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REVIEW

The role of *CYP2C19* genotyping to guide antiplatelet therapy following ischemic stroke or transient ischemic attack

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ABSTRACT

Introduction: Clopidogrel is an antiplatelet agent recommended for secondary prevention of ischemic stroke (IS) and transient ischemic attack (TIA). Conversion of clopidogrel to its active metabolite by hepatic cytochrome P450-2C19 (CYP2C19) is essential for the inhibition of the P2Y12 receptor and subsequent platelet aggregation to prevent thrombotic events. *CYP2C19* is highly polymorphic, with over 30 loss of function (LoF) alleles. This review considers whether there is sufficient data to support genotype guided antiplatelet therapy after stroke.

Areas covered: A systematic literature review retrieved articles, which describe the interaction between *CYP2C19* genotype and clinical outcomes following IS or TIA when treated with clopidogrel. The review documents efforts to identify optimal antiplatelet regimens and explores the value genotype guided antiplatelet therapy. The work outlines the contemporary understanding of clopidogrel metabolism and appraises evidence linking *CYP2C19* LoF variants with attenuated platelet inhibition and poorer outcomes.

Expert opinion: There is good evidence that *CYP2C19* LoF allele carriers of Han-Chinese ancestry have increased risk for further vascular events following TIA or IS when treated with clopidogrel. The evidence base is less certain in other populations. The expansion of pharmacogenetics into routine clinical practice will facilitate further research and help tailor other aspects of secondary prevention.

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1. Introduction

Following an ischemic stroke (IS) or transient ischemic attack (TIA) several interventions are used in combination as part of a package of care to manage acute sequelae, promote rehabilitation, and prevent further vascular events. The reported rates of stroke recurrence after a primary event vary considerably, ranging from 4.3% to 20.6% over the first year and from 16.2% to 35.3% over the first 5 years [1–4]. A 2011 global meta-analysis estimated that 11% of individuals have a recurrence within a year and 26% within 5 years [5]. The effective prevention of these secondary events has major individual and societal benefits, maintaining patient quality of life whilst reducing utilization of acute healthcare services.

2. Identifying the optimal antiplatelet regimen

The rates of secondary stroke have fallen over the past three decades, demonstrating the success of secondary prevention strategies. A population cohort study reviewing the records of patients with a first-ever stroke between 1995 and 2018 in South London found that the rates of 5-year stroke recurrence dropped from 18% (95% CI, 15%–21%) to 12% (10%–15%) between 1995 and 2005. Lifestyle interventions, including smoking cessation and more regular exercise, play a major role in preventing further stroke, as does the effective control of modifiable risk factors, such as hypertension, hyperlipidemia and diabetes mellitus [6].

A major component of any secondary preventative strategy for stroke is the prescription of antiplatelet agents. A collaborative meta-analysis in 2002 found that antiplatelets reduced the rate of further stroke by approximately 25% over the course of two years [7]. Understanding which antiplatelet regimen offers maximal reduction of secondary vascular events, whilst balancing the risk of adverse events, has long been an important area of translational research (Table 1). In 1996 a randomised placebo-controlled trial of clopidogrel versus aspirin in patients at risk of ischemic events (CAPRIE) showed that patients treated with clopidogrel had an 8.7% (95% CI 0.3–16.5) relative-risk reduction of combined vascular events compared with patients receiving aspirin. In combination with health economic analysis, these data led to clopidogrel being recommended as the first choice antiplatelet agent following IS and TIA by the National Institute for Health and Care Excellence (NICE) in the United Kingdom (UK) [8].

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Article highlights

- Clopidogrel is a commonly prescribed antiplatelet agent which requires activation by the Cytochrome P450 enzyme CYP2C19.
- Common genetic variation in the *CYP2C19* gene, resulting in reduced CYP2C19 activity, has been associated with poorer clinical outcomes for a range of conditions when treated with clopidogrel.
- The best evidence is in the context of cardiovascular disease and *CYP2C19* genotyping is increasingly performed as part of routine clinical practice in many centers.
- The literature investigating the potential usefulness of *CYP2C19* genotyping in IS and TIA is heterogenous and most evidence has been produced in populations of East Asian ancestry.
- In East Asian populations, there is sufficient evidence to support *CYP2C19* genotype guided prescribing following IS and TIA. In other populations, the evidence is less definitive and further research is required.
- *CYP2C19* genotype represents just one variable which can influence outcomes following IS or TIA, and any genetic results should be considered within the wider clinical context.

In late 1990s and early 2000s, several trials set out to investigate the most suitable antiplatelet monotherapy to prevent secondary vascular events following IS or TIA [11,12]. Around this time, compelling trial data began to emerge demonstrating the benefit of duel antiplatelet therapy (DAPT) for patients with acute coronary syndrome (ACS) or in individuals having undergone percutaneous coronary intervention (PCI) [13]. Similarly, the combination of aspirin and dipyridamole was shown to be superior to aspirin alone following IS or TIA in an individual patient data meta-analysis [14]. Comparable results have been reported for cilostazol, another phosphodiesterase inhibitor, when combined with aspirin versus aspirin alone [15]. These data raised the possibility that combining clopidogrel and aspirin may offer superior outcomes following IS or TIA, compared with clopidogrel monotherapy. The Clopidogrel in High-Risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) Trial investigated the benefit of DAPT with clopidogrel and aspirin, relative to aspirin alone, in a predominantly Chinese cohort following minor IS or TIA treated within 24 hours after the onset of symptoms. Secondary stroke occurred in 8.2% of patients in the DAPT group, as compared with 11.7% of those receiving aspirin (hazard ratio 0.68; 95% CI 0.57–0.81).

