Abstract—Genetic variations have been shown to influence drug metabolism, risk of adverse drug events, and pharmacodynamic responses for many drugs routinely used to treat patients with stroke or at risk for stroke. Examples include clopidogrel, statins, antihypertensive medications, and coumadin. Further validation studies are needed to assess the clinical utility of selecting drugs and doses based on genetic tests. Physicians, pharmaceutical companies, regulatory agencies, and health insurers continue to grapple with how best to translate this burgeoning field into effective individualized medicine. (Stroke. 2009;40:00-00.)

Key Words: ischemic stroke ■ cerebral infarction ■ genetics ■ genomics ■ pharmacology ■ pharmacogenomics ■ prevention

Pharmacogenomics offers the possibility of running genetic tests on blood, saliva, or other easily accessed sources of DNA to maximize efficacy, minimize serious adverse events, and optimize cost-effectiveness of pharmacological therapies for primary and secondary stroke prevention. Though this dream of individualized stroke prevention has yet to be fully realized, substantive progress has been made on many fronts.

Response to Antiplatelet Agents
Aspirin reduces the risk of stroke by 13% to 25%. Clinically effective doses range widely from 30 to 1300 mg per day. Aspirin acetylates serine residue 530 in the active site of cyclooxygenase-1 (COX-1), sterically inhibiting the metabolism of arachidonic acid. This ultimately leads to a reduction of thromboxane A2, a potent activator of platelets. Numerous genetic association studies of both conservative and nonconservative variants of COX-1 have been done in the hopes of identifying ones that might impart a variable response to aspirin. For example, the C50T (rs3842787) COX-1 mutation causes a single amino acid change (Pro17Leu) proximal to the signal peptide cleavage region. The variant is common in populations of European descent, but not among the Chinese.1 People with the C50T variant tend to have higher levels of urinary 11-dehydrothromboxane B2 (a metabolite of thromboxane A2) when not on aspirin.2 However, the response to aspirin seems to be the same whether or not one has the mutation.2,3 A study of 5 COX-1 single-nucleotide polymorphisms found that COX-1 haplotype significantly affected aspirin responsiveness.4

To date, no gene test or set of tests has been adequately validated with regard to predicting so-called aspirin resistance. Further, several nongenetic tests of platelet sensitivity have been shown to correlate with poor cardiovascular outcomes despite patients being prescribed aspirin.5 It might be possible in the future to use a combination of genetic information and platelet sensitivity data to differentiate biological aspirin resistance from medical noncompliance.

Like aspirin, clopidogrel has a proven ability to reduce the risk of recurrent ischemic stroke.6 Clopidogrel requires transformation into an active metabolite by cytochrome P-450 (CYP) enzymes for its antiplatelet effect. The genes encoding CYP enzymes are polymorphic. Carriers of at least one CYP2C19 reduced-function allele (about 30% of the population) have about a one third reduction of plasma exposure to the active metabolite of clopidogrel compared to noncarriers.7 This reduced exposure to active metabolite has clinical significance. Clopidogrel-treated subjects in TRITON-TIMI-38 had a relative increase of 53% in the composite outcome of the risk of stroke, MI, or cardiovascular death if they were carriers of the reduced-function CYP2C19 allele and a 3-fold increase in the risk of in-stent thrombosis.7

In a nationwide French registry, patients with two variant alleles of ABCB1 (TT at nucleotide position 3435), a gene modulating clopidogrel absorption, had a higher rate of cardiovascular events at 1 year than those with the ABCB1 wild-type genotype (CC at nucleotide 3435) (15.5% versus 10.7%).8 Patients carrying any two CYP2C19 loss-of-function alleles had a higher event rate than patients with none (21.5% versus 13.3%). For the 1535 patients who
underwent percutaneous coronary intervention during hospitalization, the rate of cardiovascular events among patients with two \textit{CYP2C19} loss-of-function alleles was 3.58 times higher than those with none.

\textit{CYP2C19} variants affect responsiveness to clopidogrel in young individuals with coronary atherosclerosis as well. In a study of patients age <45 years who survived a first MI and were exposed to clopidogrel for at least a first MI, the composite end point of MI, urgent coronary revascularization, and death occurred more frequently in carriers of the \textit{CYP2C19*2} variant than in noncarriers (hazard ratio [HR]=3.69).\textsuperscript{9} The same variant was also a risk factor for in-stent thrombosis (HR=6.02). The detrimental effect of the \textit{CYP2C19*2} genetic variant persisted from 6 months after clopidogrel initiation to the end of follow-up (HR=3.00).\textsuperscript{3}

Although it is clear that genetic differences can lead to variable responses to clopidogrel, it remains to be seen whether pharmacogenetic prescribing will prove superior or inferior to newer P2Y12 antagonists like prasugrel, ticagrelor, cangrelor, and elinogrel.\textsuperscript{10} Studies are needed to determine whether patients who undergo carotid stenting under protocols that include clopidogrel are at increased risk of ipsilateral stroke if they harbor relevant \textit{ABCB1} or \textit{CYP2C19} variants.

