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EXPRESSION OF CYTOCHROME P450 AND OTHER BIOTRANSFORMATION GENES IN FETAL AND ADULT HUMAN NASAL MUCOSA

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Abbreviations used are: CYP, cytochrome P450; PCR, polymerase chain reaction, ALDH, aldehyde dehydrogenase; FMO, flavin-containing monooxygenase; UGT, UDP-glucuronosyltransferase; and GST, glutathione S-transferase.

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ABSTRACT:

Despite recent progress in the identification and characterization of numerous nasal biotransformation enzymes in laboratory animals, the expression of biotransformation genes in human nasal mucosa remains difficult to study. Given the potential role of nasal biotransformation enzymes in the metabolism of airborne chemicals, including fragrance compounds and therapeutic agents, as well as the potential interspecies differences between laboratory animals and humans, it would be highly desirable to identify those biotransformation genes that are expressed in human nasal mucosa. In this study, a global gene expression analysis was performed to compare biotransformation enzymes expressed in human fetal and adult nasal mucosa to those expressed in liver. The identities of a list of biotransformation genes with apparently nasal mucosa-selective expression were subsequently confirmed by RNA-PCR and DNA sequencing of the PCR products. Further quantitative RNA-PCR experiments indicated that, in the fetus, ALDH6, CYP1B1, CYP2F1, CYP4B1 and UGT2A1 are expressed preferentially in the nasal mucosa, and that ALDH7, FMO1, and GSTP1 are at least as abundant in the nasal mucosa as in the liver. The nasal mucosal expression of CYP2E1 was also detected. These findings provide a basis for further explorations of the metabolic capacity of the human nasal mucosa for xenobiotic compounds.

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Biotransformation of xenobiotic compounds in the nasal mucosa may play important roles in chemical toxicity, drug efficacy, and, possibly, detection of odorants (see Ding and Dahl, 2003, for a recent review). Numerous biotransformation enzymes have been identified in the nasal mucosa in mammals, including cytochrome P450 (P450) monooxygenases, flavin-containing monooxygenases (FMO), aldehyde dehydrogenases (ALDH), alcohol dehydrogenase, carboxylesterases, epoxide hydrolases, UDP glucuronosyltransferase (UGT), glutathione transferases (GST), sulfotransferases, and rhodanese (Ding and Dahl, 2003). However, only a few of these enzymes, such as CYP2A13, have been studied in human nasal mucosa (Ding and Dahl, 2003; Ding and Kaminsky, 2003), primarily because of the lack of sufficient quantities of human nasal tissue samples for in vitro studies. A detailed knowledge of biotransformation enzyme expression in human nasal mucosa is critical for our ability to determine the roles of human nasal biotransformation enzymes in drug metabolism, chemical toxicity, and chemoreception.

Recent advances in genomic technologies have made it possible to detect and quantify gene expression using small amounts of RNA samples. In the present study, we first performed a global gene expression analysis, so as to obtain preliminary data on the biotransformation genes expressed in human fetal and adult nasal mucosa. Gene expression in fetal and adult liver was also examined and compared to that in the nasal tissues, for identification of those genes that are preferentially expressed in the nasal mucosa. The nasal expression of a list of biotransformation genes that were identified by the microarray analysis as “nasal mucosa-predominant” was subsequently confirmed by qualitative RNA-PCR and DNA sequencing. Further quantitative RNA-PCR experiments were performed to determine the relative expression levels of the apparently nasal mucosa-predominant biotransformation genes, in fetal liver and nasal mucosa. Our findings provide a basis for further explorations of the metabolic capacity of the human

nasal mucosa for xenobiotic compounds.

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Materials and Methods

Total RNA preparation. The Institutional Review Boards at the participating institutions approved the study. Human fetal liver and nasal mucosa were provided by the University of Washington Birth Defects Research Laboratory. Adult nasal biopsy samples (from the middle or upper nasal turbinate) were obtained at Shandong University, China; all subjects underwent nasal polypectomy and had normal sense of smell. The postmortem time for the fetal samples was less than 30 min, and the adult nasal biopsy samples were frozen within 30 min of dissection. Total RNA was prepared with use of Trizol reagent (Invitrogen, Carlsbad, CA). Quality of RNA was assessed by the A_{260}/A_{280} ratio and by electrophoretic analysis on denaturing gels. Autopsy liver RNA from a 27-year-old male Asian, who died suddenly of unknown causes (postmortem time unknown), was obtained from Clontech. The RNA samples were used for RNA-PCR and microarray analysis.

