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# Mouse lung CYP1A1 catalyzes the metabolic activation of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)

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**2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) carcinogenesis is initiated by $N^2$-hydroxylation, mediated by several cytochromes P450, including CYP1A1. However, the role of CYP1A1 in PhIP metabolic activation *in vivo* is unclear. In this study, *Cyp1a1*-null and wild-type (WT) mice were used to investigate the potential role of CYP1A1 in PhIP metabolic activation *in vivo*. PhIP $N^2$-hydroxylation was actively catalyzed by lung homogenates of WT mice, at a rate of 14.9 ± 5.0 pmol/min/g tissue, but <1 pmol/min/g tissue in stomach and small intestine, and almost undetectable in mammary gland and colon. PhIP $N^2$-hydroxylation catalyzed by lung homogenates of *Cyp1a1*-null mice was ∼10-fold lower than that of WT mice. In contrast, PhIP $N^2$-hydroxylation activity in lung homogenates of *Cyp1a2*-null versus WT mice was not decreased. Pretreatment with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin increased lung *Cyp1a1* mRNA and lung homogenate PhIP $N^2$-hydroxylase activity ∼50-fold in WT mice, where the activity was substantially inhibited (70%) by monoclonal antibodies against CYP1A1. *In vivo*, 30 min after oral treatment with PhIP, PhIP levels in lung were similar to those in liver. After a single dose of 0.1 mg/kg [$^{14}$C]PhIP, lung PhIP-DNA adduct levels in *Cyp1a1*-null mice, but not in *Cyp1a2*-null mice, were significantly lower ($P = 0.0028$) than in WT mice. These results reveal that mouse lung has basal and inducible PhIP $N^2$-hydroxylase activity predominantly catalyzed by CYP1A1. Because of the high inducibility of human CYP1A1, especially in cigarette smokers, the role of lung CYP1A1 in PhIP carcinogenesis should be considered. (237 words).**

## Introduction

2-Amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP), the most abundant dietary heterocyclic amine, is carcinogenic in multiple organs and in numerous species. PhIP carcinogenesis is initiated by PhIP $N^2$-hydroxylation (1), which has been reported to be primarily catalyzed by CYP1A2 (2–4). However, recent studies raise the possibility of the CYP1A2-independent pathways for PhIP metabolic activation. In humans, excretion of the glucuronide conjugate of $N^2$-hydroxy PhIP (N-OH-PhIP) in urine shows a low correlation with CYP1A2 activity (5). In a mouse carcinogen bioassay with PhIP, the incidence of lymphomas and hepatocellular adenomas is higher in *Cyp1a2*-null mice than WT mice (6). Moreover, PhIP–DNA adducts are detectable in mammary gland and colon in *Cyp1a2*-null mice (7).

Multiple *in vitro* studies have revealed that CYP1A1 catalyzes PhIP metabolic activation (8–11). cDNA-expressed human CYP1A2, CYP1A1 and CYP1B1, respectively, catalyze N-OH-PhIP formation with maximal velocities ($V_{max}$) of 90, 16 and 0.2 nmol/min/nmol P450, and apparent $K_m$ values of 79, 5.1 and 4.5 μM (12). Thus, although CYP1A2 exhibited the highest *V*max for N-OH-PhIP production, the catalytic efficiency ($V_{max}/K_m$) of CYP1A1 is 2.75-fold higher than that of CYP1A2. With CYP1B1, PhIP $N^2$-hydroxylation activity is 80-fold lower than that of CYP1A1 and formation of the non-mutagenic metabolite 4′-Hydroxy-PhIP (4-OH-PhIP) is favored ∼2-fold over that of the promutagenic metabolite N-OH-PhIP (12,13). Given the high catalytic efficiency of CYP1A1 for PhIP metabolic activation, it is important to establish the potential role of extrahepatic CYP1A1 in PhIP metabolism and carcinogenesis. Such studies are particularly important because PhIP induces tumors in several extrahepatic organs. In the current study, PhIP $N^2$-hydroxylase activity was detected in extrahepatic organs including lung, stomach, small intestine, colon and mammary gland. *Cyp1a2*-null mice were used to examine CYP1A2-independent PhIP metabolic pathways and *Cyp1a1*-null mice were used to assess the potential role of CYP1A1 in PhIP metabolic activation.

