Stroke

TOPICAL REVIEW

Section Editors: Martin Dichgans, MD, and James Meschia, MD

Pharmacogenomics in Stroke and Cardiovascular Disease: State of the Art

Stephanie Ross, MSc, PhD; Kristi Krebs, MSc, PhD; Guillaume Paré, MD, MSc; Lili Milani, PhD

ABSTRACT: There is considerable interindividual variability in the response to antiplatelet and anticoagulant therapies, and this variation may be attributable to genetic variants. There has been an increased understanding of the genetic architecture of stroke and cardiovascular disease, which has been driven by advancements in genomic technologies and this has raised the possibility of more targeted pharmaceutical treatments. Pharmacogenetics promises to use a patient's genetic profile to treat those who are more likely to benefit from a particular intervention by selecting the best possible therapy. Although there are numerous studies indicating strong evidence for the effect of specific genotypes on the outcomes of vascular drugs, the adoption of pharmacogenetic testing in clinical practice has been slow. This resistance may stem from sometimes conflicting findings among pharmacogenetic studies, a lack of stroke-specific randomized controlled trials to test the effectiveness of genetically-guided therapies, and the practical and cost-effective implementation of genetic testing within the clinic. Thus, this review provides an overview of the genetic variants that influence the individual responses to aspirin, clopidogrel, warfarin and statins and the different methods for pharmacogenetic testing and guidelines for clinical implementation for stroke patients.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: genetics ■ pharmacogenetics ■ stroke

In 2019, stroke was the second most leading cause of death worldwide and it resulted in 6.55 million deaths.[1] Antiplatelet and anticoagulant therapies are commonly used to lower the risk of recurrent strokes but there is variability in the response to these agents.[2] Recently, there has been an increased understanding of the genetic architecture of stroke, which has been driven by advancements in genomic technologies and raising the possibility of more targeted pharmaceutical treatments. The primary goal of pharmacogenetics is to use a patient's genetic profile to treat those who are more likely to benefit from a particular intervention by selecting the best possible therapy. Thus, a better understanding of stroke pharmacogenetics will help personalize treatments, and thereby, improve their safety and effectiveness. The objective of this review is to provide an overview of the genetic variants that influence the individual responses to aspirin, clopidogrel, warfarin, and statins as well as to discuss different methods for pharmacogenetic testing and guidelines for clinical implementation.

Aspirin

Aspirin is an antiplatelet drug that is commonly used in the secondary prevention of stroke.[3] Aspirin irreversibly acetylates cyclooxygenase (COX)-1 in order to reduce platelet activation and inhibit the production of thromboxane A2 from arachidonic acid.[4] Several patients treated with aspirin still experience treatment failure and have an increased risk of recurrent events. This is known as aspirin resistance,[5,6] and it can result in laboratory or clinical resistance.[4] The mechanisms of aspirin resistance are multifactorial and may result from patient compliance, inadequate dosing, drug interactions, or genetic factors.

Aspirin resistance has been associated with several genetic variants, but the most commonly studied one is

Correspondence to: Guillaume Paré MD, MSc, Department of Pathology & Molecular Medicine, Population Health Research Institute, Hamilton Health Sciences and McMaster University, David Braley Cardiac Vascular and Stroke Research Institute, 237 Barton St. East Rm. C3-103, Hamilton, Ontario, Canada L8L2X2, Email pareg@McMaster.ca or Lili Milani, PhD, University of Tartu, Institute of Genomics, Riia 23B, 51010 Tartu, Estonia, Email lili.milani@ut.ee.
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the *PIA1/A2* of the glycoprotein IIIa (GPIIIa) gene, which encodes for a fibrogen receptor and von Willebrand Factor that aids in the platelet aggregation activation.[7] Wang et al (2019) conducted a meta-analysis of 35 trials to assess the effect of the *PIA1/A2* polymorphisms on laboratory aspirin resistance and adverse outcomes in coronary artery disease (CAD) patients who were on aspirin maintenance therapy.[8] The meta-analysis showed that there was no difference in aspirin resistance depending on *P1A1/A2* carrier status (odds ratio [OR] carrier versus noncarrier=0.94 [95% CI, 0.63–1.40]; N=3077). There were no significant differences in the risk of death, myocardial infarction (MI), or target vessel revascularization between *P1A1/A2* carriers versus noncarriers (*P* value > 0.05 for all). These results are consistent with another meta-analysis, which showed that healthy *P1A1/A2* allele carriers were more likely to be aspirin resistant, but this effect was absent among individuals with cardiovascular disease (CVD).[9] These results suggest that the *PIA1/A2* polymorphism may not have a causal role in aspirin resistance among CAD patients.

