

the proneural signature may explain the good prognosis associated with *IDH1*-mutated gliomas.<sup>1</sup> *IDH1* mutations may occur in a specific cell lineage with metabolic characteristics that favor the occurrence of this mutation.

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1. Phillips HS, Kharbanda S, Chen R, et al. Molecular subclasses of high-grade glioma predict prognosis, delineate a pattern of disease progression, and resemble stages in neurogenesis. *Cancer Cell* 2006;9:157-73.

**THE AUTHOR AND A COLLEAGUE REPLY:** De Carli et al. report the *IDH* mutation status of pediatric gliomas. In fact, some of the gliomas sequenced in our study were from children. We report here that these childhood tumors included 14 WHO grade II gliomas (of which 4 contained *IDH1* mutations and 1 contained an *IDH2* mutation) and 3 WHO grade III gliomas (none of which contained *IDH* mutations). As we reported in our article, 15 pediatric glioblastomas (WHO grade IV) did not contain *IDH* mutations. Children with *IDH*-mutated gliomas were also older than the other patients in our study (median age, 17 years vs. 5 years;  $P=0.002$ ). Our findings complement those of De Carli et al., and we agree with the interpretation that adolescent gliomas may resemble adult gliomas.

Ducray et al. elaborate on an association between the proneural gene-expression signature and *IDH1* mutations in WHO grades II, III, and IV gliomas. A confounder in this analysis is tumor type, which is associated with both the molecular signature<sup>1</sup> and *IDH1* mutation status. For instance, 89% of anaplastic astrocytomas and

31% of glioblastomas in a previous analysis had a proneural signature,<sup>1</sup> and we found that 69% of anaplastic astrocytomas and 11% of glioblastomas in our study contained *IDH1* mutations. Thus, the association reported by Ducray et al. may reflect the known association of molecular subclass with tumor type and not *IDH1* status in particular. Previous studies have shown mixed results for associations between genetic alterations and expression patterns in specific tumor types. For instance, a 1000-gene complementary DNA array analysis of diffuse astrocytomas did not show any patterns associated with p53 alteration,<sup>2</sup> whereas Ducray et al. previously identified a significant association between codeletion of 1p and 19q and the proneural signature in anaplastic oligodendrogliomas, using microarray analysis.<sup>3</sup> Nevertheless, associations among proneural signature, tumor type, *IDH* status, and other genetic alterations may suggest distinct biologic properties in a subgroup of gliomas. We agree that a discrete molecular signature in *IDH1*-mutated gliomas could reflect a cell lineage of origin in these gliomas in which such mutations are favored. However, we cannot discount the non-mutually exclusive possibility that some differences in molecular signature arise later during gliomagenesis, perhaps as a phenotype of the genetic alterations with which they associate.

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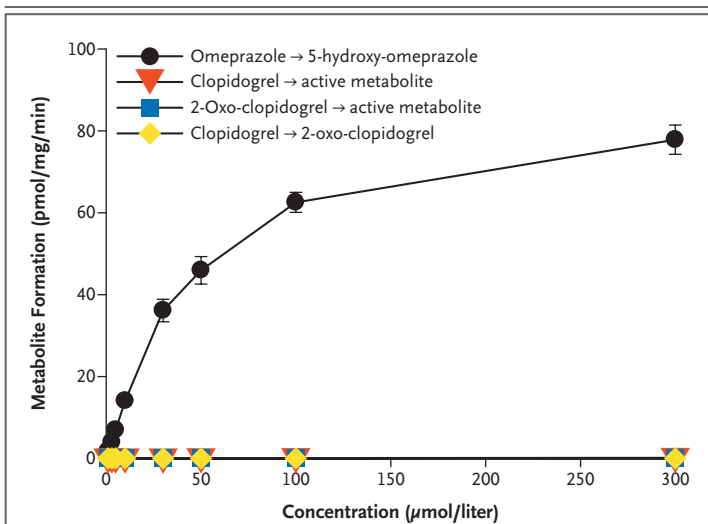
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## Cytochrome P-450 Polymorphisms and Response to Clopidogrel

**TO THE EDITOR:** On the basis of the results of a genetic association study (Jan. 22 issue),<sup>1</sup> Mega et al. conclude that reduced-function variants of the *CYP2C19* allele are responsible for lower plasma levels of the active metabolite of clopidogrel; these lower levels lead to decreased platelet inhibition and thereby increase cardiovascular risk.

However, no direct evidence of the causal involvement of the cytochrome P-450 enzyme CYP2C19 in the biotransformation of clopidogrel to its active metabolite is presented.