## Materials and methods

### *Chemicals and reagents*

PhIP and N-OH-PhIP were obtained from the National Cancer Institute Chemical Carcinogen Reference Standard Repository at the Midwest Research Institute (Kansas City, MO). [$^{14}$C]PhIP, >95% radio-pure, was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). 4-OH-PhIP was provided by Dr M. Nagao (National Cancer Research Center, Tokyo, Japan). NADPH and 6-chloromelatonin were purchased from Sigma-Aldrich (St Louis, MO). Monoclonal antibodies against CYP1A1 (mAb 1-7-1) were used to study CYP1A1 inhibition (14,15). High-performance liquid chromatography solvents and other chemicals were of the highest grade commercially available. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was obtained from Dr Steven Safe at Texas A&M University.

### *Animals and treatments*

*Cyp1a1*-null mice (16), *Cyp1a2*-null mice (17), and their corresponding wild-type (WT) mice, female, 2–4 months old, were maintained under a standard 12 h light/12 h dark cycle with water and chow provided *ad libitum*. Handling was in accordance with animal study protocols approved by the National Cancer Institute Animal Care and Use Committee. For CYP1A1

**Abbreviations:** PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; N-OH-PhIP, $N^2$-hydroxy-PhIP; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

induction, WT and *Cyp1a1*-null mice were injected *i.p.* with 10 μg/kg TCDD and killed 72 h after dosing. TheTCDD dose and time was chosen were based on earlier published studies showing maximal induction of CYP1A1 (18). For the *in vivo* PhIP distribution, WT mice were treated by oral gavage with 40 mg/kg PhIP. Thirty minutes after PhIP treatment, mice were killed by $CO_2$ asphyxiation; lung, liver, mammary gland and colon were collected and frozen at −80°C for further analysis. For PhIP–DNA adducts detection, *Cyp1a1*-null mice, *Cyp1a2*-null mice and WT mice were treated orally with 100 μg/kg of [$^{14}$C]PhIP (6.3 μCi/kg). The mice were killed 18 h after administration, and tissues were collected and frozen at −80°C for further analysis.

*PhIP metabolism in extrahepatic organs*

PhIP metabolism in extrahepatic organs was detected *in vitro* using tissue homogenates. To prepare homogenates, each organ was washed with 20 mM phosphate-buffered saline (PBS), pH 7.4, and homogenized (500 μl PBS/100 mg tissue) by a motor-driven Teflon-tipped pestle. PhIP metabolism in homogenates (20 mg tissue) were assayed in a final volume of 400 μl, containing 20 mM PBS, pH 7.4, 10 μM PhIP and 1 mM NADPH. To assess the role of CYP1A1 in PhIP metabolism, monoclonal antibodies against CYP1A1 (mAb 1-7-1) were pre-incubated for 5 min with lung homogenates at 37°C. Reactions were initiated by the addition of NADPH and terminated 10 min later by the addition of 1.0 ml ethyl acetate and 1.0 ml methyl *tert*-butyl ether. 6-Chloromelatonin (5 μl of 100 μM stock solution) was added as an internal standard. Samples were centrifuged at 3000 r.p.m. for 5 min at 4°C. The organic layer was then transferred to a new tube, dried with $N_2$ and reconstituted in 100 μl of 70% methanol and 30% $H_2O$ containing 0.1% formic acid. All reactions were performed in duplicate. PhIP metabolites were detected by LC-MS/MS.

*PhIP tissue distribution*

PhIP in various tissues was extracted 30 min after a single oral dose of PhIP administered to WT mice. Briefly, 100 mg of each tissue was homogenized in 500 μl PBS, and 300 μl of each homogenate was extracted with a mixture of 1.0 ml ethyl acetate and 1.0 ml methyl *tert*-butyl ether. 6-Chloromelatonin (5 μl of 100 μM solution) was added as an internal standard. The extracted samples were dried with $N_2$ and reconstituted in 100 μl of 70% methanol and 30% $H_2O$ containing 0.1% formic acid. An aliquot of 6 μl of each sample was used for LC-MS/MS analysis.

