymorphisms on individual cancer susceptibility. Interindividual variation of GST expression is, therefore, a factor that should be taken account of in epidemiological studies.

INTRODUCTION

The GSTs (EC 2.5.1.18) of the alpha, mu, and pi classes are regarded as enzymes of detoxification (1, 2). It has been argued, on the basis of isoenzyme substrate specificity, that GST content and expression (2, 3) or differences in specific activity (4–8). Thus, the genes responsible for these GSTs (2–4), and these genetic changes can be reflected in changes in enzyme activity that result from altered expression (2, 3) or differences in specific activity (4–8). Thus, the investigation of these genetic polymorphisms in relationship to cancer susceptibility is of considerable interest in this area of research. Only with a few isoenzymes has a genetic polymorphism been consistently related to cancer risk. Notable is the case of the GSTM1 null polymorphism, where individuals with the homozygous null allele, who are unable to express the GSTM1–1 protein, are at an increased risk of several cancers (9–11). In most cases, however, changes in GST expression or changes in enzyme-specific activity that result from genetic polymorphisms are moderate (4–8).

In previous studies of human pancreas, we have analyzed levels of exogenous (12) and endogenous (13) DNA adducts in relation to genetic polymorphisms of the GSTs. Here, we present data on the variation of expression of GSTs of the alpha, mu, and pi classes in normal pancreatic tissue to illustrate how interindividual GST protein expression can vary 6- to over 100-fold, depending on the subunit. That is, phenotypic expression of GSTs in a single organ may present greater variation in enzyme activity than that predicted from genetic polymorphisms and should be taken into account in epidemiological studies when considering the effect of genetic polymorphisms on cancer susceptibility.

MATERIALS AND METHODS

Isolation of GSTs. Samples of grossly normal human pancreas tissue were obtained from organs donated for transplant or research. After procurement, the organs were kept in cold storage solution on ice for 5–30 h. Organs preserved in this manner for up to 30 h are considered suitable for transplantation (12). Samples of tissue were snap frozen in liquid nitrogen and stored at −80°C until use. Information on age, race, gender, smoking status, and body mass index were recorded; however, details were not available for all donors (see “Results”). Cytosols and DNA were prepared from 0.5 to 5 g of tissue as described previously (12, 14) with the inclusion of the protease inhibitors, 4-(2-aminoethyl)benzenesulfonyl fluoride (0.01 m) and pepstatin A (5.0 mg/l; Sigma Chemical Co., St. Louis, MO), and stored at −80°C until use. A GST mixture was prepared from a known aliquot of each cytosol (0.2–0.4 ml, corresponding to 0.1–5.0 mg of protein) using an S-linked glutathione-agarose (Sigma) column (5-mm diameter, 10-mm length), essentially as described previously (15). Bound GSTs were eluted from the column in 100 mM Tris, 40 mM sodium phosphate, and 50 mM glutathione (pH 9.6) buffer. Under these conditions, 98% of the bound GSTs elute in 1.3 ml.

Analysis of GSTs. A portion of the GST eluate (0.250 ml) was subjected to HPLC analysis within 2 days of storage (at 4°C), essentially by the method of Oxlund-Farrants et al. (15). Significant change from this method is the use of a Phenomenex “Jupiter” 5 μm, C18 column (4.6-mm inside diameter × 250 mm; Phenomenex, Torrance, CA), which provided for better GST subunit separation. GST subunits were identified by their HPLC elution profile (16) and electrospray mass spectrometry, using a Platform single quadrupole mass spectrometer (Micromass, Altrincham, United Kingdom). For this purpose, GST subunits purified by HPLC were dried with a stream of argon and dissolved in 50% aqueous acetonitrile/0.5% formic acid. Positive ion data were acquired over the range of m/z 500–2000 in MCA mode using infusion at 5:1/min. The instrument was calibrated using the multiple-charged ion peaks from a separate introduction of horse heart myoglobin (Sigma; M-1882; 16,951.5 Da), and data were acquired under the same conditions as the GST samples. Subtracted data were processed by maximal entropy calculation to convergence using 1 Da/Channel resolution. The data were centroided to accurately determine the masses.