### Summary

Studies have yielded mixed yet to be replicated results with regard to the influence of \textit{COX-1} gene variants on aspirin resistance. Variants in \textit{ABCB1} and \textit{CYP2C19} have consistent and measurable effects on atherothrombotic clinical outcomes in patients treated with clopidogrel (Table). Cost effectiveness studies are needed to determine how this information could be used to optimize selection of an antiplatelet agent for stroke prevention.

#### Statin-Associated Myopathy

The relative risk of stroke is reduced by about 20\% for each 1-mmol/L (39 mg/dL) decrease in LDL cholesterol achieved using statins.\textsuperscript{11} Myopathy is a rare but serious complication of statin therapy. The risk appears greatest with higher doses and when statins are taken along with certain other medications.

A genome-wide association study was performed in 85 subjects with definite or incipient myopathy and 90 controls, all of whom were taking 80 mg of simvastatin daily as part of a trial involving 12 000 participants.\textsuperscript{12} Replication was tested in a trial of 40 mg of simvastatin daily involving 20 000 participants. The genome-wide scan yielded a strong association of myopathy with the rs4363657 single-nucleotide polymorphism (SNP) located within \textit{SLCO1B1} on chromosome 12 ($P=4\times10^{-15}$). The noncoding rs4363657 was in nearly complete linkage disequilibrium with rs4149056, a nonsynonymous SNP linked to statin metabolism. There was a 15\% prevalence of the rs4149056 C allele in the study population. For every copy of the C allele, the odds of myopathy increased 4.5-fold. Subjects with the CC genotype had a nearly 17-fold increased risk of myopathy relative to those with the TT genotype.

The adverse consequences of having a \textit{SLCO1B1} variant in terms of statin therapy appears to be pharmacokinetically mediated. The \textit{SLCO1B1} gene encodes for the organic anion transporting polypeptide 1B1 (OATP1B1), an uptake transporter located at the sinusoidal membrane of human hepatocytes. Variants in the \textit{SLCO1B1} gene have been shown to increase plasma concentrations of simvastatin in healthy volunteers by up to 2 fold.\textsuperscript{13}

#### Response to Warfarin

Warfarin reduces the risk of stroke and other major ischemic vascular events in patients with nonvalvular atrial fibrillation by 30\% over and above aspirin, which by itself reduces the risk by 20\%.\textsuperscript{14} The high variability in drug response and a narrow therapeutic index complicate initiation of warfarin therapy. The principal enzyme involved in warfarin metabolism is \textit{CYP2C9}, and the pharmacological target of warfarin is vitamin K epoxide reductase (\textit{VKORC1}). Variation in these genes leads to variable response to warfarin.

Polymorphisms in \textit{CYP2C9} and \textit{VKORC1} genes were genotyped in 92 patients undergoing total hip or knee replacement.\textsuperscript{15} A model for refining the dose of warfarin after the third dose had been given was developed that explained four fifths of the variability in dosages. Significant predictors included \textit{CYP2C9*3} and \textit{CYP2C9*2} genotypes, and \textit{VKORC1} (haplotypes A or B).

A study of patients receiving long-term warfarin therapy determined \textit{VKORC1} haplotype frequencies in a multiracial population, and \textit{VKORC1} messenger RNA (mRNA) expres-

### Table. Common Stroke Prevention Medications and Genes Whose Variants May Affect Pharmacokinetics, Pharmacodynamics, or Risk of Adverse Events

<table>
<thead>
<tr>
<th>Drug</th>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clopidogrel</td>
<td>Cytochrome P450, family 2, subfamily C, polypeptide 19</td>
<td>\textit{CYP2C19}</td>
<td>Affects levels of active metabolite of clopidogrel</td>
</tr>
<tr>
<td></td>
<td>ATP-binding cassette, sub-family B, member 1</td>
<td>\textit{ABCB1}</td>
<td>Affects absorption of clopidogrel</td>
</tr>
<tr>
<td>Statins</td>
<td>Solute carrier organic anion transporter family, member 1B1</td>
<td>\textit{SLCO1B1}</td>
<td>Affects statin metabolism and risk of myopathy</td>
</tr>
<tr>
<td>Warfarin</td>
<td>Vitamin K epoxide reductase complex, subunit 1</td>
<td>\textit{VKORC1}</td>
<td>This is the pharmacodynamic target of warfarin anticoagulation. Variants affect warfarin sensitivity</td>
</tr>
</tbody>
</table>

\textit{Cytochrome P450, family 2, subfamily C, polypeptide 9} \textit{CYP2C9} Affects warfarin metabolism
sion in human liver samples. A low-dose haplotype group (A) and a high-dose haplotype group (B) were identified. Mean maintenance dose of warfarin differed significantly among haplotype groups. VKORC1 haplotype groups A and B explained about 25% of the variance in dosing. Asian-Americans had a higher proportion of group A haplotypes and African-Americans a higher proportion of group B haplotypes. VKORC1 mRNA levels varied according to haplotype combination, suggesting that VKORC1-related warfarin response variability is regulated at the transcriptional level.