*Identification and quantification of PhIP and PhIP metabolites by LC-MS/MS*

PhIP metabolites were detected by LC-MS/MS, as described previously (4). Briefly, LC-MS/MS analysis was performed using a PE SCIEX API 2000 ESI triple quadrupole mass spectrometer (PerkinElmer/ABI, Foster City, CA). A Luna C18 50 mm × 4.6 mm i.d. column (Phenomenex, Torrance, CA) was used to separate PhIP metabolites. The flow rate through the column at ambient temperature was 0.25 ml/min with 70% methanol and 30% $H_2O$ containing 0.1% formic acid. The mass spectrometer was equipped with a turbo ion spray source and run in the positive ion mode. The turbo ion spray temperature was maintained at 350°C and a voltage of 4.8 kV was applied to the sprayer needle. $N_2$ was used as the turbo ion spray and nebulizing gas. Identification and quantification of PhIP metabolites and the internal standard were accomplished by multiple reactions monitoring with the transitions m/z 225.2/210.2 for PhIP, 241.2/223.2 for N-OH-PhIP, 241.2/226.2 for 4-OH-PhIP and 267.0/208.4 for 6-chloromelatonin.

*Lung Cyp1a1 mRNA detection*

Relative levels of mouse lung *Cyp1a1* RNA were quantified by real-time quantitative PCR (qPCR) using SYBR Green I chemistry. Total RNA was isolated from individual mouse lung samples obtained from untreated and TCDD-treated WT and *Cyp1a1*-null mice, using Trizol reagent (Invitrogen Life Technologies, Carlsbad, CA) and the manufacturer's protocol for tissue. The isolated RNA was treated with ribonuclease-free deoxyribonuclease followed by reverse transcription using random hexamers and murine leukemia virus reverse transcriptase to yield cDNA using the GeneAmp RNA core kit (Applied Biosystems, Foster City, CA). One microgram of total lung RNA was converted to cDNA in a final vol of 20 μl according to the manufacturer's protocol. Triplicate samples of each qPCR mixture, each containing 4 μl of SYBR Green I PCR master mix (Applied Biosystems), were transferred into separate wells of a 384-well plate and run through 40 cycles on an ABS 7900HT Sequence Detection System (Applied Biosystems) (19). Samples were initially incubated at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Dissociation curves were generated after each qPCR run to ensure that a single, specific product was amplified. Results were analyzed using the comparative $C_T$ ($\Delta\Delta C_T$) method, as described in User Bulletin 2 of the ABI PRISM 7700 Sequence Detection System. Data are graphed as relative RNA values, normalized to the 18S rRNA content of each sample. Similar results were obtained by normalization to β-actin RNA (data not shown). qPCR primers for *Cyp1a1* were as follows: forward primer ON-1582 (5′-GGT TAA CCA TGA CCG GGA ACT-3′) and reverse primer ON-1583 (5′-TGC CCA AAC CAA AGA GAG TGA), yielding an amplicon length of 122 nt. The forward primer was designed to span the junction between exons 6 and 7 of the mouse *Cyp1a1* gene (NM_009992) to eliminate amplification of any contaminating genomic DNA. Primers for β-actin (forward primer 5′-TCC ATC ATG AAG TGT GAC GTT-3′; reverse primer 5′-TGT GTT GGC ATA GAG GTC TTT ACG-3′) and 18S rRNA (forward primer 5′-CGC CGC TAG AGG TGA AA TC-3′; reverse primer 5′-CCA GTC GGC ATC GTT TAT GG-3′) were as shown. Primer specificity was verified by BLAST analysis.

*Lung PhIP–DNA adduct detection*

DNA isolation from mouse lung, and sample preparation for the quantification of DNA adduct levels by accelerator mass spectrometry (AMS) has been reported elsewhere (20). Briefly, lung tissues were homogenized then digested in lysis buffer (4 M urea, 1.0% Triton X-100, 10 mM EDTA, 100 mM NaCl 10 mM DTT, 10 mM Tris–HCl, pH 8.0) containing 0.8 mg/ml proteinase K overnight at 37°C. Undigested tissue was removed by centrifugation, and the supernatant was treated for 1 h at room temperature with RNase A, (0.5 mg/ml) and RNase T1 (5 μg/ml). DNA was extracted using Qiagen column chromatography (Qiagen, Valencia, CA) according to the manufacturer's instructions. DNA purity was determined by the A260 nm/A280 nm ratio. A ratio between 1.6 and 1.8 was considered pure. Pure DNA samples were then submitted for adduct analysis by AMS.