Quantitation of GSTs. Subunits were quantitated by absorption at 214 nm and by reference to known quantities of authentic GSTP1–1, M1b–1b, and

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3 The abbreviations used are: GST, glutathione S-transferase; HPLC, high-pressure liquid chromatography.
A1-1 standards (PanVera, Madison, WI). Those peaks for which no standards were available were quantitated using data established for peaks of similar retention time and peak width. Approximately one-third of analyses were repeated to establish the reliability of quantitation. Correlations between levels of subunit expression within each tissue sample were examined using the program SigmaStat (Jandel Corp., San Rafael, CA). Protein concentrations were determined by the dye-binding method using Bio-Rad reagent (Hercules, CA), according to the supplier’s instructions and using BSA as standard.

**Genotyping.** The *hGSTM1/M1*<sup>+</sup> polymorphism was determined by PCR using albumin as positive control, essentially as reported previously (17).

**RESULTS**

Forty-three, grossly normal pancreas samples were examined from donors of age 8–59 years (mean, 26.3). Age was not available for 11 donors. Eighteen donors were male, 14 female, and 11 had no data; 16 were nonsmokers, 4 were ex-smokers, 15 were current smokers, and 8 were of unknown status. Seventeen donors were Caucasian, and four were African American; race was not available for 22 donors.

**Confirmation of Subunit Identity.** On the basis of established HPLC elution profiles for GSTs (15, 16), subunits present in pancreas were identified as: P1, M1a, M1b, M2, M3, A1, and A2 (Fig. 1). Identity was confirmed by mass spectrometry (Table 1) for subunits P1, M1a, M2, M3, and A1. The masses of subunits A1 and M3 corresponded to the posttranslationally modified NH<sub>2</sub>-terminal acetylated derivatives (16). The P1 subunit subjected to mass spectrometry was derived from a homozygous P1*B donor (data not shown), and the predicted mass agrees with that of the Val<sup>104</sup> form (4).

The HPLC elution pattern of GST subunit A2 was complexed with the peak appearing as a “doublet” in ~50% of the analyses (Fig. 1B). Detailed examination of 13 samples showed that the later eluting material contained only a protein of mass corresponding to the reported mass of N-acetylated GSTA2-Thr<sup>111</sup> (16), and the earlier eluting material contained a protein of mass corresponding to that of the known N-acetylated A2-Ser<sup>111</sup> variant (16, 18). In addition, the earlier eluting material of several samples contained a protein of mass ~25,520 Da, corresponding to the mass of the N-acetylated product of the hGSTA2 clone reported by Röhrdanz et al. (19), which codes for a GSTA2-Ser<sup>111</sup>, Ala<sup>209</sup> variant.

Two subunits that were not readily identifiable on the basis of known elution profiles of human GSTs were present as major components in several samples (Fig. 1, C and D, peaks x and y). These were determined to be isoforms within the alpha class by their cross-reaction with antibodies raised to the GST alpha class but not to those of the mu or pi classes (results not shown). Automated Edman degradation of the HPLC-purified subunits demonstrated that they possessed blocked NH<sub>2</sub> termini; GSTP1 retained a free NH<sub>2</sub> terminus after an identical purification procedure. Their masses (Table 1) do not agree with the deduced masses for any known human alpha class GST (16, 20–22). However, the later eluting peak “y” apparently represents the GSTA4 subunit because its mass corresponds to that of putative NH<sub>2</sub> terminally acetylated hGSTA4<sub>4</sub> gene product (20, 21).

The mass of subunit “x” does not correspond to that of any known alpha class GST nor to likely posttranslationally modified products and appears to be a novel form designated here as GSTA5.