The 2018 Platelet-Oriented Inhibition in New TIA and Minor Ischemic Stroke (POINT) Trial, which had a similar design to CHANCE, but was performed largely in North America and Europe, similarly found an additional benefit from DAPT. Patients with minor IS or high-risk TIA were randomized to receive either clopidogrel plus aspirin, or aspirin monotherapy. Combined major ischemic events occurred in 5.0% of patients receiving clopidogrel plus aspirin, compared to 6.5% receiving aspirin alone (hazard ratio 0.75, 95% CI 0.59–0.95), with most events occurring in the first week after the initial event [16]. In contrast to CHANCE, the POINT trial found an increased rate of hemorrhage in the DAPT arm.

With the awareness that two antiplatelet agents were superior to one, it was hypothesized that the addition of a third antiplatelet might add further benefit. The Triple Antiplatelets for Reducing Dependency after Ischemic Stroke (TARDIS) Trial set out to investigate this, comparing the safety and efficacy of intensive versus guideline-based antiplatelet therapy in patients with acute non-cardioembolic IS or TIA [17]. Participants were randomized in a 1:1 ratio to receive loading doses and then 30 days of intensive, triple antiplatelet therapy (combined aspirin 75 mg, clopidogrel 75 mg, and dipyridamole 200 mg twice daily) or guideline-based therapy (comprising either clopidogrel alone or combined aspirin and dipyridamole). In total, 3096 participants had been recruited to the TARDIS trial when the data monitoring committee advised that the study should stop prematurely due to an increase in major bleeding rates in the intensive antiplatelet arm. Intensive antiplatelet therapy was not shown to be associated with a significant reduction in the primary outcome.

The findings from TARDIS suggested that the addition of a third antiplatelet may not be an appropriate way to further optimize secondary prevention strategies following stroke. Another approach might be to utilize newer antiplatelet agent, such as ticagrelor. The Acute Stroke or Transient Ischemic Attack Treated with Ticagrelor and Aspirin for Prevention of Stroke and Death (THALES) Trial recruited patients with non-cardioembolic, non-severe IS or high-risk TIA [18]. This compared ticagrelor and aspirin DAPT versus aspirin alone and found that the risk of stroke or death within 30 days was lower with ticagrelor–aspirin (5.5%) than with aspirin (6.6%) alone (hazard ratio 0.79; 95% CI 0.68–0.93). A re-analysis of the THALES data demonstrated that this risk reduction applied to disabling as well as non-disabling strokes at 30 days [19].

The reduction in secondary stroke risk from antiplatelet therapy needs to be balanced against the increased risk of bleeding from the same medicines. Within the TARDIS trial, intensive antiplatelet therapy resulted in more bleeding and bleeding of greater severity (OR 2 · 54, 95% CI 2.05–3.16) [17]. The POINT trial found that hemorrhage occurred in 0.9% of patients receiving DAPT (clopidogrel plus aspirin) but only in 0.4% of patients receiving aspirin alone (HR 2.32; 95% CI, 1.10–4.87) [16]. There is some evidence, mostly from the cardiovascular literature, to suggest that ticagrelor has a higher bleeding risk as compared with other antiplatelet agents [20–22]. Within the stroke literature, the THALES trial found that severe bleeding occurred in 0.4% of patients receiving ticagrelor-aspirin compared to 0.1% of patients receiving aspirin alone (HR 3.26; 95% CI 1.40–7.59), although this effect is likely to be at least partially related to the comparison between DAPT and monotherapy. The Acute Stroke or Transient Ischemic Attack Treated with Aspirin or Ticagrelor and Patient Outcomes (SOCRATES) Trial compared ticagrelor against aspirin for the prevention of major vascular events in patients with non-severe IS or high-risk TIA [23]. In this trial ticagrelor monotherapy was not found to be superior to aspirin and the study also found no difference in bleeding rates between the two antiplatelet medicines.

Over the past two decades there has been extensive investigation to optimize secondary stroke prevention and identify the best antiplatelet regimen. Given the findings from TARDIS which showed that the use of three antiplatelet agents confer an unacceptably high rate of bleeding, it may be that DAPT represents a treatment ceiling with the currently available

Table 1. Randomized control trials investigating antiplatelet treatment regimens following stroke and TIA.