A retrospective cohort study of patients receiving long-term warfarin therapy for various indications was conducted at 2 anticoagulation clinics. Among 185 patients with analyzable data, 31.4% had at least 1 variant CYP2C9 allele and 68.6% had the wild-type (*1/*1) genotype. Mean maintenance dose varied significantly among the 6 genotype groups. Patients with at least 1 variant allele had an increased risk of above-range INRs. The variant group also required more time to achieve stable dosing. Subjects with a variant genotype also had more than twice the risk of a serious bleeding event.

A retrospective genome-wide association study was designed to identify polymorphisms that could explain a large fraction of the dose variance. White patients from an index warfarin population (n = 181) and 2 independent replication patient populations (n = 374) were studied. The most significant independent effect was associated with VKORC1 polymorphisms (P = 6.2 x 10^-15) in the index patients.

A study of 297 patients starting warfarin therapy assessed CYP2C9 genotypes (CYP2C9 *1, *2, and *3), VKORC1 haplotypes (designated A and non-A), clinical characteristics, response to therapy (as determined by INR), and bleeding events. Compared to patients with the non-A/non-A haplotype, patients with the A/A haplotype of VKORC1 had a decreased time to the first INR within the therapeutic range and to the first INR > 4. The CYP2C9 genotype was a significant predictor of time to the first INR > 4 (P = 0.03). Both the CYP2C9 genotype and VKORC1 haplotype had a significant influence on the required warfarin dose after the first 2 weeks of therapy.

Gene test-informed dosing algorithms have been compared to the empirical fixed-starting-dose approach. Current algorithms may show racial differences in performance. In one example, 259 subjects who started using warfarin were followed until they reached maintenance dose. The model for patients of European ancestry predicted 51% of the doses to within 1 mg of the observed dose. The algorithm outperformed the 5 mg/d fixed-dose approach, which predicted 29% of the doses to within 5 ± 1 mg. The model for patients of African ancestry predicted 37% of the doses to within 1 mg of the observed dose, representing a small improvement compared with 5 mg/d, which predicted 34% of the doses to within 1 mg of 5 mg/d.

The clear influence of genetics on response to warfarin therapy has led to the development of several commercially available platforms for pharmacogenetic dosing. The assays take 3 to 8 hours to complete, and accuracies range from 99% to 100%. Whether these assays should be used in routine clinical practice depends on the degree to which pharmacogenetically guided dosing improves on the current standard of practice as it relates to initiation of warfarin therapy and on whether these gains justify the added expense of testing. Randomized comparisons are required to address these questions with proper scientific rigor. One such trial of 206 individuals being initiated on warfarin randomized subjects to pharmacogenetically-guided versus standard dosing. DNA was genotyped for CYP2C9 *2 and CYP2C9 *3 and VKORC1 C1173T. Standard dosing followed an empirical protocol, whereas pharmacogenetic-guided dosing followed a regression equation including the 3 genetic variants, age, sex, and weight. A research pharmacist unblinded to treatment strategy adjusted doses of warfarin. Patients were followed for up to 3 months. Pharmacogenetic-guided predictions of doses more accurately approximated stable doses (P < 0.001), resulting in smaller (P = 0.002) and fewer (P = 0.03) dosing changes. Multiple variant allele carriers were at increased risk of an INR ≥ 4 (P = 0.03). However, the primary end point of percent out-of-range INRs did not differ significantly between arms.

Summary
Response to warfarin therapy is under genetic control (Table). However, routine use of commercial testing cannot be advocated until the cost effectiveness of such testing proves clinically superior to intensive INR monitoring and empirical “trial-and-error” dosing.

Response to Antihypertensive Medications
In patients with stroke, nearly 9 of 10 have hypertension, but only about a third of those being treated achieve a blood pressure of 140/90 mm Hg. For stroke clinicians the imperative to treat elevated blood pressure aggressively is obvious, whereas how best to attain this goal is less so.

