

CORRESPONDENCE

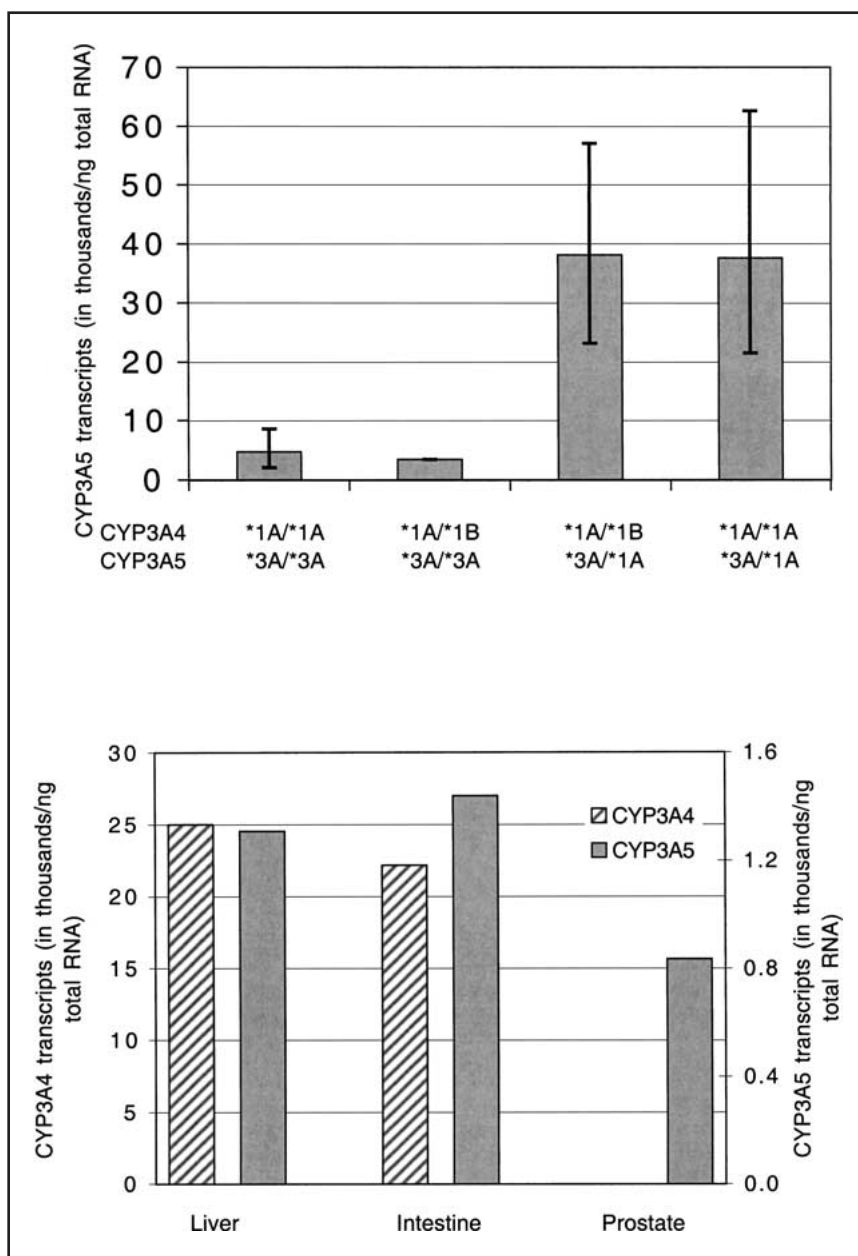
Re: Modification of Clinical Presentation of Prostate Tumors by a Novel Genetic Variant in CYP3A4

The variable expression and activity of CYP3A isozymes observed in the population has been discussed as a factor that affects both response to therapies and the individual cancer predispo-

sition. The CYP3A4-V gene variant in the 5'-regulatory region of the CYP3A4 gene (herein referred to as CYP3A4*1B) was associated with high-grade prostate cancer (1) and with a reduced risk for treatment-related leukemia (2). It has been postulated that CYP3A4*1B might reduce CYP3A4 expression and thereby decrease both steroid metabolism in the prostate and production of DNA-damaging chemotherapeutic metabolites (1,2). However, although the single nucleotide polymorphism disrupts a putative regulatory element in the CYP3A4 promoter, several studies did not detect any substantial effect of CYP3A4*1B on CYP3A4 expression or activity [(3) and references therein].

We noticed a tight linkage between CYP3A4*1B and CYP3A5*1A, a recently described marker of the CYP3A5 polymorphism (4,5). In 230 DNA samples isolated from Caucasians with a variety of medical conditions, the allelic frequencies of CYP3A4*1B and CYP3A5*1A were 3.7% (95% confidence interval [CI] = 2% to 5.4%) and 5.4% (95% CI = 3.3% to 7.5%), respectively. Three of 230 individuals were heterozygous for the CYP3A4*1B allele, 12 individuals were heterozygous for the CYP3A5*1A allele, and 12 individuals were heterozygous for both alleles. One individual was homozygous for CYP3A4*1B and heterozygous for CYP3A5*1A. This distribution was statistically significantly