*Statistical analysis*

All values are expressed as the mean ± SD and analyzed by Student's *t*-test. $P < 0.05$ was regarded as significantly different between the compared groups.

## Results

### *PhIP metabolism in extrahepatic organs*

Extrahepatic PhIP metabolism was investigated using tissue homogenates prepared from WT mouse lung, stomach, small intestine, colon and mammary gland. The velocity of PhIP $N^2$-hydroxylation was highest in the lung, 14.9 ± 5.0 pmol/min/g tissue, whereas it was below 1 pmol/min/g tissue in stomach and small intestine, and almost undetectable in mammary gland and colon (Figure 1A). PhIP 4′-hydroxylation, a non-mutagenic metabolic pathway, exhibited similar tissue specificity. PhIP $N^2$-hydroxylation in lung is NADPH- and enzyme(s)-dependent, as N-OH-PhIP was not detected in the absence of NADPH or tissue homogenate (Figure 1B).

### *PhIP metabolism in lung is CYP1A1-dependent*

PhIP metabolism was assayed in lung homogenates prepared from *Cyp1a1*-null mice, *Cyp1a2*-null mice and WT mice. There was a marked decrease in PhIP $N^2$-hydroxylation and 4′-hydroxylation activities in *Cyp1a1*-null mouse lung homogenates (Figure 2B) compared to WT mice (Figure 2A). The ∼10-fold lower PhIP $N^2$-hydroxylase activity in *Cyp1a1*-null mouse lung homogenates contrasts with a ∼50% higher PhIP $N^2$-hydroxylase activity in *Cyp1a2*-null mouse lung homogenates (Figure 2C). The increase of PhIP $N^2$-hydroxylase activity in *Cyp1a2*-null mouse lung homogenates can be significant inhibited (∼90%) by monoclonal antibody (mAb) against CYP1A1. These data indicate that PhIP hydroxylation activity in lung is largely mediated by CYP1A1, rather than CYP1A2.

### *Effect of CYP1 inducer on PhIP hydroxylase activity in lung*

In lung homogenates of WT mice pretreated with the CYP1 inducer TCDD, PhIP $N^2$-hydroxylase activity was increased ∼50-fold, to 636 ± 167 pmol/min/g tissue, compared

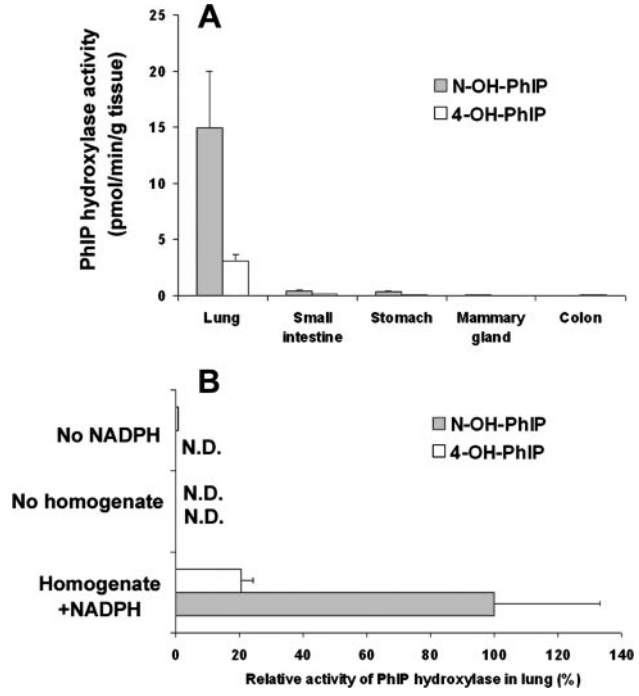


**Fig. 1.** PhIP hydroxylase activity in extrahepatic organs of WT mice. (**A**) PhIP hydroxylase activity in homogenates of lung, small intestine, stomach, mammary gland and colon. (**B**) Relative PhIP hydroxylase activity in lung homogenates. The incubations were carried out with or without homogenates (20 mg tissue) and/or 1 mM NADPH. PhIP metabolites were detected by LC-MS/MS. Data are expressed as mean ± SD ($n = 3$).