GeneChip microarray analysis. The Affymetrix GeneChip Instrument System was used to perform global expression analysis at the Wadsworth Center Microarray Core facility. Prior to microarray analysis, RNA samples were further purified with an RNeasy kit from Qiagen (Chatsworth, CA). The purified RNA samples were used to prepare double-stranded cDNAs, which in turn were used to synthesize biotin-labeled cRNAs. Fragmented cRNAs were hybridized to Affymetrix GeneChips (U95Av2) according to standard protocols established by the manufacturer. The U95Av2 chip contains probe sets for all full-length genes represented in Build 95 of the UniGene database. The GeneChips were washed and then stained with streptavidin-phycoerythrin using a fluidics station, and the hybridization signals were detected with a laser scanner. The data were analyzed using the Affymetrix MicroArray Suite software (version 5.0). Normalized signal intensity values were used for estimating the relative expression levels of a given transcript in differing samples. The “absolute call” (present, marginal, or

absent) for the expression of an individual transcript was determined according to statistical significance of the differences in hybridization intensity between perfect-match probe and mismatch probes for each probe set. RNA quality and labeling efficiency were confirmed by first hybridizing a portion of the labeled cRNA samples to an Affymetrix Test3 chip, prior to hybridization of the U95Av2 Chip.

Qualitative RNA-PCR. RNA-PCR was performed in a PE9600 PCR machine (PE Applied Biosystems, Foster City, CA) with Thermoscript RNase H^- reverse transcriptase (Invitrogen). First-strand cDNAs were synthesized at 50 ^oC, with the use of 2 μg total RNA, 2.5 μM oligo-$(dT)_{16}$ primer, and 5 mM $MgCl_2$, in a total volume of 20 μl. Two microliters of the reverse transcription product were added to PCR mixtures, which contained 0.5 μM each of the two primers, 0.2 mM each of dNTPs, 2 mM $MgCl_2$, 1X PCR buffer A (Promega, Madison, WI), and 2.5 U of *Thermus aquaticus* polymerase, in a total reaction volume of 50 μl. PCRs were performed for 35 cycles with the following conditions: denaturation at 95 ^oC for 30 s, annealing (at the temperature indicated in Table 1) for 30 s, and elongation at 72 ^oC for 45 s. PCR products were analyzed by electrophoresis on agarose gels, purified using the QIAquick Gel Extraction Kit (Qiagen), and sequenced by the Molecular Genetics Core facility of the Wadsworth Center, to confirm PCR specificity. Negative control reactions (no template) were routinely included, to monitor potential contamination of reagents.

For detection of CYP2E1 in human fetal nasal mucosa, the reverse transcription reaction was carried out using SuperScript III First-Strand Synthesis System (Life Technologies, Carlsbad, CA). PCR amplification was carried out in a LightCycler (Roche Diagnostics, Mannheim, Germany). Each reaction mixture, in a total volume of 10 μl, contained 1 μl of reverse transcription product, 0.8 μl of 25 mM $MgCl_2$, 0.4 μM of each primer, and 1 μl of

LightCycler FastStart DNA Master Reaction Mix SYBR Green I (Roche Applied Science, Indianapolis, IN). Gel-purified PCR product was sequenced for confirming the detection of CYP2E1.

Quantitative PCR. Real-time PCR analyses were performed in a LightCycler according to the instructions in the LightCycler Kit (Roche Applied Science). Primers (Table 2) were used at 0.25 μM in a 20-μl reaction volume. Two microliters of the reverse transcription product (diluted with water or used directly) were added per capillary, and 40 PCR cycles were monitored. At the end of the PCR cycles, melting curve analysis was performed by heating to 95°C, followed by cooling to 65°C, and gradual heating to 92°C at 0.2°C/s.

For each primer set, a serial dilution of the reverse transcription products was made, so that amounts equivalent to 0.2 μg ($1/10^{th}$) to 0.2 ng ($1/10{,}000^{th}$) of the initial total RNA used in the reverse transcription step were used to perform quantitative PCR. This was to ensure that the amounts of PCR products detected in the experimental groups fall within the linear range of PCR amplification. For all of the biotransformation enzymes studied, an amount of reverse transcription product equivalent to 0.04 μg RNA, and a final Mg^{2+} concentration of 4.0 mM, gave the best results. Therefore, these parameters were applied to each reaction. All reactions were performed in duplicate. For monitoring of the extent of potential contributions of contaminating genomic DNA templates to the final PCR products, additional control reactions were performed by omitting reverse transcriptase during the reverse transcription step.