Minor components eluting in the region of subunits M4 and M5 (16) were inconsistently expressed in most samples (labeled “M” in Fig. 1). The peak from one sample that contained the greatest amount of such material gave a mass corresponding to that of the deduced M5 sequence quoted by Rowe et al. (Ref. 16; although not that of the observed mass for testicular GSTM5). This region of the HPLC profile was variable between samples, and because of the small amounts of protein present, we have not been able to investigate the identity of these minor components further. Two further minor components (* of Fig. 1) appear to represent anomalous modification of subunits P1 and M3 after storage of GST pools, because during repeat analysis, they increased in height as the P1 and M3 peaks decreased in height. Their masses (i.e., mass of adjacent peak plus ~300 Da, results not shown) are consistent with the formation of mixed protein-SG disulfides, as suggested by Rowe et al. (16).

**Variation of Expression of GST Subunits.** Distributions of data for GST subunit expression are presented in Fig. 2, and variation of expression is summarized in Table 2. Total GST expression among the samples varied by 7.3-fold. GST subunits identified in all samples were P1, M2, M3, and A1 (Figs. 1 and 2). All samples expressed a form of GSTA2. Of these A2 forms, A2-Thr<sup>111</sup> alone was expressed.

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**Fig. 1.** Separation of GST subunits by reverse-phase HPLC. Approximately 1–10 μg of protein from a GST pool prepared by glutathione-agarose affinity chromatography was subjected to reverse-phase HPLC on a Phenomenex “Jupiter” column (4.6 × 250 mm) and eluted with a 60-min linear gradient of 35–65% acetonitrile (plus 0.04% trifluoroacetic acid)-water (plus 0.06% trifluoroacetic acid). Absorption was monitored at 214 nm. A, profile typical of most samples. B, a GSTM1b-positive sample illustrating the partial resolution of GSTA2 subunit forms. C, a GSTM1a-positive sample illustrating a high level of expression of GST subunit α-“y” (GST subunit A4, see “Results”). D, a GSTM1-negative sample illustrating a high level of expression of GST subunit α-“x” (designated subunit A5, see “Results”). Peaks marked * are the apparent mixed GS-protein disulfides that elute slightly earlier than the parent subunits. The peak labeled A2 of the Thr<sup>111</sup> Glu<sup>209</sup> form; those of C and D are the Ser<sup>111</sup>Glu<sup>209</sup> form. Those of B include both the Thr<sup>111</sup>, Glu<sup>209</sup> (later eluting) and the Ser<sup>111</sup>, Ala<sup>209</sup> (earlier eluting) forms. The peak labeled M is a probable mu class minor component.
in 13 samples; a mixture of A2-Thr\(^{111}\) plus either A2-Ser\(^{111}\) or A2-Ser\(^{111}\)/Ala\(^{209}\) was expressed in 20 samples, and either A2-Ser\(^{111}\) or A2-Ser\(^{111}\)/Ala\(^{209}\) was expressed in 10 samples. Although unambiguous assignment to subunits A2-Ser\(^{111}\) or A2-Ser\(^{111}\)/Ala\(^{209}\) was not made in all cases, tissues were identified that expressed only one of the three A2 forms or any two of them. Because of this complexity, GST subunit A2 forms have been grouped together for analysis.

Levels of expression varied 6–100-fold, according to subunit (Table 2). GST subunits P1 and A2 form the bulk of GSTs present (52–92% of total GSTs) and were the least variable, both in terms of absolute expression and percentage of total GSTs (Table 2). Superimposed on this pattern were subunits M1a, M1b (Fig. 1, B and C), A4 (Fig. 1C) and A5 (Fig. 1D). Twelve donors were determined to be of the GSTM1-positive genotype, and 17 were GSTM1-null (genotype not available for 14 tissues). An additional nine donors, for which genotype was not available, were determined to be GSTM1-positive on the basis of presence of the M1 subunit. No GSTM1 subunit was detected in tissue from the GSTM1-null individuals; and, within the