	Study Characteristics				Study Outcomes		
Study [Reference]	Recruiting Location	Eligibility Criteria	Treatment and/or Comparator	n	Primary Outcome	Secondary and/or Safety Outcomes	Findings
European Stroke Prevention Study (1987) [9]	Europe	TIA, RIND or IS	[Aspirin + Dipyridamole] vs Placebo	2,500	Stroke recurrence or death	Side effects and bleeding	33% benefit in favor of the treatment group ($p < 0.001$).
European Stroke Prevention Study-2 (1996) [11]	Europe	TIA or IS	Aspirin vs Dipyridamole vs [Aspirin + Dipyridamole] vs Placebo	6,602	Stroke recurrence or death	TIA, MI, and other vascular events. Bleeding.	Stroke risk reduced by 18% with aspirin, 16% with dipyridamole, and 37% with combination therapy.
CAPRIE (1996) [12]	Global	IS, MI or Symptomatic PAD	Clopidogrel vs Aspirin	19,185	Recurrence of IS, MI, PAD.	Amputation, major bleed, all-cause mortality	Primary outcome relative-risk reduction of 8 · 7% in favor of clopidogrel [95% CI 0 · 3–16 · 5]
SPS3 (2012) [79]	Global	Symptomatic small subcortical stroke/TIA within 6 months	[Aspirin + Clopidogrel] vs Aspirin	3,020	Stroke Recurrence	Rate of cognitive decline and major vascular events, Bleeding	No significant difference in primary outcome between treatment groups.
CHANCE (2013) [10]	China	Minor IS or High-Risk TIA	[Clopidogrel + Aspirin] vs [Aspirin]	5,170	Stroke recurrence	Composite vascular events	HR for primary outcome with DAPT: 0.68 [95% CI 0.57–0.81]
SOCRATES (2016) [23]	Global	Minor Stroke or High-Risk TIA	Ticagrelor vs Aspirin	13,199	Time to composite outcome of IS, MI, or Death	Composite vascular events. Major bleeding.	No significant difference in primary outcome between treatment groups.
TARDIS (2018) [17]	Denmark, Georgia, New Zealand, and the UK	TIA or IS	[Aspirin + Clopidogrel + Dipyridamole] vs [Clopidogrel or [Aspirin + Dipyridamole]]	3,096	Stroke or TIA recurrence	ADLs, cognition, MMSE score, mood. Hemorrhage.	No significant difference in primary outcome between standard and intensive treatment groups.
POINT(2018) [16]	North America & Europe	Minor Stroke or High-Risk TIA	[Clopidogrel + Aspirin] vs Aspirin	4,881	Composite outcome of IS, MI, or Death	Bleeding	HR for primary outcome with DAPT: 0.75 [95% CI 0.59–0.95]
PRINCE (2019) [70]	China	Minor Stroke or High-Risk TIA	[Ticagrelor + Aspirin] vs [Clopidogrel + Aspirin]	675	High Platelet Reactivity at 90 Days	Stroke Recurrence and Composite Vascular outcomes. Bleeding	HR for primary outcome with [Ticagrelor + Aspirin] = 0.40 [95% CI 0.28 to 0.56]
THALES (2020) [18]	Global	Minor Stroke or High-Risk TIA	[Aspirin + Ticagrelor] vs [Aspirin]	11,016	Stroke recurrence or death	IS and incidence of disability within 30 days. Severe bleeding.	HR for primary outcome with DAPT: 0.79 [95% CI 0.68–0.93]
CHANCE-2 (2021) [68]	China	Minor Stroke or High-Risk TIA and CYP2C19 LoFA carriers	[Ticagrelor + Aspirin] vs [Clopidogrel + Aspirin]	6,412	Stroke Recurrence	Bleeding	HR for primary outcome with [Ticagrelor + Aspirin]: 0.77 [95% CI 0.64–0.94]

IS = Ischemic Stroke. TIA = Transient Ischemic Attack. RIND = Reversible Ischemic Neurologic Deficit. MI = Myocardial Infarction. MMSE = Mini Mental State Examination. HR = Hazard Ratio. PAD = Peripheral Arterial Disease. ADL = Activities of Daily Living, LoFA = loss of function allele.

medicines. Therefore, maximizing the benefit from those two antiplatelet agents is of critical importance. The many trials discussed above demonstrate concerted efforts to identify the optimal antiplatelet regimen for secondary prevention of IS. However, this strategy may well have diminishing returns as it is iterated. The fine-tuning of DAPT is an important area of study, but the effect size across a population is likely to be less impactful as the optimal strategy is approached.

An alternative strategy is to personalize antiplatelet therapy. Rather than choosing antiplatelet regimens based on which treatment has greatest efficacy across a large population, there is increasing evidence to support tailored, individualized prescribing based on a patient's genetic profile, a concept known as pharmacogenetics [24]. For stroke medicine, this is highly relevant for the prescription of clopidogrel, a medicine which requires activation by the hepatic P450 cytochrome system before it can confer its antiplatelet effect.

3. Pharmacogenetics and drug metabolism

The United States (US) Food and Drug Administration (FDA) defines pharmacogenetics as the study of variation in DNA sequence as related to drug response [25]. Pharmacogenetics is intrinsically related to pharmacokinetics, the study of drug absorption, distribution, metabolism, and excretion [26]. The synergistic functioning of these processes ensures that an appropriate concentration of the medicine's active metabolite

is in the appropriate body space, at the appropriate time, to deliver a clinically relevant effect. Take, for example, clopidogrel which, prescribed as an oral formulation, is not metabolically active and requires processing by hepatic enzymes before it can irreversibility inhibit the $P2Y_{12}$ subtype of the ADP receptor, delivering its antiplatelet activity [27].

First, clopidogrel needs to be absorbed by the body, specifically by the ABCB1 transporter on the apical surface of intestinal cells, and then transported into the hepatic system for metabolization by the P450 Cytochrome system (Figure 1). The metabolism of clopidogrel takes place in two sequential steps involving several enzymes, the most clinically relevant of which is Cytochrome P450-2C19 (CYP2C19). Disrupted activity of the ABCB1 transporter, the CY2C19 enzyme, or other P450 cytochrome enzymes could all, theoretically, disturb the normal pharmacokinetics of clopidogrel, impacting its activity.

The proper functioning of pharmacokinetic proteins, such as ABCB1 and CYP2C19, is associated with variation in their genetic sequence. Many of these genes are highly polymorphic, meaning there are many sequence variants which can occupy the same genomic position (alleles) within a given population. Much of this variation will have very little impact on protein function, but some genetic variation can disrupt the activity of the protein product. In some cases, just one sequence variant in a gene related to drug metabolism is capable of rendering the medicine ineffective [28]. These pharmacogenetic variants are common in the general healthy population and can contribute significantly to differences observed in drug efficacy and can markedly increase the risk of adverse drug reactions (ADRs).

There are an increasing number of gene-medicine pairs where awareness of genetic variation should result in a change in prescribing behavior. The Clinical Pharmacogenetics Implementation Consortium (CPIC) creates international consensus recommendations for pharmacogenetic guided prescribing and there are currently peer reviewed guidelines for 26 gene drug pairs (www.cpicpgx.org/guidelines). One of the earliest of these publications related to the prescription of clopidogrel based on *CYP2C19* genotype.

3.1. CYP2C19 genotype and clopidogrel

Clopidogrel is a thienopyridine prodrug which requires hepatic biotransformation to form an active metabolite that selectively and irreversibly inhibits platelet aggregation [27]. As discussed above, conversion of clopidogrel to its active form requires two sequential oxidative steps in the liver involving several cytochrome P450 enzymes, including CYP2C19 (Figure 1).