to untreated WT controls (Figure 3A). Similar results were obtained for PhIP 4′-hydroxylation. PhIP $N^2$-hydroxylation in lung homogenates of TCDD-treated *Cyp1a1*-null mice was ~80% lower than that of TCDD-treated WT mice (Figure 3B). mAb against CYP1A1 inhibited PhIP $N^2$-hydroxylation in WT lung by ~70%, but had no significant effect on that of *Cyp1a1-null* mice (Figure 3B). Consistent with the activity increase, lung *Cyp1a1* mRNA was increased ~50-fold in WT mice after TCDD treatment. Moreover, *Cyp1a1* mRNA was readily detectable in untreated WT mouse lung at ~2–2.5% the level of TCDD-treated WT mouse lung (Figure 4). While CYP1A1 protein was readily detected in TCDD-treated mouse lung, the protein was not detected in untreated mouse lung tissues, even though PhIP $N^2$-hydroxylation activity was readily detected. This is due to the limited sensitivity of western blotting compared to the high sensitivity of the LC-MS for detection of the $N^2$-hydroxy metabolite of PhIP.

### *PhIP distribution in mouse lung*

Thirty minutes after oral PhIP treatment (40 mg/kg), mouse lung, liver, mammary gland and colon tissue were collected, and PhIP distribution was assayed by LC-MS/MS. Liver had the highest PhIP concentration at 46.4 nmol/g tissue (Figure 5). Interestingly, lung had similar PhIP level as liver (41.9 nmol/g tissue). PhIP concentration in mammary gland and colon was lower than that of liver and lung (22.1 and 29.4 nmol/g tissue, respectively). The high level of PhIP in lung raises the possibility of localized PhIP metabolic activation by lung CYP1A1. There was no significant difference in PhIP tissue distribution between WT, *Cyp1a1*-null and *Cyp1a2*-null mice (data not shown).