<table>
<thead>
<tr>
<th>Subunit</th>
<th>P1</th>
<th>A1(^{a})</th>
<th>A2(^{a})</th>
<th>A3(^{a})</th>
<th>A5(^{c})</th>
<th>M1c</th>
<th>M2</th>
<th>M3</th>
<th>All GST</th>
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<tbody>
<tr>
<td>Range</td>
<td>1.1–6.5</td>
<td>0.18–5.1</td>
<td>0.72–10.8</td>
<td>0.02–2.2</td>
<td>0.02–1.1</td>
<td>0.04–0.5</td>
<td>0.01–0.33</td>
<td>0.12–0.91</td>
<td>2.8–20.3</td>
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<tr>
<td>Mean</td>
<td>2.78</td>
<td>1.44</td>
<td>4.26</td>
<td>0.13</td>
<td>0.15</td>
<td>0.17</td>
<td>0.07</td>
<td>0.34</td>
<td>9.24</td>
</tr>
<tr>
<td>Fold variation</td>
<td>6.13</td>
<td>28.3</td>
<td>15.0</td>
<td>~100</td>
<td>~55</td>
<td>12.5</td>
<td>16.5</td>
<td>7.58</td>
<td>7.25</td>
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<td>Composition (%)</td>
<td>15.3–67.3</td>
<td>1.36–41.0</td>
<td>18.7–62.5</td>
<td>0.10–18</td>
<td>0.25–20</td>
<td>0.55–7.98</td>
<td>0.28–4.66</td>
<td>1.24–8.0</td>
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<tr>
<td>Mean</td>
<td>32.2</td>
<td>15.3</td>
<td>43.2</td>
<td>1.59</td>
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<td>30.1</td>
<td>3.34</td>
<td>~180</td>
<td>~80</td>
<td>14.5</td>
<td>16.6</td>
<td>6.45</td>
<td></td>
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</table>

\(^{a}\)Expressed as \(\mu g\) GST subunit/mg cytosolic protein.

\(^{b}\)The lower limit is set at the lower limit of reliable detection (see "Results").

\(^{c}\)Excludes GSTM1-null samples.
GSTM1-positive tissues, expression varied 12.5-fold, from 0.04 to 0.5 \(\mu g/\text{mg cytosolic protein}\) (Table 2). Thirteen samples showed expression of only subunit M1a, one M1a and M1b (in approximately equal amounts), and six M1b only. Subunits A4 and A5 are highly variable in expression (Fig. 2; Table 2). Subunit A4 was apparently absent in 34 samples (set at the lower limit of detection in Fig. 2), expressed at low levels (0.1–0.7 \(\mu g/\text{mg cytosolic protein}\)) in eight samples and highly expressed in only one sample, in which it represented a major component accounting for 18% of the total (Table 1 and Fig. 1C). A5 was expressed at low levels of 0.02–0.25 \(\mu g/\text{mg cytosolic protein}\) in all but four samples (Fig. 2) in which it formed a major component accounting for 9–20% of the total (e.g., Fig. 1D).

The lower limit of quantitation of GSTP1 and mu class GSTs was 0.01 \(\mu g/\text{mg cytosolic protein}\), and for GSTs of the alpha class, 0.02 \(\mu g/\text{mg cytosolic protein}\). Repetition of analyses established that quantitation was reliable to within ± 8%; HPLC analyses were reproducible to within ± 3%.

**Correlations between GST Subunit Expression.** For each sample, the total amount of GST subunits did not correlate with age, smoking status, body mass index, or gender (results not shown). Nor was there any significant correlation between amounts of any one GST subunit and these variables (results not shown).

Comparisons between levels of the least variable major component, the GSTP1 subunit, with total GSTs, GSTA2 or GSTM3, for each of the samples established a correlation between expression of the subunit pairs (Table 3 and Figs. 3 and 4). Excluding M1-null individuals, there was also a significant correlation between GSTs P1 and M1 (Table 2; Fig. 3B) and M3 and M1 (Fig. 3D). In addition, there was a trend for higher expression of total GSTs and of subunits A1 or A2 to be associated with higher expression of the minor GST components; subunits M1 (in M1-positive samples) and M2 (\(r < 0.5; P = 0.01–0.03\)). Those samples that expressed GSTs A4 and A5 at high levels (Fig. 1, C and D; Fig. 2) did not, otherwise, exhibit an anomalous pattern of GST expression. Expression of these subunits was not correlated with that of other alpha class GSTs nor with each other.