Like many other members of the CYP450 superfamily, the *CYP2C19* gene is highly polymorphic with greater than 25 known alleles. The combination of two alleles one inherited from each parent, (the diplotype), determines an individual's metabolizer status, and a significant proportion of the population are considered to have 'poor' or 'intermediate' CYP2C19 function (Table 2) [27,29]. In pharmacogenetics the star (*) allele format is the nomenclature of choice, allowing for easier notation of diplotype status. In the case of *CYP2C19, *1* is the normal or 'wild-type' allele with a Western-European allele frequency of 0.63. **2* and **3* are the most common loss of function alleles (LoFA) and carriers of one of these (i.e. **1/*2* or **1/*3*) are labelled as 'intermediate' metabolizers and so produce less of clopidogrel's active metabolite [30]. Meanwhile, any individual who carries two LoFA is a 'poor' metabolizer. Conversely, **17* is the commonest increased function allele and is associated with enhanced conversion to active clopidogrel.

An individual's CYP2C19 metabolizer phenotype, derived from their *CYP2C19* genotype, is associated with their ability to process clopidogrel. The population frequency of CYP2C19 phenotypes varies across different biogeographical groups. Whereas 26% of patients of European decent are considered intermediate metabolizers, that figure rises to 45% of individuals with East Asian ancestry [30]. When considering the population frequency of poor metabolizers, individuals with severely reduced CYP2C19

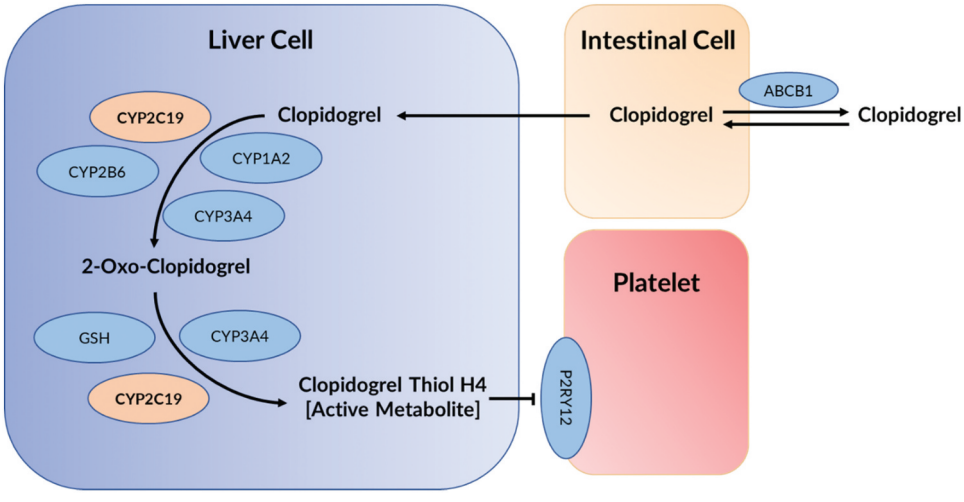

Figure 1. Absorption and metabolization of clopidogrel to form an active metabolite. Clopidogrel requires activation by the P450 cytochrome system before it can have its antiplatelet effect. Clopidogrel is absorbed into the intestinal cells by the ABCB1 transporter before being converted into its active metabolite by a series of enzymatic processes. Clopidogrel Thiol H4, the activated form of clopidogrel, is able to inhibit the P2RY12 subtype ADP receptor, preventing platelet activation. GSH = Glutathione. Figure adapted from [30].

Table 2. Assignment of CYP2C19 phenotype status based on genotype plus prescribing recommendations.

Likely Phenotype	Genotype	Examples of Diplotype	Clopidogrel Prescribing Implications
Normal Metabolizer	An individual carrying two functional (*1) alleles	*1/*1	Use label recommendations
Intermediate Metabolizer	An individual carrying 1 functional (*1) and 1 LoFA	*2/*1, *3/*1, *8/*1	Alternative antiplatelet should be considered
Poor Metabolizer	An individual carrying 2 LoFA	*2/*2, *2/*3, *4/*8A	Alternative antiplatelet should be considered
Ultra-Rapid Metabolizer	An individual carrying at least one increased activity allele (*17) and no LoFA.	*17/*1, *17/*17	Use label recommendations

Adapted from the Clinical Pharmacogenetic Implementation Consortium Guidelines for *CYP2C19* Genotype and Clopidogrel Therapy: 2022 Update [30]

activity, the discrepancy is even more marked. Whereas 13% of individuals of East Asian ancestry are predicted to be poor metabolizers, just 2.3% of individuals of European ancestry have the same status [30]. This is thought to be a major contributor to the 'East Asian Paradox,' a phenomenon whereby patients of East Asian Ancestry have reduced anti-ischemic benefits and increased bleeding risk during antithrombotic therapy. These population differences may have ramifications when considering optimal treatment strategies, as well as on the impact and relevance of *CYP2C19* testing within a population.

3.2. CYP2C19 genotyping in cardiovascular disease

Most of the clinical evidence for *CYP2C19* guided antiplatelet therapy has been produced in the context of cardiovascular disease, specifically ACS and PCI. Retrospective analyses have repeatedly demonstrated that *CYP2C19* LoFA carriers have worse outcomes after PCI when treated with clopidogrel [31–34]. However, despite this relatively robust evidence, there have only been limited attempts to implement these findings in the clinical setting. One barrier to implementation is likely to be because trials over the past two decades have demonstrated the superiority of other antiplatelet agents, namely ticagrelor and prasugrel, over clopidogrel after PCI following acute myocardial infarction (AMI) [35,36]. As such, national and international guidelines were adapted to recommend ticagrelor or prasugrel as first line antiplatelet agents in AMI, despite being more expensive and conferring an increased risk of bleeding than clopidogrel [35–39].