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Results

The Affymetrix GeneChip® U95Av2 Array contains a comprehensive (although still incomplete) collection of human genes. A manual inspection identified a number of biotransformation genes represented on this chip, including genes for 6 alcohol dehydrogenases, 11 ALDHs, 31 CYPs, 2 epoxide hydrolases, 2 carboxylesterases, 6 FMOs, 12 GSTs, 13 oxidases, 17 reductases, 2 rhodaneses, 3 sulfatases, 16 sulfotransferases, and 9 UGTs. Initial screening of available human RNA samples identified four that were of suitable quality for gene array analysis (Table 3). Array analysis detected transcripts of many, but not all, of the biotransformation genes in the nasal mucosal RNA preparations, with a generally good agreement between the results from the fetal and the adult nasal mucosa (data not shown). Although the array data are preliminary, since only a single sample was analyzed for each group, a comparison of the signal intensity in liver and nasal mucosa for the biotransformation genes identified eight that are apparently expressed more abundantly in the nasal mucosa than in the liver, in both fetal and adult samples. The signal intensity values as well as the GenBank accession numbers for this subset of genes are shown in Table 3. Notably, the "absolute calls" (*absent, marginal*, or *present*), shown in Table 3, were not used as the criterion for gene selection, partly because many genes with relatively high signal intensity values were called *absent*. The list of identified genes included two ALDHs, three CYPs, one FMO, one GST, and one UGT; the latter, UGT2A1, was reported previously to be nasal mucosa-specific (Jedlitschky et al., 1999). CYP2A13, which is known to be predominantly expressed in the nose (Su et al., 2000), is not included in this list, because the CYP2A probe sets on the gene chip could not distinguish among the three known human CYP2A genes, CYP2A6, CYP2A7, and CYP2A13.

The preliminary data obtained with the gene arrays were confirmed by RNA-PCR with RNA samples from adult liver, fetal liver, and fetal nasal mucosa. Adult nasal mucosa RNA

samples were not available for these studies. Specificity of the primers used was confirmed by BLAST analysis of the GenBank database, by the electrophoretic detection of a single band of expected size, and by sequence analysis of purified PCR products (results not shown). As shown in Figure 1, transcripts of each of the eight genes were detected in total RNA preparations from fetal nasal mucosa. For fetal and adult liver, RNA-PCR products from CYP2F1, CYP4B1, and UGT2A1 were barely detected. However, such tissue selectivity was less obvious for the other five genes.

The sequences of the PCR products matched the reference sequences, except for CYP4B1. The sequence of the PCR product amplified from the nasal CYP4B1 mRNA corresponded to the splice variant found in seminal vesicles (Byland et al., 1999) and lung (Carr et al., 2003a), with a Ser207 insertion. Unlike the "wild-type" human CYP4B1 protein (Zheng et al., 1998), this CYP4B1 splice variant appears to be functional, as indicated by studies on CYP4B1(Ser207)-transgenic mice (Imaoka et al., 2001). However, although a recombinant fusion protein of CYP4B1(Ser-207) with NADPH-cytochrome P450 reductase was functional (Imaoka et al., 2001), in vitro evidence for the function of a natural CYP4B1 enzyme has yet to be obtained (e.g., Carr et al., 2003a). Sequence of the hepatic CYP4B1 PCR products was not determined, because the products were detected at very low abundance (Fig. 1). In addition, the sequence of CYP2F1 PCR product agreed with the one reported by Chen and coworkers (2002) and Carr and co-workers (2003b) (GenBank accession number NM_000774), with respect to the 12–bp segment in exon 5 that was previously found in a different position (Nhamburo et al., 1990), and the dinucleotide differences at positions 468 and 469 of the CYP2F1 cDNA.

We performed quantitative, real-time RNA-PCR to further evaluate the tissue selectivity of the expression of these eight genes in fetal liver and nasal mucosa. For most genes, new sets of PCR primers that were suitable for specific amplification in the LightCycler were designed

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(Table 2). For all primers, specificity was confirmed by melting curve analysis, by the detection of a single band of expected size on agarose gels, and by sequencing of PCR products (results not shown). Freshly prepared RNAs from the liver and nasal mucosa of two fetuses (#15777, Caucasian; #16005, African American; both male and at gestational day 91) were used for quantitative analysis. The level of β-actin mRNA was also quantified in each RNA sample, and the results were used to correct for the variability in the actual amounts of RNA added to the reactions. The primers for β-actin amplify a 396-bp product from β-actin cDNA and a 491-bp product from β-actin genomic DNA. The fact that the 491-bp product was not detected in any of the RNA samples analyzed (data not shown) indicates the absence of significant contamination by genomic DNA. In additional control experiments (not shown), in which the reverse transcriptase was omitted during first-strand cDNA synthesis, PCR products derived from contaminating genomic DNA, which contains processed β-actin pseudogenes (Hurteau and Spivack, 2002), were detected in the RNA samples. However, the amounts of genomic DNA-derived β-actin PCR product were less than 0.1% as plentiful as cDNA-derived β-actin PCR product.