The mean levels of M3 expression in the two groups “M1a phenotype” and “M1-null or M1b phenotype” were not significantly different in terms of \(\mu g\) M3/mg cytosolic protein (0.37 ± 0.21 and 0.32 ± 0.14, respectively) nor in percentage composition

<table>
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<tr>
<th>Subunit</th>
<th>A1</th>
<th>A2&lt;sup&gt;a&lt;/sup&gt;</th>
<th>A4</th>
<th>A5</th>
<th>M1&lt;sup&gt;a,b&lt;/sup&gt;</th>
<th>M2</th>
<th>M3&lt;sup&gt;b&lt;/sup&gt;</th>
<th>All GST&lt;sup&gt;c&lt;/sup&gt;</th>
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<tr>
<td>(n)</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>21</td>
<td>43</td>
<td>43</td>
<td>43</td>
</tr>
<tr>
<td>(r)</td>
<td>0.18</td>
<td>0.75</td>
<td>0.12</td>
<td>0.13</td>
<td>0.72</td>
<td>0.27</td>
<td>0.73</td>
<td>0.72</td>
</tr>
<tr>
<td>(P)</td>
<td>0.24</td>
<td>&lt;0.001</td>
<td>0.45</td>
<td>0.42</td>
<td>&lt;0.001</td>
<td>0.09</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
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<sup>a</sup> Indicates a significant correlation.

<sup>b</sup> Omitting M1-null samples.
(3.83 ± 1.7% and 3.90 ± 1.4%, respectively). However, when normalized to GSTP1 expression, there was a relatively higher GSTM3 expression in the GSTM1a-positive individuals than the GSTM1b or M1-null individuals (Fig. 4).

**DISCUSSION**

**Definition and Variation of the GST Phenotype in Pancreas.** This study of GSTs in normal human pancreas shows that, despite the wide variation in expression, the general pattern of expression of GST subunits P1, A1, A2, M2, and M3 is conserved. Total GST content varied 7-fold, and levels of expression among these five subunits varied 6–30-fold. Within this variation, there is a “core” of GSTs, subunits P1, A2, and M3, that makes up the bulk of GST content of pancreas and in which variation, both in absolute terms and composition, is less, i.e., 6–15-fold in amounts and 3–7-fold in composition. These appear to be constitutive elements of the pancreas. Thus, the pancreatic GST phenotype is defined by a 7-fold variation in GST subunits P1 and M3 and 15–30-fold variation in subunits A2 and A1. Although tissue-specific expression of GSTs in pancreas has been described on the basis of a few tissue samples (16, 23), the physiologic and genetic basis for this is still unknown. Additionally, the phenotype appears to be independent of age (8–59 years), gender, and smoking status. However, it should be noted that GST composition is highly variable within different cell types of the pancreas (24).

Superimposed on this pattern is the expression of GST subunits M1a, M1b, A4, and A5. Although the expression of GSTM1 depends on the genotype, within the group of GSTM1-positive individuals, low level of M1 expression is also part of the pancreas phenotype.