Unlike clopidogrel, ticagrelor and prasugrel do not require activation by CYP2C19. In 2009, the Platelet Inhibition and Patient Outcomes (PLATO) trial demonstrated the superiority of ticagrelor over clopidogrel after PCI following AMI. A genetic substudy of this trial, including 10,285 patients, investigated whether the increased benefit of ticagrelor may be related to the poor outcomes in the LoFA carriers within the clopidogrel groups [33]. This analysis found that ticagrelor was a more efficacious treatment for ACS than clopidogrel, irrespective of *CYP2C19* and *ABCB1* genotype. In the clopidogrel group, the event rate at 30 days was higher in patients with one or two *CYP2C19* LoFAs compared to wild type individuals (5 · 7% vs 3 · 8%), leading to earlier separation of event rates between treatment groups.

A similar genetic reanalysis of the TRITON–TIMI 38 Study, which in 2007 found a benefit from prasugrel over clopidogrel in patients with acute coronary syndrome, further highlights the relevance of considering CYP2C19 genotype when deciding upon an antiplatelet regimen [40]. Individuals with at least one *CYP2C19* LoFA were estimated to have a substantial reduction in the risk of the composite cardiovascular outcome (cardiovascular death, myocardial infarction, or stroke) whilst taking prasugrel as opposed to clopidogrel. The same reduction in risk was not observed in individual's who did not carry at least one LoFA.

Over the past decade the management of coronary artery disease (CAD) has progressively moved away from the use of clopidogrel and towards the use of other agents. This is partly because the initial results from the PLATO and TRITON-TIMI studies were so compelling and partly because, even if genotype guided therapy might be beneficial, identifying those patients in the acute setting prior to PCI was not a realistic proposition. Until recently, having an individual's *CYP2C19* status recorded within a patient's medical records or being able to generate that information via a point of care test (POCT) was not a realistic proposition. Historically, a range of genetic testing approaches have been applied in different centers to genotype *CYP2C19*, including Sanger sequencing, pyrosequencing, and SNP array genotyping platforms. All of these tests have a turnaround time measured in days or weeks, rather than minutes or hours [41]. However, genotyping technology has advanced significantly over the past decade and point of care genetics is now beginning to emerge as a realistic proposition [42].

In 2019 a randomized, assessor-blinded trial compared a genotype-guided dosing strategy for oral antiplatelet against ticagrelor in patients undergoing PCI [22]. In the genotype-guided cohort, patients with LoFAs were prescribed ticagrelor in lieu of clopidogrel. The hypothesis being that the additive value from ticagrelor was derived from LoFA carriers not responding to clopidogrel. The authors proposed that, in patients who appropriately metabolized clopidogrel, the efficacy of clopidogrel would be comparable to ticagrelor. The trial demonstrated that genotype-guided therapy was non-inferior to ticagrelor and, importantly, the risk of bleeding was lower. In summary, this work demonstrated that genetics could be applied acutely to guide prescribing, improve medicines safety, and potentially support the use of less expensive antiplatelet medicines, saving money for the healthcare system. Since the publication of this article a meta-analysis synthesized seven randomized control trials (RCTs), including the large TAILOR-PCI trial, and concluded that the effect of ticagrelor compared with clopidogrel in reducing ischemic events in

patients with CAD is based primarily on *CYP2C19* LoFA carrier status [34,43].

Data within the cardiovascular disease literature supports the use of genotype guided antiplatelet therapy following PCI and several leading pharmacogenetic centers perform *CYP2C19* testing as part of their routine clinical practice [44,45]. Although the evidence is less mature than that found in the cardiovascular literature, there have been several studies exploring the impact of *CYP2C19* testing to inform antiplatelet prescribing following IS or TIA. An approach which may offer a strategy to further optimize secondary prevention.

4. The evidence for CYP2C19 genotyping after stroke or TIA

A structured literature review was undertaken on 2 January 2022 to identify studies which investigated the impact of *CYP2C19* genotype on outcome following stroke or TIA. These were identified via a Boolean search of Medical Subject Heading (MeSH) Terms within Web of Science; MEDLINE (via OVID); Embase (via OVID); and PubMed Central. The following search [((((Pharmacogenetics) OR (Pharmacogenomics) OR (CYP2C19) OR (Genotyping)) AND ((Ischemic Stroke) OR (Stroke) OR (Transient Ischemic Attack)))] was used. No date limits were used and the final literature pool was agreed upon by all authors. For each article, relevance was determined by review of the title and abstract. 28 unique manuscripts were retrieved. The identified articles varied considerably in their design and quality and are summarized narratively herein (Table 3). The early literature consists mainly of cohort studies but, in recent years, several retrospective analyses of large RCTs have been published, significantly strengthening the evidence base.

One of the first studies to investigate the impact of *CYP2C19* genotype on clinical outcomes was published by Jia et al in 2013 [55]. In this prospective cohort study, 259 patients with acute IS were recruited and treated with 75 mg clopidogrel once daily. Participants were genotyped for *CYP2C19, CYP3A4* and *P2Y12* variants and then followed up at 7 days, 3 months, and 6 months. Clopidogrel response was also assessed by the change in adenosine diphosphate-induced platelet aggregation before and after 7-day treatment. The primary outcome of interest was not well defined, but the outcome considered most was whether the modified Rankin scale (mRS) score was less than or equal to 2 (termed 'good') at each time point. Participants were grouped by metabolizer status and comparisons were then made. The results showed significant differences by metabolizer status at 6 months, with 86% of normal, 77.8% of intermediate, and 65.6% of poor metabolizers with 'good' mRS scores. *CYP2C19* genotype or metabolizer status was not shown to interact with stroke recurrence risk.

In the following year three further cohort studies were published, two from China and one from the United States [56–58]. As is common across the *CYP2C19* stroke literature, the inclusion criteria within these studies varied considerably. Spokoyny et al undertook a retrospective cohort study using a database at a large comprehensive stroke center in the US, recruiting patients who had a recorded IS or TIA and if they already had their CYP2C19 status recorded in their medical records. Notably, even in 2014, all IS/TIA patients routinely underwent *CYP2C19* testing when being prescribed clopidogrel at their center, demonstrating the global variation in the utilization of pharmacogenetics. This study was likely underpowered and did not identify a statistically significant association between recurrent events and metabolizer status, although the authors argued that there was a trend toward normal metabolism conferring a protective effect (HR 0.23, 95% CI 0.04–1.14).