The COX enzymes are responsible for the formation of prostaglandins, prostacyclin, and thromboxane.[4] Some studies suggest that the variability in aspirin response may be due to COX-1 (Prostaglandin-endoperoxide synthase [PTGS1]) genetic variants because they impact platelet function and aspirin response.[10–12] However, there is uncertainty in the genetic effect of *COX-1* on stroke outcomes due to a lack of well-powered studies.

The COX-2 is an inducible enzyme that is upregulated in inflammatory states. COX-2 was thought to be protective but some randomized controlled trials (RCTs) showed that selective COX-2 inhibitors increased the risk of adverse events.[13–15] Although animal models have demonstrated that inhibition of the COX-2 enzyme reduced the risk of atherosclerosis, they have also shown an increased risk of thrombosis.[16–19] Thus, the role of *COX-2* remains controversial in CVD. However, a meta-analysis of 49 232 patients from 6 prospective RCTs showed that rs20417 (*COX-2/PTGS2*) carrier status was associated with a reduced risk of vascular outcomes (OR, 0.78 [95% CI, 0.70–0.87]).[20] In addition, aspirin use (*P* for interaction: 0.004) and previous CAD (*P* for interaction: 0.015) modified the effect of rs20417 carrier status on the risk of adverse outcomes.[20] Another genetic analysis sought to identify common variants in genes or gene products that interact with COX-2 inhibitors (coxibs), which included rs7270354 (matrix metallopeptidase 9 [*MMP9*]) and rs4888383 (breast cancer anti-estrogen resistance protein 1[*BCAR1*]).[21] These results suggest that there may be other potential pathways that impact the coxib-associated risk of CAD.

Clopidogrel

Clopidogrel is a prodrug that inhibits the $P2Y^{12}$ receptor on the surface of platelets.[22] Patients with acute ischemic stroke or transient ischemic attack are treated with dual antiplatelet therapy of clopidogrel and aspirin to help manage the risk of recurrent strokes.[23–25] However, the response to clopidogrel varies among these patients, and some have a higher risk of recurrent vascular events.[26]

Most studies have focused on the hepatic cytochrome (CYP) P450 2C19 (CYP2C19) enzyme because it acts to biotransform clopidogrel into its active metabolite.[27] Carriers of the *CYP2C19*2* or *CYP2C19*3* loss-of-function (LOF) alleles have a poorer response to clopidogrel and higher risk of vascular events,[28] whereas carriers of the *CYP2C19*17* gain-of-function allele have a better response to clopidogrel but a higher risk of bleeds.[29]

A meta-analysis of 4762 patients with acute ischemic stroke or transient ischemic attack assessed the effect of LOF (*CYP2C9*2*, **3*, and **8*) and gain-of-function (*CYP2C19*17*) alleles on the outcomes of clopidogrel treatment.[30] LOF carriers had an increased risk of stroke relative to the noncarriers (relative risk [RR], 1.92 [95% CI, 1.57–2.35]; *P*<0.001). However, this analysis included observational studies and studies without control groups. Therefore, it is difficult to determine whether the risk of stroke is due to the effect of *CYP2C19* carrier status on clopidogrel metabolism or if it is due to some underlying biological pathways that are independent of clopidogrel. Thus, Paré et al (2010) explored the effect of LOF and gain-of-function alleles using a subset of 1156 patients from the ACTIVE A trial (Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events).[31] There was no interaction between the effect of clopidogrel and LOF carrier status on vascular outcomes (Heterogeneity *P*: 0.73) or bleeds (Heterogeneity *P*: 0.16). Similar results were observed for gain-of-function carriers on vascular events.[31] These results were consistent with another meta-analysis of 4 placebo-controlled RCTs (N=11 477).[32]