The amounts of PCR products for the four RNA samples were all within the linear range of the standard curves (not shown), except in cases where no product was detected. Values obtained from duplicate reactions were consistent, with differences less than 5% of the means in threshold cycle numbers. For the standard curves, Pearson's correlation coefficients from linear regression were always greater than 0.95. As shown in Table 4, the relative mRNA levels were consistent between the two fetuses examined. Intra-individual comparisons of mRNA levels in the two tissue types indicate that ALDH6, CYP1B1, CYP2F1, CYP4B1, and UGT2A1 were expressed at much higher levels in the nasal mucosa than in the liver. On the other hand, FMO1

and GSTP1 appear to be expressed at similar levels in the two tissues. For ALDH7, a relatively large inter-individual difference was found, primarily because of differences in the levels of hepatic ALDH7 mRNA; the tissue expression ratios were 10 and 2.9, respectively, with higher levels in the nasal mucosa than in liver.

A comparison of the results in Table 3 and Table 4 indicates that some transcripts, such as CYP2F1, which had been classified as *marginal* or *absent* in the gene array analysis (Table 3), were actually expressed in the nasal mucosa (Table 4). The same situation was found for the expression data for CYP2E1; this enzyme was called *absent* in the nasal mucosa, but was detected by qualitative RNA-PCR and DNA sequencing (data not shown). These discrepancies likely reflect the differing specificities and sensitivities of the two techniques.

The fetal nasal mucosa tissue used in this study was known to contain the olfactory region, because the entire middle and superior turbinates had been used for RNA preparation. The adult nasal biopsies also likely contained olfactory mucosa, since, of the three samples pooled, two were from the superior turbinate, and one was from the upper part of the nasal septum. Similarly dissected tissues have previously been found (Chen et al., 2003) to express olfactory marker protein (OMP), a well-known marker for the olfactory neuroepithelium (Buiakova et al., 1994). Notably, although the OMP gene was not included on this chip, several neuronal transcripts, such as human olfactory receptor OLF3 (OR2F1, accession no. U56421), were *present* in the nasal mucosa, but *absent* in liver (data not shown), a finding that further supports the presence of olfactory mucosa in the tissue used here.

Discussion

Little is known about the metabolic capacity of the human nasal mucosa for numerous xenobiotic compounds. The lack of sufficient quantities of human nasal tissue samples has made it very difficult to directly determine the metabolic activity of nasal biotransformation enzymes. The knowledge of the transcripts expressed, and their relative levels in differing nasal mucosal samples, will facilitate future studies to characterize the expression of the corresponding proteins, which is at present a more difficult task than studies on RNA expression. Once the expression of a biotransformation gene has been confirmed, we may be able to predict the metabolic capacity of the nasal mucosa based on the results of in vitro studies that use heterologously expressed enzymes.

In this study, using a combination of gene array analysis and RNA-PCR, we have identified transcripts for nine biotransformation genes in human nasal mucosa, namely, ALDH6, ALDH7, CYP1B1, CYP2E1, CYP2F1, CYP4B1, FMO1, GSTP1, and UGT2A1. Furthermore, using quantitative RNA-PCR, we have shown that ALDH6, CYP1B1, CYP2F1, CYP4B1, and UGT2A1 are expressed at much higher levels in the nasal mucosa than in the liver of the same fetuses. These findings, combined with previous detection of CYP2A6, CYP2A13, CYP2B6, CYP2C, CYP2J2, CYP3A, NADPH-cytochrome P450 reductase, microsomal epoxide hydrolase, GSTA, GSTP1, and UGT2A1 in human nasal mucosa (Ding and Dahl, 2003; Ding and Kaminsky, 2003), provide strong support for the idea that human fetal as well as adult nasal mucosa plays an active role in the biotransformation of numerous xenobiotic compounds.