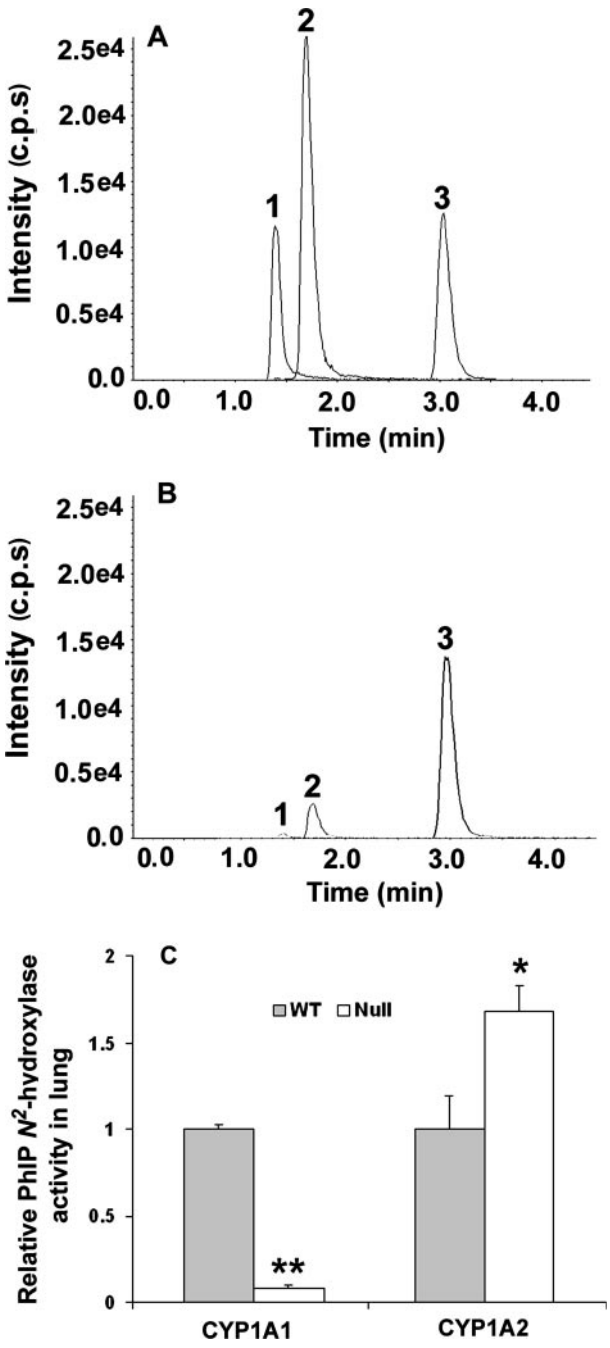


**Fig. 2.** PhIP metabolism in lung homogenates. (**A**) Typical chromatography of PhIP metabolites in lung homogenates of WT mice. (**B**) Typical chromatography of PhIP metabolites in lung homogenates of *Cyp1a1*-null mice. PhIP metabolites were detected by LC-MS/MS, 241.2/226.2 for 4-OH-PhIP (1), 241.2/223.2 for N-OH-PhIP (2), and 267.0/208.4 for 6-chloromelatonin (3, as an internal standard). (**C**) Relative quantification of PhIP $N^2$-hydroxylase activity in lung homogenates of *Cyp1a1*-null mice and *Cyp1a2*-null mice. PhIP $N^2$-hydroxylase activity in WT mouse lung homogenates was set as 1.0. Data are expressed as mean ± SD ($n = 3$). $^{**}P < 0.01$ and $^{*}P < 0.05$ compared with WT mice.

### *PhIP-DNA adducts in lung*

PhIP-DNA adducts in lung of *Cyp1a1*-null mice, *Cyp1a2*-null mice and WT mice were analyzed after a single dose of 100 μg/kg [$^{14}$C]PhIP (6.3 μCi/kg). PhIP-DNA adducts were detected by AMS. PhIP-DNA adducts were significantly lower (46%) in *Cyp1a1*-null mouse lung than that in WT

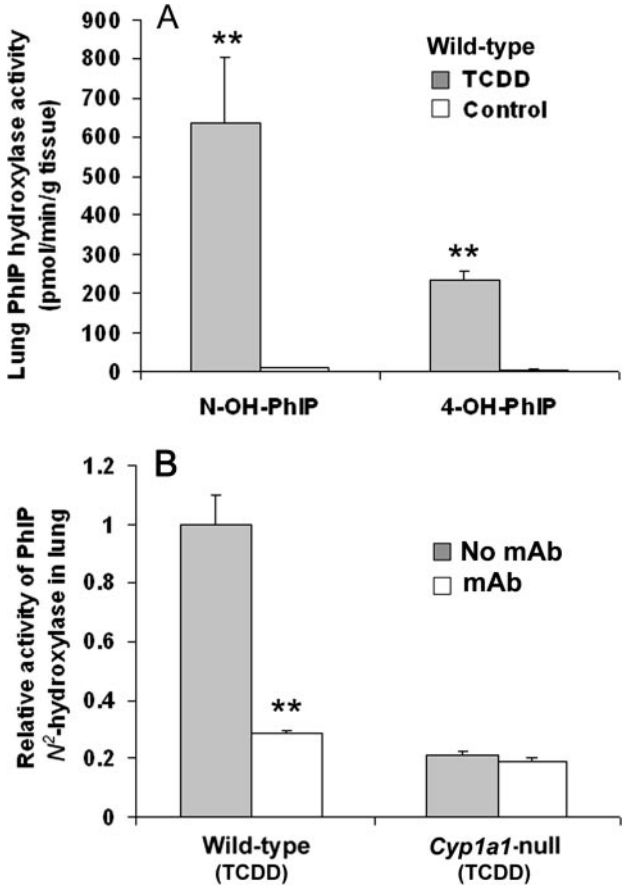


**Fig. 3.** PhIP hydroxylase activity in lung homogenates of WT versus *Cyp1a1*-null mice, with or without TCDD pretreatment. (**A**) Comparison of PhIP hydroxylase activity in lung homogenates of untreated versus TCDD-treated WT mice. Data are expressed as mean ± SD ($n = 3$). $^{**}P < 0.01$ compared with that of untreated WT mice. (**B**) Comparison of PhIP $N^2$-hydroxylase activity in lung homogenates of TCDD-treated WT versus *Cyp1a1*-null mice. PhIP $N^2$-hydroxylase activity in WT lung homogenates without monoclonal antibodies (mAb) against CYP1A1 was set as 1.0. Data are expressed as mean ± SD ($n = 3$). $^{**}P < 0.01$ compared to that of WT without mAb.

