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Characterization Of Cyp2a13.1 And Cyp2a13.2, A Variant Protein From The Cyp2a13*2 Allele Associated With Decreased Incidences Of Lung Adenocarcinoma In Smokers.

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CYP2A13, expressed mainly in the respiratory tract, has high efficiencies in metabolically activating tobacco-specific nitrosamines. In our previous study, we found that an exon 5 3375C>T (Arg257Cys) mutation leads to decreases in the activity of the 2A13 protein. However, more recent studies indicate that the 2A13.2 variant contains both the 3375C>T mutation and an exon 1 (74 G>A) mutation, and the exon 5 mutation is linked with the exon 1 mutation in most individuals. Thus, in this study, we have obtained the CYP2A13.1 and CYP2A13.2 proteins through site-directed mutagenesis, and heterologous expression in insect Sf9 cells. The activities of these two proteins were compared using a number of known CYP2A13.1 substrates, including the tobacco-specific procarcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Our results indicate that CYP2A13.2 is only slightly less active than CYP2A13.1 with NNK and other substrates tested. In additional studies, we developed a method for distinguishing the CYP2A13.1 and CYP2A13.2 proteins by chemical cleavage followed by either SDS-PAGE or MS analysis of peptide fragments. This method should allow for detection of the translated proteins in vivo. Finally, we have tested the ability of the CYP2A13.1 protein to metabolize selected environmental compounds to expand the library of CYP2A13 substrates. Our results indicate that we have identified seven potential new substrates for CYP2A13, all of which are or have the potential to be respiratory tract toxicants. These findings support the role of CYP2A13 proteins in respiratory tract toxicity of environmental compounds.