Whilst Spokoyny et al assessed records of patients with classical IS and TIA, another study that year by Lin et al conducted a retrospective analysis of 90 patients who had undergone vertebral artery stenting and were treated with clopidogrel and aspirin [57]. This demonstrated an association between CYP2C19 status and outcome, demonstrating that LoFA carriers had higher rates of in-stent restenosis (HR 2.96, 95% CI 1.33–6.61). This was followed by Zhang et al who, as part of a prospective cohort trial, found that the **2* variant was associated with both laboratory and clinical clopidogrel resistance [58]. Although the **3* variant was associated with laboratory clopidogrel resistance, this did not result in higher rates of stroke in this study.

These three trials from 2014 serve to demonstrate the heterogenous design of studies which report the interaction between *CYP2C19* genotype and outcome. Critically, caution must be taken when attempting to interpret these studies in combination, as was attempted in a 2017 meta-analysis by Pan et al [59]. This identified 15 studies of 4,762 patients with stroke or TIA treated with clopidogrel between 2013 and 2016. Of note, three of these studies recruited patients who had undergone some form of vascular stenting following an IS. The first of these was produced by Lin et al, as discussed above, which revealed an association between *CYP2C19* genotype and vertebral artery stent restenosis [57]. Zhu et al subsequently found that *CYP2C19* LoFA carriers were more likely to experience secondary vascular events following carotid artery stenting than non-carriers, if treated with clopidogrel [60].

The third study included in the meta-analysis assessed patients with cerebrovascular stenosis receiving stenting and DAPT (clopidogrel 75 mg and Aspirin 100 mg) [61]. Li and colleagues undertook a prospective cohort trial of 268 patients and over 12 months there were 39 events. The study did not identify a relationship between *CYP2C19* genotype and clinical outcome. However, it did find an association between rare variants in three other genes, *P2Y12, COX1*, and *PON1*. There is conflicting evidence regarding the impact of these genes on clopidogrel resistance, but there is certainly not sufficient data to consider testing for these in clinical practice to guide therapy or to support prognostication [62,63].

The process by which in-stent restenosis occurs is not directly comparable to the pathophysiology of atherosclerosis or thrombosis in a native vessel. Both might precipitate stroke and share certain pathological hallmarks, but there are likely to be certain distinct pathophysiological processes at play. Therefore, the interaction of *CYP2C19* genotype on outcome, and the resulting efficacy of clopidogrel, is also likely to be non-identical between these distinct conditions. As such,

Table 3. Characteristics of studies describing the interaction between CYP2C19 genotype and ischemic stroke.

		Study Characteristics					Study Outcomes		
Study	Reference	Study Type	Country	Eligibility Criteria	Treatment and/or Comparator	n	Primary Outcome	Secondary and/or Safety Outcomes	Interaction Between *CYP2C19* LoFA carrier status and Primary Outcome?
Jia et al (2013)	[55]	Cohort	China	IS	Clopidogrel	259	Stroke Recurrence	mRS >2	No. [HR 3.10; 95% CI 0.37–26.1]
Spokoyny et al (2014)	[56]	Cohort	USA	IS or TIA	Clopidogrel	53	Stroke Recurrence	N/A	No. Measured protective effect of extensive metabolism. [HR 0.23; 95% CI 0.04–1.14]
Lin et al (2014)	[57]	Cohort	China	IS or TIA with VAS	Clopidogrel + Aspirin	90	In-stent Restenosis	N/A	Yes. [HR 2.96; 95% CI 1.33–6.6]
Zhang et al (2014)	[58]	Cohort	China	IS	Clopidogrel	136	Stroke Recurrence	LCR, Increase in NIHSS Score, Additional Vascular Events	No. [3.17; 95% CI 0.96–10.51]
Qiu et al (2015)	[46]	Cohort	China	IS	Clopidogrel	211	MRS >2 at 6 Months	LCR, Composite Vascular Outcome	Yes. [3.01; 95% CI 1.23–7.38]
Sun et al (2015)	[47]	Cohort	China	First IS	Clopidogrel	625	Composite Vascular Outcome	Bleeding	Yes. [HR 2.31; 95% CI 1.39–3.84]
Jeong et al (2015)	[64]	Cohort	Korea	IS caused by large artery atherosclerosis	Clopidogrel	76	Early Ischemic Lesion Recurrence on Imaging	N/A	Yes. For Poor Metabolizers. [HR 19.3; 95% CI, 3.15–117.56]
Han et al (2015)	[71]	Cohort	China	IS	Clopidogrel	345	Composite Vascular Outcome	LCR, Bleeding	No. [HR 1.78; 95% CI 0.84–3.78]
SPS3 (2015)	[81]	Post-Hoc Analysis of RCT	Global	Symptomatic small subcortical stroke/ TIA within 6 months	[Aspirin + Placebo] vs [Aspirin + Clopidogrel]	522	Stroke Recurrence	Rate of cognitive decline and major vascular events, Bleeding	No. [HR 1.81; 95% CI 0.76–4.30] *Interaction identified in white participants. [HR 5.19; 95% CI 1.08–24.9*
Fang et al (2015)	[72]	Cohort	China	IS	Clopidogrel	114	Stroke Recurrence	N/A	Yes. [HR = 2.31; 95% CI 1.20–4.46]
Hoh et al (2016)	[75]	Cohort	USA	IS	Clopidogrel + Aspirin	188	Composite Outcome of TIA, IS, MI, or Death	Secondary Composite Endpoint of TIA, IS, or death	Yes, but direction of effect is opposite to what one would expect. [HR 0.13; 95% CI 0.03–0.62]
Li et al (2016)	[61]	Cohort	China	IS undergoing stenting	Clopidogrel + Aspirin	268	Composite Outcome of TIA, IS, MI, and death	N/A	No. [HR 0.49; 95% CI 0.17–1.46]
Yi et al (2016)	[62]	Cohort	China	IS	Clopidogrel	375	Composite outcome of Stroke Recurrence, MI, and death	MRS Score at 6 months	Yes. [HR 1.98; 95% CI 1.23–4.17]
Zhu et al (2016)	[60]	Cohort	China	IS and treatment with CAS	Clopidogrel	959	Stroke Recurrence	Hemorrhagic Stroke	Yes. [HR 2.13; 95% CI 1.067–4.26]
CHANCE (2016)	[82]	Post-Hoc Analysis of RCT	China	Minor IS and High-Risk TIA	[Clopidogrel + Aspirin] vs [Aspirin + Placebo]	2933	Stroke Recurrence	Composite Vascular Events	Yes. Only LoFA non-carriers received benefit from DAPT. [Non carriers: HR 0.51; 95% CI 0.35–0.75] Vs [Carriers: HR 0.93; 95% CI 0.69–1.26]
Wang et al (2016)	[48]	Cohort	China	IS	Clopidogrel	321	Composite Vascular Outcome	N/A	No. [HR 1.49; 95% CI 0.89–2.29]
Chen et al (2018)	[49]	Cohort	China	IS	Clopidogrel or Aspirin	634	Composite Vascular Outcome or MRS >2	N/A	No. [HR 1.125; 95% CI 0.62–2.04]