**Complexity of the Alpha Class GSTs in Pancreas.** Particularly notable and complex is the high level of expression of GSTs of the alpha class in pancreas, where four alpha class GSTs (subunits A1, A2, A4, and A5) are expressed. Concerning GSTA2, three variants are known, two on the basis of the amino acid substitution Thr-111→Ser (18, 25) and the third on the basis of the GSTA2 gene isolated by Röhrdanz et al. (19), in which the base substitutions at 401 and 695 bp code for the amino acid changes Thr-111→Ser, Glu-209→Ala. Our assignment to these forms is based on the observed masses of the HPLC-purified subunits, which agree with the predicted amino acid substitutions, taking into account the known posttranslational modification of human alpha class GSTs (removal of the NH2-terminal methionine and acetylation of the resultant alanine; Ref. 16). The genomic GSTA2 sequence of Röhrdanz et al. (19) apparently differs from that of Klöne et al. (25) in the approximate lengths of introns 1 and 6, and it has been suggested, on this basis, that the two GSTA2 clones represent distinct genes. The gene for the A2-Ser111 variant has not been reported. It is not clear, therefore, whether the three A2 proteins are the products of separate gene loci or whether they represent allelic variation within the A2 locus. Despite the reported differences in intron length between the known A2 genes, they are identical in the 5′ upstream region for 310 bp (26) and may, therefore, be subject to coregulation. Thus they may behave as allelic forms, even if they are distinct genes. Because of these ambiguities, we have regarded the A2 proteins as the products of allelic variation, which is consistent with their pattern of expression in pancreas. Whatever their nature, GSTA2 protein is clearly subject to polymorphism, and there are individuals who appear to be homozygous or heterozygous within the three forms.

In addition to GSTs A1 and A2, DNA sequences are available for GSTA3 and GSTA4. As mentioned above, the late-eluting HPLC peak “γ” appears to represent the NH2 terminally acetylated protein.
product of the GSTA4 gene. GSTA4 is known to be expressed in pancreas on the basis of the presence of its mRNA (20) but has not, previously, been observed as a native protein. The available clones for GSTA3 are incomplete cDNAs (21, 22), and an accurate, deduced mass is not available. However, the mass of subunit A5 does not correspond to the amino acid sequence deduced from the purported full-length GSTA3 cDNA (21), nor to likely posttranslationally modified products. On this basis, GSTA5 appears to be a novel gene product. Whether subunits A4 and A5 are related to the basic GSTs of human skin (27) or lung (28) is unclear. However, their highly variable pattern of expression, independent of that of subunits A1 and A2, suggests that they are products of highly inducible gene expression.

All known human class alpha GSTs have peroxidase activity (2, 20, 21, 28, 29). Additionally, GSTA4 has high activity toward the genotoxic hydroxyalkenal products of lipid peroxidation (21, 29). Because a high-energy diet, possibly resulting in high levels of lipid peroxidation products, is a risk factor in pancreatic cancer (30, 31), variation between individuals in type and levels of alpha class GSTs may be of considerable importance in pancreatic disease.

**Regulation of GSTs.** The genes for the alpha mu and pi classes of GSTs are located on separate chromosomes (2, 32), and a molecular basis for the apparent coregulation of expression of GSTs P1, A2, and M3 in the pancreas is not clear. In the case of GSTP1, the gene is known to contain a complex of regulatory elements between −95 to 55 bp [summarized in Hayes and Pulford (2)]). These include an nuclear factor-κB-like (silencer) element and ARE/TRE (AP-1 recognition site) consensus motifs contained in the “C1-region” (33). This last is absolutely required for GSTP1 transcription. Activation of the C1 region (e.g., by binding of the Fos-Jun complex) is apparently cell type specific (34, 35). GSTP1 expression is also enhanced by insulin (36). Alpha class GST mRNAs and protein levels are selectively inducible in human hepatocytes in primary culture by diihthiothiones, phenobarbital and 3-methylcholathrene (37). In addition, a daily intake of Brussels sprouts also leads to selective GST alpha class protein induction in humans (38). Variation in diet may, therefore, be the cause of the wide variation of expression of GST subunits A1, A4, and A5 in the pancreas. Although potential AP1, AP2, GRE, and HNF1 regulatory elements have been identified in the 5′-upstream regulatory region of human GST alpha class genes (26, 39, 40), no elements corresponding to ARE or XRE consensus sequences have been revealed, and the mechanism by which inducers act is not known. Also, there are no similarities between the 5′-upstream regions of the human GST alpha and Pi genes (26), nor between those of GSTM1 and M3 (41). It has been suggested that SP1 elements in the GSTM3 gene are responsible for low levels of expression of M3 in most tissues (41). Whether such sites offer a mechanism for induction is not clear.