The implementation of *CYP2C19* testing to guide clopidogrel therapy in patients undergoing primary percutaneous coronary intervention (PCI) has been slow due to conflicting results across studies. A meta-analysis of 11 RCTs and 3 observational studies in 20 743 patients reported that genotype guided antiplatelet therapy reduced the risk of major cardiovascular events (RR, 0.78 [95% CI, 0.63–0.95]), but it did not have an effect on the risk of bleeds (RR, 0.88 [95% CI, 0.77–1.01]).[33] These results conflict with the TAILOR-PCI open-labeled trial (Tailored Antiplatelet Therapy Following PCI; NCT01742117).[34] TAILOR-PCI randomized 5302 patients undergoing PCI for acute coronary syndrome or stable CAD to either point-of-care genotyping (POC) (N=2652) or conventional therapy (N=2650). In the POC group, *CYP2C19* LOF carriers (*CYP2C19*2* or **3*) were treated with ticagrelor and noncarriers received clopidogrel, whereas all patients in the conventional therapy group received clopidogrel and were then genotyped 12 months later. Among the

1849 *CYP2C19* LOF carriers in the trial, 85% assigned to the POC arm received ticagrelor (N=764/903) and 99% in the conventional therapy arm received clopidogrel (N=932/946). Among *CYP2C19* LOF carriers, there were no differences between the POC or conventional therapy on major vascular events (hazard ratio [HR], 0.66 [95% CI, 0.43–1.02]). Another noninferiority, open-label RCT assessed the effect of *CYP2C19* POC testing (N=1242) or treatment with either ticagrelor or prasugrel (N=1246) in patients with ST-segment–elevation myocardial infarction who underwent PCI.[35] In the POC arm, *CYP2C19* LOF carriers received ticagrelor or prasugrel, and noncarriers received clopidogrel. There was no difference between treatment groups on the composite outcome (5.1% versus 5.9%), but there was a lower risk of major bleeds in the POC group when compared with the conventional therapy group (HR, 0.78 [95% CI, 0.61–0.98]). Regardless of these results, clopidogrel is now off-patent and it may be more costeffective to treat patients who are unlikely to respond to clopidogrel with a different nonpatented agent. Overall, effective implementation *CYP2C19* testing will depend on the whether the choice of therapy (ie, clopidogrel or an alternative agent) differs by genotype carrier status[36]; however, there is still a need for large-scale prospective RCTs conducted in stroke patients.

Warfarin

Warfarin is an oral anticoagulant that is used in the primary and secondary prevention of atrial fibrillation patients.[37] Optimal warfarin treatment requires regular monitoring using the international normalized ratio.[37] Approximately, 30 to 35% of the variability in warfarin response is due to genetic variants, which is more than clinical variables alone. Therefore, further insight into the interplay of these clinical and genetic variants may help improve the management of warfarin and help reduce the risk of stroke events.

The metabolism of warfarin is dependent on CYP2C9[38] and *CYP2C9* LOF alleles (*CYP2C9*2* and *CYP2C9*3*) have been associated with over-anticoagulation and an increased risk of bleeds.[39] Warfarin targets the vitamin K epoxide reductase complex subunit 1 (VKORC1) enzyme to inhibit vitamin K metabolism[40] and rs9923231 (*VKORC1*) carriers have an increased risk of adverse events, whereas carriers of the rare *VKORC1* mutation have an increased risk of adverse ischemic events.[41,42] Finally, the CYP4F2 gene encodes for an enzyme that is involved in vitamin K metabolism and carriers of the rs2108622 (*CYP4F2*) require an increased warfarin dose.[43]

A few RCTs have assessed the effect of genotype-guided dosing of warfarin relative to standard dosing. The first trial was the COAG trial (Clarification of Optimal Anticoagulation Through Genetics) which tested the effect of a genotype-guided dosing algorithm in 1015 patients with a target international normalized ratio of 2 to 3 during the first 5 days of warfarin therapy.[44] There was no difference in the mean percentage of time in the therapeutic range at 4 weeks in either treatment groups (genotype-guided: 45.2% versus clinically-guided: 45.4%; *P*=0.91). The second trial was the EU-PACT trial (European Pharmacogenetics of Anticoagulant Therapy) which assessed the effect of genotype-guided dosing when compared with standard dosing in 455 patients during the first 5 days of warfarin therapy.[45] In contrast to the COAG trial, there was a higher mean percentage of time in the therapeutic range at 4 weeks in the genotype-guided group (67.4%) versus the standard dosing group (60.3%; *P*<0.001). The third trial was the GIFT trial (Genetics Informatics Trial) which explored the effect of genotype-guided dosing to clinically guided warfarin dosing on days 1 to 11 and to target either an international normalized ratio of 1.8 or 2.5 in 1650 patients undergoing elective hip or knee arthroplasty.[46] The genotype-guided dosing group reduced the risk of the composite end point when compared with clinically guided dosing group (RR, 0.73 [95% CI, 0.56–0.95]). The only significant difference in the composite was episodes of elevated international normalized ratio (RR, 0.71 [95% CI, 0.51–0.99]). However, there was no difference in the treatment groups for hard clinical outcomes, such as major bleeding within 30 days (RR, 0.24 [95% CI, 0.05–1.15]) or death within 30 days (no deaths).