(*Continued*)

Table 3. (Continued).

		Study Characteristics					Study Outcomes		
Study	Reference	Study Type	Country	Eligibility Criteria	Treatment and/or Comparator	n	Primary Outcome	Secondary and/or Safety Outcomes	Interaction Between *CYP2C19* LoFA carrier status and Primary Outcome?
Tornio et al (2018)	[76]	Cohort	UK	IS	Clopidogrel or Clopidogrel + Aspirin	94	Composite Outcome of Stroke Recurrence or Death	N/A	Yes. [HR 2.23; 95% CI 1.17–4.24]
Yi et al (2018)	[50]	RCT	China	First IS	[Clopidogrel + Aspirin] vs Aspirin	570	Early Neurologic Deterioration	Composite Vascular Outcome, Bleeding	Yes. LoFA carriers benefited from DAPT vs Aspirin monotherapy. [HR 0.78; 0.58–0.96]
Lin et al (2018)	[51]	Cohort	China	First IS	[Clopidogrel + Aspirin] or Aspirin Monotherapy	375	Early Neurologic Deterioration	Composite Vascular Outcome, Bleeding	Yes. [HR 2.82; 95% CI 1.34–6.96]
PRINCE (2019)	[70]	Post-Hoc Analysis of RCT	China	Minor Stroke or High-Risk TIA	[Ticagrelor + Aspirin] vs [Clopidogrel + Aspirin]	675	High Platelet Reactivity at 90 Days	Stroke Recurrence and Composite Vascular Outcome, Bleeding	Yes. PMs and IMs randomised to Ticagrelor had decreased platelet reactivity at 90 days compared to those taking clopidogrel. This interaction was not observed for extensive metabolizers.
POINT (2020)	[83]	Post-Hoc Analysis of RCT	North America & Europe	Minor Stroke or High-Risk TIA	[Clopidogrel + Aspirin] vs [Aspirin + Placebo]	932	Composite Outcome of IS, MI, or Death	Bleeding	No. [HR 0.49; 95% CI 0.12–1.95]
Lv et al (2021)	[52]	Cohort	China	IS	Clopidogrel or Aspirin	485	Composite Vascular Outcome	Bleeding	Yes [HR 2.05; 95% CI 1.30–3.25 P = 0.002]
Lin et al (2021)	[53]	Cohort	China	First IS	Clopidogrel	122	Recurrent Stroke	Bleeding	Yes [HR: 7.56; 95% CI 1.35–42.78, P = 0.022].
Pilling et al (2021)	[78]	Cohort	UK	Patients in UK-Biobank Prescribed Clopidogrel	Clopidogrel	7483	IS	MI, Composite Vascular Outcome	Yes. [HR 1.53; 95% CI 1.04–2.26
CHANCE-2 (2021)	[68]	RCT	China	Minor Stroke or High-Risk TIA and LoFA carriers	[Ticagrelor + Aspirin] vs [Clopidogrel + Aspirin]	6412	Recurrent Stroke	Bleeding	Unable to assess. Risk of primary event lower in Ticagrelor arm.
Patel et al (2021)	[77]	Cohort	USA	ICAD	Clopidogrel + Clopidogrel DAPT	337	IS	N/A	Yes. [HR 3.4; 95% CI 1.40–8.20]
Patel et al (2022)	[54]	Cohort	USA	CAS	Clopidogrel and Aspirin	1110	First Time IS	Intracranial Hemorrhage	Yes. [1 LoFA – HR 2.3; 95% CI 1.10–4.70] and [2 LoFAs – HR 10.20; 2.80–36.80].

IS = Ischemic Stroke. TIA = Transient Ischemic Attack. LoFA = Loss of Function Allele. ICAD = Intracranial Atherosclerotic Disease. CAS = Carotid Artery Stenosis. PM = Poor Metabolizer. IM = Intermediate Metabolizer. EM = Extensive Metabolizer. HR = Hazzard Ratio. VAS = Vertebral Artery Stenting. N/A = Data Not Available. LCR = Laboratory Clopidogrel Resistance. MRS = Modified Rankin Scale. NIHSS = NIH Stroke Scale/Score.

including these studies within the same meta-analysis without explicit sub-group analysis is somewhat problematic.