**GSTs and Cancer Susceptibility.** Much attention has been focused on GSTs of the mu and pi classes because they have activity toward the carcinogenic polycyclic aromatic hydrocarbon metabolites that are a consequence of smoking and dietary exposure (1, 2), and there are also genetic polymorphisms in these classes that are reflected in levels of enzyme expression or specific activity (2–8). The most marked of these is the M1-null polymorphism, where GSTM1-null individuals cannot express the GSTM1 protein. However, in this case, correlation between genotype and cancer susceptibility is highly dependent on the organ under study (11). In the case of pancreas, GSTM1-null genotype does not appear to be a risk factor for pancreatic cancer, although possession of the GSTM1*B allele may confer risk for pancreatic disease (42). Nevertheless, our studies illustrate several factors concerning the complexity of the GSTM1-positive phenotype in the pancreas. Although the M1-positive genotype is well defined, the M1-positive phenotype is not, because protein levels vary by 12.5-fold and may consist of the M1a and/or M1b subunits. Further complexity is introduced by the M1*A allele being in linkage with the M1*B allele and the suggestion that a regulatory element in the latter could cause reduced expression of the M3 subunit in M1*A individuals compared with M1*0 or M1*B individuals (3). However, in the lung, M1-positive individuals were shown to have higher levels of expression of GSTM3 than M1-negative individuals (43). This is also seen in the pancreas in the case of GSTM1-positive samples, but only when GSTM3 expression is normalized to GSTP1 levels. Thus, the effect of GSTM1-deletion is compounded by the tendency toward lower levels of expression of GSTM3.

In GSTP1, at least three alleles are known (4, 5) in which mutations in exonic sequence lead to proteins that differ in their catalytic properties. However, these differences appear to be moderate. For example, the kcat of the P1a-1a and P1b-1b homodimers toward the carcinogenic benzo(a)pyrene metabolite, (+)-anti-benzo(a)pyrene-7,8-diol-9,10-oxide, determined using cell expression protein, are 1.3 and 4.4 s−1, respectively (5). This 3.4-fold variation could be masked by variation in expression, such as the 6-fold variation of GSTP1 seen in this study. For example, two samples that were homozygous for the P1*A allele exhibited levels of expression of 1.5-1a of 4.1 and 5.3 μg/mg cytosolic protein, and two that were heterozygous, P1*A/ P1*B, express P1a and P1b subunits (presumably as a mix of homo- and heterodimers) at 1.2 and 1.7 μg/mg cytosolic protein. Assuming subunit activity to be independent of protein dimer (i.e., a 2.7-fold difference in activity between P1a-1a and P1a-1b), the catalytic activity of the cytosols would be the reverse of that predicted from genotype.

**Concluding Remarks.** The data presented here show that although pancreas-specific expression of GSTs is highly regulated, variability of phenotypic expression of GSTs in the pancreas should be taken into account when assessing the possible effects of genetic polymorphisms in the GSTs. The pancreas is not unique in this respect. Similar ranges of variation of GST expression is known to be a property of many human organs and of both normal and tumor tissue, e.g., breast (44), colon (45–47), endometrium (48), liver (49, 50), lung (43, 47), kidney (47, 49), stomach (46, 49), and hair follicles (51). In addition, the wide variation in expression of GSTs A1, A4, and A5 suggest that they are subject to inducibility beyond the ranges of normal phenotypic expression. Our results also show the complexity of alpha class GSTs in pancreas, a complexity that also exists in other human tissues (27, 28) and the significance of which remains to be determined. Despite the wide range of variation of expression of GSTs, the effect of minor regulatory elements on expression (e.g., that of GSTM3) are discernible by “normalizing” protein levels to those of the least variable component of the phenotype, GSTP1. This may be a useful approach to examine other factors responsible for GST induction in vivo.

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