These inconsistent trial results may be due to differences in patient populations or trial designs. For instance, the EU-PACT and GIFT trials included European patients, whereas COAG included North American patients with European and African American ancestry. Secondly, unlike the other genotype guided RCTs, the GIFT trial incorporated the *CYP4F2*3* genotype into the dosing algorithm and the algorithm was used for the first 11 days of warfarin dosing, which is longer than the other trials that only used 5 days. The trials also used different dosing algorithms, which may not be generalizable across studies, as well as different control groups, which may not represent clinical practice and could potentially underestimated effect sizes. There were also differences in the length of follow-up among the 3 trials, which ranged from 28 to 90 days. This suggests that dosing algorithms could have a greater clinical impact over time or it could reflect differences in clinical care across the 3 studies. Finally, all of the trials used a surrogate outcome to assess the efficacy of genetically guided dosing algorithms.

Despite these results, the use of warfarin is starting to diminish after the introduction of direct oral anticoagulants because they are easier to use and they do not require monitoring. For instance, warfarin prescribing

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dropped over the last 10 years from 77 to 12% in specific indications like atrial fibrillation.[47] Based on these discrepancies and the reduced use of warfarin, it is important that future trials testing the effectiveness of warfarin pharmacogenetic algorithms in stroke patients select appropriate patient populations, outcomes and trial designs.

Statins

Statins reduce the levels of low-density lipoprotein cholesterol and are used as a first-line therapy for CAD prevention.[48] Statins inhibit 3-hydroxy-3-methylglutaryl-coenzyme A reductase, a key enzyme in cholesterol synthesis. Numerous RCTs have demonstrated that the use of statins substantially reduces the risk of cardiovascular events.[49,50] However, adverse effects that are associated with the use of statins will often alter drug adherence and may lead to discontinuation of treatment. The most common adverse effect of statins is myotoxicity that appears as fatigue, muscle pain or weakness or in more severe cases as myonecrosis to rhabdomyolysis.[51] In 2008, the SEARCH Collaborative Group performed a GWAS in 85 patients with definite or incipient simvastatin-myopathy and 90 matched controls, and detected a strong association (OR, 4.50 [95% CI, 2.60–7.70]) between a SNP in the *SLCO1B1* gene and the onset of myopathy.[52] *SLCO1B1* encodes a transporter that aids the hepatic uptake of all statins and decreased function of this transporter leads to reduced drug transport to the liver, which can lead to build-up of the active form of simvastatin.[53] The SEARCH study gave start to numerous pharmacogenetic (PGx) studies investigating statin induced myopathy, of which several also replicated the *SLCO1B1* association. Most of these relevant studies on statin induced myotoxicity are covered in a thorough review by Kee et al.[54] An RCT done by Vassy et al in 2020 studied the impact of delivering *SLCO1B1* genetic results of statin myopathy risk to patients.[55] The trial included 408 patients, of which 193 were randomized to the intervention group and 215 were in the control group, and explored 1-year change in low-density lipoprotein-C level as a primary outcome and physician-documented statin-associated muscle symptoms as one of the secondary outcomes. Importantly, clinical testing and reporting of *SLCO1B1* results did not have poorer low-density lipoprotein cholesterol reductions after 1 year, compared with patients who received usual care, thus providing some reassurance about possible unintended harms of using *SLCO1B1* results. Statin-associated muscle symptoms were documented for 2 (1.0%) and 3 (1.4%) cases in the intervention and control groups, respectively, reporting non-significant difference. Furthermore, a recent meta-analysis of 10 European studies (1433 cases of myopathy and 2878 controls) aiming to determine the pooled genotypic effect of rs4149056 at the *SLCO1B1* gene locus on myopathy in patients with statin demonstrated that CC (OR, 2.90 [95% CI, 1.59, 5.34]) and TC (OR, 1.60 [95% CI, 1.20, 2.16]) genotypes had a significantly higher risk of myopathy than those who carried TT genotype.[56]