To test the hypothesis of Mega et al., we incubated clopidogrel, the inactive metabolic intermediate 2-oxo-clopidogrel,<sup>2</sup> and the known CYP2C19



**Figure 1. Results of Tests for the CYP2C19-Dependent Biotransformation of Clopidogrel, 2-Oxo-Clopidogrel, and Omeprazole into Metabolites.**

Clopidogrel, 2-oxo-clopidogrel, and omeprazole were incubated for 5 minutes at 37°C with microsomes preparations from CYP2C19 (National Center for Biotechnology Information sequence accession number, NM\_000769.1) transfected human embryonic kidney 293 cells (200 µg of protein containing 7.6 pmol of CYP2C19 enzyme and equimolar concentrations of purified recombinant human NADPH-cytochrome P-450 reductase and cytochrome b<sub>5</sub>) in 50 mM sodium phosphate buffer (pH 7.4), 10 mM magnesium chloride, and 2 mM NADPH (final volume, 200 µl). Incubations were performed with eight different concentrations, ranging from 1 to 300 µM. The formation of metabolites was measured in supernatants extracted by 600 µl acetonitrile by means of liquid chromatography–tandem mass spectrometry in positive electrospray mode (TSQ Quantum, Thermo Fisher Scientific). Each metabolite was detected by monitoring the mass-to-charge ratio (m/z) for the transition of the parent ion [M+H]<sup>+</sup> into selected fragment ions. The transition of m/z 338 to m/z 183 was used for quantitation of 2-oxo-clopidogrel, m/z 356 to m/z 183 for the active thiol metabolite of clopidogrel, and m/z 362 to m/z 214 for 5-hydroxy-omeprazole. Each incubation experiment was performed three times with the use of individual microsome preparations. Arithmetic means and standard deviations are shown.

substrate omeprazole<sup>3</sup> with human microsome preparations expressing CYP2C19 (Fig. 1). Although omeprazole was transformed into 5-hydroxy-omeprazole, no significant biotransformation of clopidogrel into 2-oxo-clopidogrel and active metabolite or of 2-oxo-clopidogrel into active metabolite was observed. Hence, CYP2C19 does not appear to contribute to the biotransformation of clopidogrel.

It is possible that the CYP2C19 polymorphisms represent only tags for the true causal gene variant involved in clopidogrel activation. Moreover, the CYP2C19 polymorphisms may directly affect the risk of cardiovascular events. Genomewide association studies may help resolve these discrepancies.

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**TO THE EDITOR:** Simon and colleagues (Jan. 22 issue)<sup>1</sup> assessed the relation between allelic variants of genes modulating clopidogrel and the risk of adverse events, and they found a significantly higher event rate among carriers of CYP2C19 loss-of-function alleles than among noncarriers. They also found a modestly increased rate of events among carriers of the ABCB1 variant. The authors genotyped multiple known functional variants, including ABCB1, CYP2C19, CYP3A5, P2RY12, and ITGB3, but some functional genetic variants such as CYP3A4 (modulating the metabolic activation of clopidogrel)<sup>2</sup> and ITGA2 (modulating expression of platelet glycoprotein Ia/IIa receptor) were not assessed.<sup>3,4</sup> Since variations in these genes may also have an effect on the responsiveness to clopidogrel associated with ischemic events,<sup>2-4</sup> the study cannot rule out the possibility of the influence of these genetic factors on the response to clopidogrel and the events.

In addition, although the relationship between single-gene polymorphisms and events was analyzed, the role of coexisting polymorphisms in ischemic events remains unclear. Analysis of patients carrying different genetic variants may help us to better understand the complex nature of the genetic cause of outcomes in patients who are receiving clopidogrel treatment.<sup>5</sup>

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**DR. MEGA AND COLLEAGUES REPLY:** We agree with Taubert and colleagues that our genetic association study cannot rule out the possibility that variants in *CYP2C19* were in linkage disequilibrium with other causal variants. However, the evaluated *CYP2C19* variants are known to have functional consequences. Moreover, other *in vitro* studies have shown that *CYP2C19* does convert clopidogrel to its active metabolite.<sup>1</sup> It may be that the active metabolite was not detected by Taubert et al. because the incubation conditions were suboptimal for clopidogrel, the active metabolite was not stabilized,<sup>2</sup> or clopidogrel was largely metabolized to inactive, unmeasured metabolites by esterases present in microsomes.<sup>3,4</sup> It would have been helpful to have seen evidence that their approach involving *in vitro* microsomal preparation and

liquid chromatography–tandem mass spectrometry can generate and detect the active metabolite of clopidogrel. Finally, it is unlikely that *CYP2C19* directly affects cardiovascular risk, since that would not explain the differences in the active metabolite of clopidogrel and platelet inhibition; moreover, we have shown that variants in this gene are not associated with outcomes in patients treated with prasugrel.<sup>5</sup>

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## Geographic Atrophy in Age-Related Macular Degeneration and *TLR3*

**TO THE EDITOR:** Yang et al. (Oct. 2 issue)<sup>1</sup> describe the association between a variant of the toll-like receptor 3 gene (*TLR3*) and protection from geographic atrophy, a major cause of blindness. They also show that the *TLR3* genotype affects the sensitivity of human retinal pigment epithelial cells and the retinal pigment epithelium of mice to the proapoptotic effects of long double-stranded RNA (dsRNA), a recognized ligand of *TLR3*. The dsRNA that they used in these experiments is roughly 100 times the length of small interfering RNA (siRNA) molecules, and yet they conclude that the “results suggest a role of viral dsRNA in the development of geographic atrophy and point to the potential toxic effects of short-interfering-

RNA therapies in the eye.” I am not persuaded that this conclusion is supported by their data.

Those of us developing small dsRNA molecules for therapy are aware of their potential to activate components of the innate immune system. That said, the remark by Yang et al.<sup>1</sup> about the risks of RNA-based therapeutic agents may be misleading.

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