The nasal expression of human ALDH6, ALDH7, CYP1B1, CYP2E1, CYP2F1, CYP4B1, and FMO1 had not been reported previously. GSTP1 and UGT2A1 were previously detected in adult human nasal mucosa (Krishna et al., 1995; Jedlitschky et al., 1999), but their fetal expression had not been examined. In human adults, GSTP1 immunoreactivity was detected

in the supporting cells in the olfactory mucosa by immunohistochemical analysis (Krishna et al., 1995). UGT2A1 was cloned from a human nasal mucosal cDNA library, and was characterized following heterologous expression (Jedlitschky et al., 1999). Expression of UGT2A1 mRNA was not detected in adult human liver or several other tissues by RNA-PCR, implying that this gene is expressed in a tissue-specific fashion. The nasal mucosa specificity was found for UGT2A1 expression in human fetuses in the present study.

It should be noted that, although we have selected the potentially nasal mucosa-predominant transcripts for characterization in this study, those nasal biotransformation genes that are expressed at similar levels in the liver and in the nasal mucosa could also be important for nasal metabolism. To that end, the present microarray analysis detected the expression of many other biotransformation genes, as well as genes in other functional categories, in fetal as well as adult human nasal mucosa. These results, although preliminary, should serve as a valuable reference for future studies on gene expression in fetal and adult human nasal mucosa. A survey of current literature found several other studies that used gene array methods to identify genes expressed in human nose. In one study, gene expression was compared between nasal polyp tissues and normal nasal mucosal samples from sphenoid sinuses (Liu et al., 2004). The gene expression profiles in nasal polyps before and after local treatment with fluticasone were compared in another study (Benson et al., 2004). In an earlier study, gene expression profiles in nasal mucosa from patients with allergic rhinitis were determined, and compared between patients with nasal polyps and those without (Fritz et al., 2003). In these studies on adult nasal tissues, the expression of many biotransformation genes, including the nine genes identified in the present study, was suggested by the array data, although further confirmation was not carried out. It would be important to examine the impact of nasal diseases, such as rhinitis, on the expression of biotransformation enzymes in differing nasal tissues, as local metabolism of

nasally administered drugs could influence efficacy, or could potentially lead to adverse drug reactions.

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Footnote

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Legend for figure

Fig. 1. *Qualitative RNA-PCR analysis of biotransformation gene expression in fetal nasal mucosa and liver and adult liver.*

RNA-PCR was performed as described in Materials and Methods, with primer pairs and annealing temperatures shown in Table 1. PCR products (10 μl each), generated from the same RNA samples as were used for microarray analysis (lanes 1-3, for fetal nasal mucosa, fetal liver, and adult liver, respectively), were analyzed on agarose gels and were visualized by staining with ethidium bromide. The identities of the nasal mucosal PCR products were confirmed by sequencing. The positions of selected size markers are indicated; M, a 100-bp molecular weight marker.

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TABLE 1

Primers used in qualitative RNA-PCR

Primers were designed according to the sequences in GenBank and are numbered according to the reference sequence associated with the indicated accession number.

Gene	Primers	Sequence (5'-3')	Product size (bp)	Annealing temperature (^{o}C)	Position (5')	Accession No.
ALDH6	Sense	agcccagagagcttag	405	58	2274	U07919
	Antisense	tgaagaccgaggtcct			2678	
ALDH7	Sense	aggattgagggattgaggg	510	59	2245	U10868
	Antisense	agagagacaggagacggaac			2754	
CYP1B1	Sense	gacatgatggacgcctttat	569	55	1217	U03688
	Antisense	agctgcatcttagaaagttc			1785	
CYP2E1	Sense	actatgggatggggaaacag	353	57	439	NM_000773
	Antisense	ctccttcacccttcagaca			791	
CYP2F1	Sense	tggggaagagaagcattgag	486	55	465	J02906
	Antisense	cgccaaagagcaggttatgt			950	
CYP4B1	Sense	taaggcccctgatgtgtatg	369	57	339	J02871
	Antisense	tggtactggaaggacacaag			707	
FMO1	Sense	gttactcagactttccattcc	579	57	301	M64082
	Antisense	ttatctttcgctccatcaacc			879	
GSTP1	Sense	acgtggcaggagggctcactc	485	57	125	U62589
	Antisense	tactcaggggaggccaggaa			609	
UGT2A1	Sense	ttaccacggagtccct	360	60	1209	AJ006054
	Antisense	gccgttgtcacacaga			1568	

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TABLE 2

Primers used in quantitative RNA-PCR

Primer pairs for ALDH6, ALDH7, and CYP4B1 were the same as in Table 1. The accession number for β-actin is BC009275.