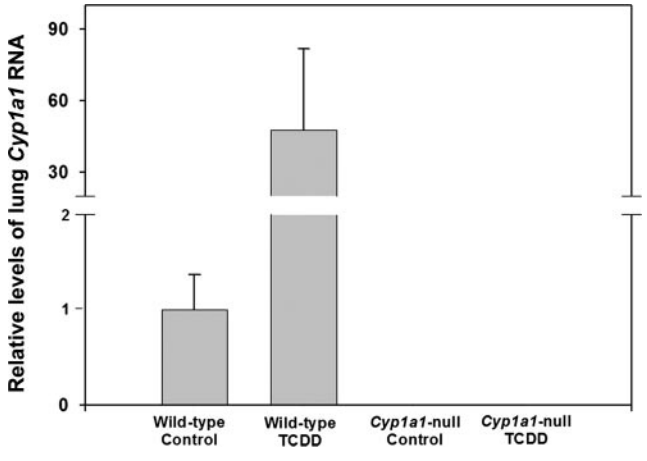


**Fig. 4.** Relative levels of lung *Cyp1a1* mRNA in untreated and TCDD-treated WT and *Cyp1a1*-null mice. Lung *Cyp1a1* RNA was quantified by real-time quantitative PCR (qPCR) using SYBR Green I chemistry. Data are graphed as relative values, normalized to the 18S rRNA content of each sample, and expressed as the mean ± SE ($n = 6$ for untreated, $n = 2$ for TCDD-treated). Lung *Cyp1a1* RNA in untreated WT group was set as 1.0.

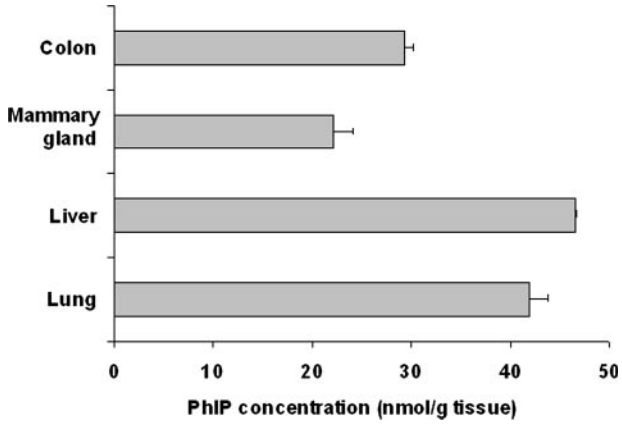


**Fig. 5.** PhIP tissue distribution in WT mice, following oral administration of PhIP (40 mg/kg). Thirty minutes after PhIP treatment, mouse lung, liver, mammary gland and colon were collected. PhIP concentration in various tissues was analyzed by LC-MS/MS. Data are expressed as mean ± SD ($n = 3$).

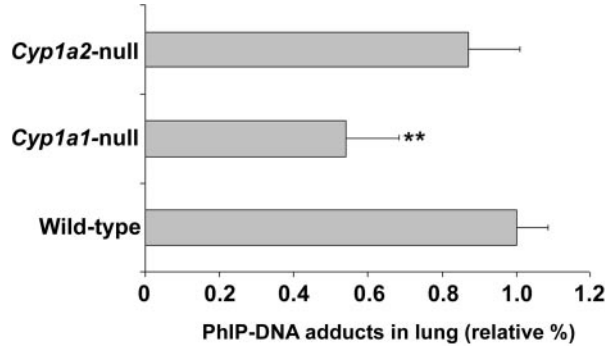


**Fig. 6.** PhIP-DNA adducts in lung. *Cyp1a1*-null mice, *Cyp1a2*-null mice and WT mice were treated orally with 100 μg/kg [$^{14}$C]PhIP (6.3 μCi/kg). The mice were killed 18 h after administration, and lung DNA was isolated for PhIP–DNA adducts detection by accelerator mass spectrometry (AMS). PhIP-DNA adducts in WT lung were set as 1.0. Data are expressed as mean ± SD ($n = 4$). $^{**}P < 0.01$ compared with WT.

mouse lung ($P = 0.0028$). In contrast, there was no significant change in PhIP-DNA adduct levels in *Cyp1a2*-null lung ($P = 0.177$) (Figure 6).