Although most pharmacogenetic studies of statins have focused mainly on simvastatin and specifically on the association with *SLCO1B1* gene, other statins also require further research to evaluate the effect of genetic variants in *SLCO1B1* and other genes on drug concentrations and less commonly occurring side-effects. For example, there are studies exploring the ATP-binding cassette subfamily G member 2 (ABCG2) and *CYP2C9* enzyme activity on statin outcomes. Systematic literature review done by the Clinical Pharmacogenetics Implementation Consortium (CPIC) has implicated sufficient evidence for association of *ABCG2* and rosuvastatin levels and the effect of *CYP2C9* genotypes on fluvastatin pharmacokinetics,[57] but further research is needed to support the association with other statins.

Guidelines for the Implementation of Pharmacogenetics

The CPIC[57] and the Dutch Pharmacogenetics Working Group (DPWG)[58,59] were developed to facilitate and provide guidance on the use of PGx genotype information in the clinic. Table 1 lists the CPIC or DPWG guidelines for 25 drugs that are related to CVD and/or stroke treatment, based on evidence from the Pharmacogenomics Knowledge Base. However, only 14 of the drugs have testing guidelines with recommendation for change in treatment, which has been concluded to be necessary based on the thorough review of the level of evidence of previous studies. Both the CPIC and the DPWG provide guidelines for clopidogrel and *CYP2C19*; simvastatin and *SLCO1B1*; atorvastatin and *SLCO1B1*; warfarin and *CYP2C9*, *CYP4F2*, *VKORC1*. DPWG additionally lists acenocoumarol and *VKORC1*; flecainide and *CYP2D6*; metoprolol and *CYP2D6*; phenprocoumon and *VKORC1*; propafenone and *CYP2D6,* and CPIC additionally lists rosuvastatin and *ABCG2*, *SLCO1B1*; lovastatin, pitavastatin, pravastatin, fluvastatin, and *SLCO1B1*.

The associations with highest evidence are for clopidogrel, warfarin, and simvastatin. The 2022 updated CPIC guideline of clopidogrel recommends an alternative antiplatelet therapy for *CYP2C19* poor or intermediate metabolizers.[60] The 2022 updated guideline of *SLCO1B1* extends the recommendations from simvastatin to all statins, and it also contains *ABCG2* recommendations specific to rosuvastatin and recommendations for fluvastatin based on *CYP2C9* genotype.[61] The warfarin guidelines were updated in 2017 and it is now recommended that warfarin dosing should be done using one of the pharmacogenetic dosing algorithms provided in EU-PACT trial.[45] Guidelines for dosing are both for adults

Table 1. CPIC and DPWG Guidelines for Stroke Medications

Drug action	Drug active agent	Gene association in CPIC guideline*	Gene association in DPWG guideline*
Antiarrhythmic	Amiodarone, disopyramide, quinidine, sotalol	X	CYP2D6 03/28/2022† (no recommendation)
	Flecainide, propafenone	X	CYP2D6 03/28/2022
Antihypertensive	Clonidine	X	CYP2D6 03/28/2022 (no recommendation)
Antithrombotic	Acenocoumarol, phenprocoumon	X	CYP2C9 03/28/2022 (no recommendation); VKORC1 04/05/2022 (testing guidance)
	Clopidogrel	CYP2C19 01/20/2022	CYP2C19 04/05/2022 (testing guidance)
	Prasugrel, ticagrelor	X	CYP2C19 03/28/2022 (no recommendation)
	Warfarin	CYP2C9, CYP4F2, VKORC1 05/28/2021	CYP2C9 03/28/2022; VKORC1 03/28/2022
	Aspirin	CYP2C9 12/10/2021 (no recommendation)	X
Beta blocking agent	Atenolol, bisoprolol, carvedilol	X	CYP2D6 03/28/2022 (no recommendation)
	Metoprolol	X	CYP2D6 03/29/2022
Lipid modifying agent	Atorvastatin, simvastatin	SLCO1B1 02/23/2022;ABCG2 02/24/2022 (no recommendation)	SLCO1B1 04/05/2022 (testing guidance)
	Fluvastatin	CYP2C9, SLCO1B1 02/23/2022;ABCG2 02/24/2022 (no recommendation)	SLCO1B1 03/28/2022 (no recommendation)
	Lovastatin, pitavastatin, pravastatin	SLCO1B1 02/23/2022;ABCG2 02/24/2022 (no recommendation)	X
	Rosuvastatin	ABCG2, SLCO1B1 02/23/2022	X

CPIC indicates Clinical Pharmacogenetics Implementation Consortium; and DPWG, Dutch Pharmacogenetics Working Group. X indicates no published guideline or recommendation.