A few randomized antihypertensive trials like INVEST and ALLHAT have incorporated genotyping of candidate genes within the study. The 6-adducin (ADD1) Gly460Trp polymorphism was studied in a subset of subjects enrolled in the INVEST, a trial involving patients over 55 years of age with hypertensive coronary artery disease to either a verapamil SR– or atenolol-based multidrug antihypertensive strategy. Patients received trandolapril with or without hydrochlorothiazide as needed for blood pressure control. Black ADD1 variant carriers showed a 2.6-fold excess risk for a primary outcome event and an 8-fold increase in the risk of death. Contrary to expectation, diuretic responsiveness was unaffected by gene status. This was in keeping with the results of GenHAT. The NPPA gene, which codes for the precursor of atrial natriuretic polypeptide, was studied in more than 38 000 subjects with hypertension enrolled in ALLHAT (the substudy is known as GenHAT). Subjects randomly received chlorthalidone, amloidine, lisinopril, or doxazosin. Minor C allele carriers experienced more favorable cardiovascular disease outcomes when randomized to receive a diuretic, whereas TT allele carriers had more favorable outcomes when randomized to receive a calcium channel blocker. Depending on genotype, the stroke rate varied from 9.6 to 15.4 per 1000 person-years. GenHAT also showed that
study subjects with the FBG-455 allele of the fibrinogen beta gene had a higher risk of stroke when treated with lisinopril compared to amlodipine. These exploratory genetic sub-studies need replication in prospective randomized trials dedicated to testing the value of drug selection based on genotype.

Genome-wide pharmacogenomic studies of antihypertensive treatment are currently underway. The advantage of such an approach is that it is hypothesis-free, thus overcoming the difficulty of low pretest probability of selecting the relevant candidate gene in a genetic association study.

Summary

There appear to be genetic factors imparting differential responses to angiotensin converting enzyme inhibitors versus calcium channel blockers and other genetic factors imparting differential responses to diuretics versus calcium channel blockers. None of these factors has emerged as an essential guidepost to selecting the optimum antihypertensive agent.

Cigarette Smoking Cessation

Genotypes that modulate smoking status, initiation, cessation, quantity, and treatment response have been identified. Many genes relate to dopamine reuptake, metabolism or binding to D2 receptors or to nicotine metabolism. Genetic testing could conceivably identify candidates for more intensive cessation programs. However, it is probably best to encourage smoking cessation uniformly. Pharmacogenomics may yet have a role if it were to be able to help clinicians choose among alternative strategies for promoting smoking cessation.

Conclusions

Future development of drugs for treatment and prevention of stroke may take into account that there are often genetic factors influencing response of drug target to drug, metabolism of drug, and likelihood of adverse effects. Allele frequencies can differ significantly across diverse race-ethnic groups (eg, COX-1 C50T). This, along with environmental differences, may help to explain race-ethnic differences often observed in clinical trials. In the past there was a tendency to medicalize race. Examples include enrichment trials that enroll individuals of a single race-ethnic group. The combination pill composed of isosorbide dinitrate and hydralazine (BiDil) was the first drug to be approved by the U.S. FDA for a specified single race-ethnic group. (ie, use for congestive heart failure in African-Americans) largely on the basis of an enrichment trial. The field of stroke research has had its own pall examples of enrichment trials base on race-ethnicity. A prominent example is the African-American Antiplatelet Stroke Prevention Study, which emerged from subgroup analysis of prior phase 3 trials in diverse populations.

Molecular genetics may refine future enrichment trials along more biological lines. A successful example of a molecular genetic enrichment trial led to the approval of trastuzumab (Herceptin) for treating metastatic breast cancer in patients whose tumors overexpress the HER2 protein. Trastuzumab is a humanized monoclonal antibody to the human epidermal growth factor receptor 2 (HER2) expressed on the surface of tumor cells. The FDA simultaneously approved trastuzumab and an accompanying test for the HER2 protein (the simultaneous development and approval of a therapy and a diagnostic test is sometimes referred to as “theragnostics”).

Getting new drugs to market that reflect the hope and promise of individualized medicine will require overcoming complex issues of trial design, pharmacoeconomics, and regulatory matters. The BiDil paradigm will probably eventually be replaced by the Herceptin paradigm. However, the field of oncology research has shown that the Herceptin approval paradigm has not been easily replicated. There is expense associated with screening for individuals expected to respond to a given therapy based on a molecular profile. Presently pharmaceutical companies bear this expense when performing trials of their product. Who will bear the expense when the trial ends and the drug is approved? While it may be easier to establish efficacy for targeted therapies, there may also be financial disincentives to develop such therapies. The market for a targeted drug will tend to be smaller than the market for a “one-size-fits-all-more-or-less” blockbuster drug. To go from where we are at present with stroke therapy to a more individualized approach, there will need to be more public support. This support could take the form of routine incorporation of biomarker assays in future publicly funded phase 3 treatment trials. The health care system will also have to support comparative effectiveness research related to comparing targeted versus nontargeted therapies for common medical problems like stroke prevention. Such support could be cost-saving in the long run by sparing patients from treatments that are ineffective or only marginally effective.

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None.

References

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