Primer	Sequence (5'-3')	Size of PCR product (bp)	Annealingtemperature (^{o}C)	Position (5')
ALDH6F2274	agcccagagagcttag	405	67	2274
ALDH6R2678	tgaagaccgaggtcct			2678
ALDH7F2245	aggattgagggattgaggg	510	65	2245
ALDH7R2754	agagagacaggagacggaac			2754
β-actinE3F	cctgactgactacctcatg	396	60	1105
β-actinE4R	tccttctgcatcctgtcggca			1500
CYP1B1F1	cccagtcgtcatcagat	221	62	4304
CYP1B1R1	cattctctctccggtaga			4524
CYP2F1F1	gattctacggaatttcggg	266	62	445
CYP2F1R1	tgtcgtacaactcgcc			710
CYP4B1F	taaggcccctgatgtgtatg	369	60	339
CYP4B1R	tggtactggaaggacacaag			707
FMO1F1363	tgtgctactgcaaggc	227	60	1363
FMO1R1589	cttgaatgttcggtccc			1589
GSTp1F1	agtcttcgccaccatgccg	375	60	1
GSTp1R1	ttcacatagtcatccttgcccac			375
UGT2A1F78	ttggccaatggaaggtagtc	208	60	138
UGT2A1R285	ccatgtcgaaacgaagtcct			345

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TABLE 3

Gene array analysis of comparative expression of biotransformation genes in adult and fetal human liver and nasal mucosa

RNA samples were from the liver (FL) and nasal mucosa (FN) of a Caucasian female fetus at the gestational age of 115 days (#16029), autopsy liver tissue from a 27-year-old Asian male (AL), and nasal mucosa (AN) from pooled biopsy samples from three male Asian subjects (32, 52, and 66 years old, respectively). Only genes with apparently selective expression in the nasal mucosa are shown. The absolute call was *present*, unless indicated otherwise.

Probe Set	Gene	Accession No.	Signal Intensity (Arbitrary units)			
			FN	FL	AN	AL
36686_at	ALDH6	U07919	1050	129[a]	1490	248[a]
40685_at	ALDH7	U10868	2640	380[a]	2800	708[a]
40071_at	CYP1B1	U03688	1810	163[a]	4360	270[a]
859_at	CYP1B1	U03688	1260	98[a]	3150	95[a]
1517_at	CYP2F1	J02906	5730[b]	635[a]	3070[a]	1530[a]
1667_s_at	CYP4B1	J02871	7370	1700[a]	5120	2850[a]
31806_at	FMO1	M64082	625	59[a]	512	99[a]
33396_at	GSTP1	U12472	19600	8570	14500	6020
829_s_at	GSTP1	U21689	26100	12000	17700	5410[a]
31646_at	UGT2A	AJ006054	2110	58[a]	1510	171[a]

[a] *Absolute call: absent*

[b] *Absolute call: marginal*

TABLE 4

Relative mRNA levels in fetal nasal mucosa and liver

Quantitative RNA-PCR was performed as described in Materials and Methods, with use of primers and annealing temperatures shown in Table 2. RNA preparations were from liver and nasal mucosa of two fetuses (#15777 and #16005), both at gestational day 91. The relative levels of each transcript in the four samples were determined with standard curves generated using serial dilutions of pooled nasal RNA. Experiments were performed in duplicate, and the results were corrected on the basis of the levels of β-actin mRNA present in each preparation.

Gene	Relative mRNA level (arbitrary units)[a]				Ratio, nasal mucosa:liver		
	#15777		#16005		#15777	#16005	Average
	Nasal mucosa	Liver	Nasal mucosa	Liver			
ALDH6	5.0	0.27	2.5	0.27	18	9.2	14
ALDH7	1.6	0.16	1.7	0.59	10	2.9	6.4
CYP1B1	1.8	0.10	1.5	0.091	18	16	17
CYP2F1	10	0.20[b]	11	0.24[b]	50	46	48
CYP4B1	15	0.09	6.7	0.07	167	96	132
FMO1	1.6	1.8	1.7	2.3	0.9	0.7	0.8
GSTP1	2.6	1.0	2.1	1.1	2.6	1.9	2.2
UGT2A1	12	0.61	7.3	0.41	20	18	19

[a] For comparisons among the four samples for each gene.

[b] No product was detected in one of the two reactions.

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Figure 1