*All dates indicate the most recent guideline publication date.

and pediatric patients specific to continental ancestry and are based on genotypes from *CYP2C9*, *VKORC1*, *CYP4F2*, and rs12777823 (in the CYP2C cluster region near CYP2C18).[62]

There are numerous examples of implementation programs where usage of CVD/stroke PGx associations in the clinic has been studied and CPIC guidelines were used. A study exploring the 12 implementation programs where *CYP2C19* genotype-guided antiplatelet therapy is implemented concluded that all institutions used CPIC guidelines for phenotype translation.[63] The loading-dose algorithm that was developed in the EU-PACT trial mentioned above is also recommended as part of the CPIC guideline.[62] Furthermore, Mayo Clinic conducted a proof-of-concept study with 82 healthy individuals that underwent genotyping of 9 PGx genes to explore the implementation of PGx in patient care, including also clopidogrel, simvastatin, and warfarin. They demonstrated that pre-emptive PGx testing offered medication improvement opportunities in 56% of the participants.[64] Importantly, pharmacists provided evidence-based (CPIC and DPWG) PGx recommendations for past, current, and future medications for the participants. A large-scale PGx implementation initiative in Europe called Ubiquitous Pharmacogenomics (U-PGx) studied delivering recommendations based on the DPWG guidelines for >40 drugs associated with 1 or more of 13 different genes, including clopidogrel, atorvastatin, simvastatin, warfarin, and others.[65] As of March 2021, personalized PGx reports had been produced for 6884 genotyped samples.[66]

In addition to clopidogrel, simvastatin, and warfarin, there are promising PGx associations that are not currently listed and are under evidence review in the CPIC guidelines, but have testing guidance in DPWG. β-Adrenergic receptor antagonists, or β-blockers, like metoprolol, are indicated for a variety of CVD and are primarily metabolized by the CYP2D6 enzyme. A meta-analysis by Blake et al in 13 studies with a total sample size of 264 participants demonstrated an effect of *CYP2D6* metabolizer phenotype on metoprolol pharmacokinetics. Pooled analysis indicated a 5.3-fold difference in peak dose-normalized plasma metoprolol concentration between UMs and PMs.[67] Furthermore, a systematic review and meta-analysis performed by Meloche and colleagues of 15 studies, including a total of 1146 individuals, indicated that patients with inactive *CYP2D6* phenotype have increased clinical effects and bradycardia with metoprolol, compared with those with an active *CYP2D6* metabolic capacity.[68] Also, class 1 antiarrhythmic drugs, such as propafenone and flecainide, are metabolized by *CYP2D6* and DPWG recommends reducing the flecainide and propafenone doses to 50% and 30% of the standard dose, respectively, in *CYP2D6* PMs.

METHODS FOR PHARMACOGENETIC TESTING

Although genome sequencing has become affordable for precision medicine programs focusing on cancer and the diagnosis of rare diseases, its costeffectiveness has not been

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proven yet for broader application such as population scale implementation of pharmacogenetics. Targeted sequencing of pharmacogenes with short or long-read sequencing[69] are more within reach, and particularly the latter has several advantages over other methods that cannot uniquely align reads or probes in the high homology regions of the Cytochrome P450 family of genes.[70] For population-scale programs, microarrays remain important for the rapid and ideally pre-emptive screening of millions of individuals at a low cost. The Axiom Precision Medicine Diversity Research Array from Thermo Fischer covers >850 000 genetic variants, and >5000 PGx markers in >1100 genes, including coverage of core and extended ADME genes across categories 1–4 in Pharmacogenomics Knowledge Base.[71] When used in conjunction with the Axiom 2.0 Plus Assay, which uses a gene-specific amplification, critical star alleles of highly homologous genes, including CYP1A2, CYP2D6, CYP2B6, CYP2A6, CYP2C18, and CYP2C8 can be detected. Recently, Illumina released the Infinium Global Diversity Array with Enhanced PGx Content,[72] with an improved coverage of high-priority pharmacogenes, and a special protocol for the difficult to discern genes like CYP2D6, CYP2D7, and CYP2B6. Overall, the array covers >1.9M markers, including >44 000 PGx variants. Importantly, both platforms also provide bioinformatic solutions for star allele calling, and Illumina also reports the corresponding metabolizer status, which simplifies the adoption of the test at clinical laboratories. However, as with most other platforms, these arrays are currently "for research use only," and further certifications are required before they can be widely adopted for clinical use. An overview of the characteristics of methods for pharmacogenetic testing is provided in Table 2.