Fig. 1. Upper panel: Expression of CYP3A5 messenger RNA (mRNA) in liver biopsy specimens obtained from individuals with a variety of medical conditions was quantified with the use of CYP3A5-specific polymerase chain reaction (TaqMan assay) and expressed as a function of CYP3A4 and CYP3A5 variant alleles. The number of transcripts was determined by including a CYP3A5 complementary DNA (cDNA) calibration curve in the TaqMan assay. **Bars** represent mean values, and **vertical lines** the range of expression. **Lower panel:** Expression of CYP3A4 and CYP3A5 mRNA in pooled livers (n = 2), intestines (n = 6), and prostate glands (n = 47) obtained from healthy individuals and assessed using CYP3A4-specific and CYP3A5-specific polymerase chain reaction TaqMan assays. The number of transcripts was determined as described above. The mRNAs were pooled by the manufacturer before the TaqMan assay.



different ($P < .001$, Fisher's exact test) from that expected for independently recombining alleles and indicated that, despite a physical distance of approximately 110 kb (6), CYP3A5*1A and CYP3A4*1B constitute a haplotype in double-heterozygous individuals.

These frequencies and their distribution predicted that approximately 80% of carriers of CYP3A4*1B alleles would exhibit increased CYP3A5 messenger RNA (mRNA) expression. In agreement with this prediction, CYP3A5 mRNA expression was increased in four of five available liver samples that were heterozygous for CYP3A4*1B alleles (Fig. 1, upper panel). The increase in CYP3A5 expression was restricted to samples that were simultaneously heterozygous for the CYP3A5*1A allele. By contrast, the presence of CYP3A4*1B alleles alone was not necessary for the increased CYP3A5 expression. These data show that CYP3A5 expression is increased in individuals carrying CYP3A4*1B alleles if they are simultaneously carriers of CYP3A5*1A alleles.

In conclusion, in approximately 80% of Caucasians carrying CYP3A4*1B, this allele is associated with increased CYP3A5 expression because of its linkage with CYP3A5*1A. The reported association between the CYP3A4*1B allele and high-grade prostate cancer (1) may, therefore, be caused by increased CYP3A5 expression rather than by altered expression or activity of CYP3A4. This hypothesis is strongly supported by the observation that, unlike its expression in the liver and the intestine, CYP3A5 but not CYP3A4 is expressed in the prostate (Fig. 1, lower panel). The reported association between CYP3A4*1B and a reduced risk of treatment-related leukemia (2) could be caused by CYP3A5-specific metabolic reactions. CYP3A proteins participate in the metabolism of most chemotherapeutic drugs currently in use. Reactions catalyzed exclusively by CYP3A5 have been reported for several substances, including the cancer drug irinotecan and the liver carcinogen aflatoxin B1 (7). Together, our observations provide an explanation for the association between prostate cancer and CYP3A4*1B expression. In addition, they suggest that differences between catalytic activities of CYP3A4 and CYP3A5 should be investigated in a systematic manner.

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RESPONSE

Wojnowski et al. provide a welcome and provocative insight into the conflicting data regarding the functional rel-

evance of CYP3A4*1B and its effect on disease endpoints. As these authors suggest, two explanations are commonly given when a genotype-disease association is observed. First, the allele under study may be in linkage disequilibrium (LD) with a truly causative allele. Wojnowski et al. suggest that this may be the case for CYP3A4. This finding is consistent with the report by Chang et al. (1), who showed that alleles at CYP3A3, CYP3A4, CYP3A5, CYP3A7, CYP3A43, CYP3AP1, and CYP3AP2 on chromosome 7q21-q22 are in LD. Therefore, the effects suggested by Wojnowski et al. may not be limited to CYP3A4 and CYP3A5 but could involve multiple genes in the CYP3A family. Second, associations (and the lack of consistency among reports) may be explained by the fact that relevant interactions among genes and between genes and exposures have not been studied. Wojnowski et al. suggest that CYP3A4*1B is associated with CYP3A5 expression. This finding is also analogous to that of Chang et al. (1), who reported that the CYP3AP1-t369g variant is associated with increased CYP3A5 activity. Given overlap in substrate specificities, induction of expression, and other biologically relevant events that may be shared among members of the CYP3A family, studies that consider complex interactions among these genes may be required to understand their effects in disease etiology and drug metabolism.

The reports of Wojnowski et al. and Chang et al. (1) do not fully explain whether associations involving the CYP3A multigene family are a result of LD alone, of multigene interactions in overlapping metabolic pathways, or of some combination of LD and multigene interactions. To evaluate these hypotheses, a complete characterization of LD between the CYP3A alleles on chromosome 7 should be undertaken. This should include formal statistical evaluation of multilocus genotypes or haplotypes. In addition, relationships of multilocus genotypes or haplotypes with phenotypic characteristics must be studied. These studies should consider the effect of potential confounders such as exogenous exposures. In each situation, large sample sizes may be required to evaluate the potential interaction effects among multilocus CYP3A genotypes. Finally, it will be of interest to evaluate

these effects across ethnic groups because allele frequencies at CYP3A4 vary significantly more by race than allele frequencies at CYP3A5 (2,3). These studies could help to clarify the effects of LD versus functionally relevant interactions involving CYP3A genes.

The phenomena discussed here not only may be of interest to those who study the CYP3A multigene family but also may represent a paradigm for genotype–disease association studies involving germline variants more generally in complex metabolic pathways. It is possible that both LD and multigene interactions may influence associations. These issues will only be resolved by appropriate molecular epidemiologic investigations that include careful characterization of LD among loci; knowledge of genotype–phenotype relationships; and well-designed, adequately powered association studies.

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