## Discussion

PhIP $N^2$-hydroxylation is considered as the initiating step in PhIP carcinogenesis (1). Previous studies showed PhIP metabolic activation by lung microsomes, using mutagenicity as an endpoint (21). In the present study using *Cyp1a1*-null versus *Cyp1a2*-null mice, PhIP $N^2$-hydroxylase activity in lung was shown to be CYP1A1-dependent. Expression of CYP1A1 in human lung has been well characterized (22,23); a centrilobular expression of *CYP1A1* mRNA and CYP1A protein was observed in peripheral lung (24). Both constitutive and inducible lung CYP1A1 have been quantified, and the median levels of CYP1A1 were 15.5 pmol/mg microsomal protein in smokers, 6.0 pmol/mg microsomal protein in non-smokers and 19.0 pmol/mg microsomal protein in ex-smokers (25). Lung CYP1A1 activity is increased ~100-fold in smokers as compared with that of non-smokers (26,27). In the current study, lung CYP1A1 expression and PhIP

$N^2$-hydroxylase activity were readily detectable in untreated WT mice. After TCDD treatment, lung CYP1A1 RNA and PhIP $N^2$-hydroxylase activity were both markedly induced. These results raise the possibility of localized CYP1A1-mediated PhIP metabolic activation in lung that is of importance due to different routes of PhIP exposure to lung. The diet is considered the most common source of PhIP, and orally administered PhIP has been shown to localized in lung (28). In the present study with oral PhIP treatment, lung had a PhIP concentration similar to that in liver. Besides oral exposure, inhalation is another important pathway for PhIP exposure to the lung. PhIP is detected in all brands of filter-tipped cigarettes, and the mean level of PhIP in cigarette mainstream smoke has been estimated at 16.4 ng/cigarette (29). PhIP is also detected in airborne particles, diesel-exhaust particles and incineration ash from garbage-burning plants (30). PhIP-DNA adduct is considered a reliable biomarker for PhIP exposure, and it has been detected in lung in several previous studies. In monkeys after a single oral dose of PhIP, DNA adducts were highest in the liver followed by the lung (31). PhIP-DNA adducts in mouse lung has also been reported, and these levels were significantly decreased by inhibiting the activity of CYP1A1 and CYP1A2 (32). In the present study, lung PhIP-DNA adducts were significant lower in lung tissue of *Cyp1a1*-null mice, but not *Cyp1a2*-null mice, compared to WT mice, which highlights the importance of CYP1A1 in PhIP metabolic action in lung. These data also demonstrate the utility of comparing various knockout mouse lines instead of relying on CYP inhibitor studies (33). Compared to the *in vitro* data supporting a role for lung CYP1A1 (~90%) in PhIP $N^2$-hydroxylation, the apparent contribution of CYP1A1 (~50%) to PhIP-DNA adduct formation is not particularly dominant. Perhaps other factors or enzymes may also contribute to the formation of PhIP-DNA adducts, including *N*-acetyltransferases, prolyl-tRNA synthetases, phosphorylases, sulfotransferases, UDP-glucuronyltransferase and enzymes involved in DNA repair (34,35).

Lung cancer is the leading cause of death among women worldwide, and cigarette smoking and secondhand smoking are regarded as the predominant risk factor (36–38). However, lung cancer also strikes women in the absence of smoking (39). For example, Chinese women have a high incidence of lung cancer despite a low smoking prevalence, and domestic exposure to cooking fumes is proposed as an important risk factor (40). Associations between Chinese-style cooking and lung cancer has been confirmed in several epidemiological investigations (41–44). Procarcinogens, including PhIP and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), were identified in smoke condensates from the frying meat (45). In the present study, localized procarcinogen metabolic activation by CYP1A1 in mouse lung has been demonstrated which may be an important risk factor for occupationally relevant human lung cancer. Because of the high efficiency of CYP1A1 in PhIP $N^2$-hydroxylation and high inducibility of human CYP1A1, especially in smokers, the role of lung CYP1A1 in PhIP carcinogenesis should be considered.

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*Conflict of Interest Statement:* None declared.

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