One challenge that is often raised in discussions of barriers of implementation of pharmacogenetic testing is long turnaround time for obtaining test results.[73,74] The impact of the time delay varies depending on the required medication. For instance, *CYP2C19* genotyping prior to clopidogrel prescription in relation to a scheduled PCI versus warfarin dosing adjustment, or medications being prescribed in emergency care settings. However, several studies have proposed and shown proof-of-concept solutions to this challenge. One point-of-care *CYP2C19* genotyping device with a turnaround time of an hour was successfully evaluated for *CYP2C19**2 testing in the clinic to assist clopidogrel dosing.[75] In another study, point-of-care genetic testing was demonstrated for the implementation of genotype-guided dosing of warfarin in 3 UK clinics, with test results available within 45 minutes.[76] Ultimately, with the advancement and broader adoption of genome-wide genotyping or whole-genome sequencing and electronic health records, preemptive pharmacogenetic testing will become accessible by integrating decision support software that can interpret existing genotype data into treatment recommendations and minimize both the cost and inconvenience of additional tests and long turnaround times for reporting test results.[77–79]

Future Directions

Although tremendous progress has been made in pharmacogenetics in the last years, great challenges remain for its widespread adoption. First, there is a need for stroke-specific RCTs to test the effectiveness of genetically-guided therapies to prevent and treat stroke. Indeed, many recommendations are based on extrapolation of results in CAD patient populations to stroke prevention. However, there is likely to be important differences in patient characteristics that could influence the performance of pharmacogenetic testing. Second, tailoring of stroke treatment according to molecular characteristics would benefit from recent advances in multi-omics technologies. Multi-omics approaches aim to integrate information from multiple comprehensive and agnostic detection modalities such as genomics, transcriptomics, epigenomics, proteomics, metabolomics, and microbiome. This approach can enable accelerated discoveries by leveraging the strengths of each modality and providing a continuum of information from DNA sequence to protein products and metabolites. Pioneering work in this area have shown promising results using microbiome to predict the effect statins and epigenetics to predict stroke outcome.[80] Although these remain to be further validated, combination of multiple "omics" modalities is a promising avenue for tailoring of treatment.

CONCLUSIONS

Pharmacogenetics has the potential to provide stroke and CVD patients with safer, better and more cost-effective drugs. There are numerous studies indicating strong evidence for the effect of specific genotypes on the outcomes of cardiovascular drugs, supporting further PGx implementation in the clinic, most covered also in this review. However, the adoption of pharmacogenetics in clinical practice has been slow. This slow transition may be due to the limited amount of evidence to support the use of pharmacogenetics in CVD patients or due to other barriers in implementation like finding technical

Table 2. Comparison of Methods for Pharmacogenetic Testing

Technology	Cost	Coverage	Sample throughput	Speed	Novel variants	Computational load
Real-time PCR	Low	Low	High	3–4 h	No	Low
Microarrays	Low	High	High	3 d	No	Low
Targeted sequencing	Medium	High	Medium	4–5 d	Yes	Medium
Whole-genome sequencing	High	High	Low	4–5 d	Yes	High

PCR indicates polymerase chain reaction.

solutions for genotyping and demonstrating their cost-effectiveness. There is still a need for more evidence-based approaches to assess the effect of genetic testing in both stroke and CVD patients. Furthermore, a better understanding of the genetics of CVD across individuals with different ancestry will be necessary to derive the full benefit from pharmacogenetic testing.

ARTICLE INFORMATION

Affiliations

Department of Clinical Epidemiology & Biostatistics, McMaster University, Hamilton, Ontario, Canada (S.R., G.P.). Estonian Genome Centre, Institute of Genomics, University of Tartu, Estonia (K.K., L.M.). Population Health Research Institute, McMaster University, Hamilton, Ontario, Canada (G.P.). Department of Pathology and Molecular Medicine, McMaster University, Hamilton, Ontario, Canada (G.P.). Thrombosis and Atherosclerosis Research Institute, Hamilton Health Sciences and McMaster University, Hamilton, Canada (G.P.).

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