Most studies included in the meta-analysis by Pan et al recruited patients with IS or TIA and treated with clopidogrel, either as monotherapy or as part of DAPT. Several of these found an association between *CYP2C19* genotype and clinical outcome, though the exact clinical outcomes varied between the studies. Several used recurrent stroke or composite vascular outcomes as their primary measures of interest. An outlier from this approach was the work by Jeong et al which recruited 76 Korean patients with acute IS who were treated with clopidogrel [64]. At 5-days after the index event, diffusion-weighted imaging (DWI) was used to detect the presence of ischemic lesions, which was used as the primary outcome measure. The study found that early lesion recurrence on DWI was more common in *CYP2C19* LoFA carriers than in non-carriers. The study did not report whether these lesions were associated with the rates of secondary vascular events, noting that a longer follow up time would be required to assess this.

By synthesizing the findings of the 15 studies identified within their literature review, Pan et al found that *CYP2C19* LoFA carriers were at increased risk of further stroke (12.0% vs 5.8%) and composite vascular events (13.7% vs 9.4%) in comparison with *CYP2C19* non-carriers. A fixed effects analysis found the relative risk for stroke to be 1.92 (95% CI, 1.57–2.35) in *CYP2C19* LoFA carriers compared to non-carriers. Notably, the analysis showed that individuals who carried two *CYP2C19* LoFAs (poor metabolizers) were at greater risk of stroke than those carrying one LoFA (intermediate metabolizers). The study did not find an association between *CYP2C19* genotype and bleeding risk. This was an important observation as several studies have suggested that the *CYP1C19*17* allele increases CYP2C19 activity and could lead to higher concentrations of clopidogrel's active metabolite, precipitating bleeding [65]. Of note, in 2021 a large multi-center collaboration assessed the records of 3,342 patients who had undergone PCI and CYP2C19-guided antiplatelet therapy. This analysis also found that the CYP2C19 *17 allele did not confer an increased risk of bleeding.

In their meta-analysis, Pan et al created funnel plots of the studies which were asymmetrical, indicating possible publication bias with an artificial deficiency of neutral studies. The addition of three hypothetical studies to correct for the bias reduced the summary relative risk for stroke to 1.80 (95%CI 1.47–2.21). Of the 15 studies identified 11 were from China, one was from South Korea and only three included patients of European ancestry. A subgroup analysis showed that the increased risk of stroke remained significant in patients with European ancestry, though the confidence intervals were broad (RR 2.46; 95% CI, 1.06–5.72).

The fact that the majority of publications investigating the interaction between *CYP2C19* genotype and outcomes following IS or TIA originate from China is unsurprising. Firstly, stroke is the third highest cause of death in China, accounting for 1.57 million deaths in 2018 [66]. The 2016 Global Burden of Disease (GBD) study estimated that the country had the highest estimated lifetime risk of stroke of any nation after age 25 years, up to 39.3% (95% CI, 37.5–41.1) [67]. This compares to lifetime risk of 22.7% (95% CI 21.4–23.9) in Western Europe. As such, there is a particularly urgent need to optimize stroke outcomes in China. Secondly, as discussed above, the frequency of LoFA carriers is far greater in East Asia than in other populations.

Numerous cohort studies in Chinese populations support the use of *CYP2C19* genotyping to tailor antiplatelet therapy following IS and TIA. This evidence was further bolstered by a 2016 reanalysis of the CHANCE trial. The work by Wang et al found a significant interaction between LoFA status and the impact of DAPT (clopidogrel and aspirin) on the rate of new stroke in the first 90 days. Only those without a LoFA received treatment benefit from the addition of clopidogrel [82]. Here, 6.7% of non-carriers had a new stroke with clopidogrel-aspirin compared to 12.4% with aspirin monotherapy (HR 0.51, 95% CI, 0.35–0.75). This is in comparison to carriers, in whom 9.4% of individuals had a new stroke taking clopidogrel-aspirin and 10.8% had a further stroke taking aspirin (HR 0.93, 95% CI, 0.69 to 1.26). Similar rates were observed for the secondary composite outcome measure.

Of the 2,933 patients included in the CHANCE sub-analysis, 1726 (58.8%) of participants were *CYP2C19* LoFA carriers. Furthermore, just under 10% of the cohort carried two *CYP2C19* loss of function (LoF) variants, although these poor metabolizers were not found to have increased rates of stroke compared to their intermediate counterparts. The proportion of LoFA carriers found in CHANCE is greater than had been expected based on previous estimates [27]. The spectrum of CYP2C19 activity in this cohort is skewed towards reduced activity, which means the finding may not be directly comparable to populations who are not of Han-Chinese descent, where there is a relative greater frequency of normal *CYP219* metabolism.

Spurred by the findings from the sub-analysis of CHANCE, the CHANCE-2 trial set out to test whether DAPT with ticagrelor and aspirin was superior to clopidogrel and aspirin in reducing the risk of further stroke amongst *CYP2C19* LoFA carriers [6,68,69]. This randomized, double-blind, placebo-controlled trial was conducted at 202 centers in China. A rapid POCT *CYP2C19* genotyping system was used to screen 11,255 patients who presented with minor stroke (NIHSS <3) or a high-risk TIA according to the ABCD2 criteria. 57% of patients were found to be LoFA carriers and therefore eligible for inclusion, similar to the prevalence of *CYP2C19* genotypes identified in CHANCE. The study demonstrated that the risk of stroke at 90 days was modestly lower with ticagrelor than with clopidogrel (6.0% vs 7.6) in patients with LoFAs (HR 0.77, 95% CI 0.64–0.94).

Although CHANCE-2 was well-designed, there was no comparator cohort of patients who did not carry *CYP2C19* LoF variants. As such, independently, the study does not answer whether ticagrelor plus aspirin is superior to clopidogrel plus aspirin because of the intrinsic superiority of ticagrelor to clopidogrel, or because clopidogrel was ineffective due to the population having reduced CYP2C19 activity. This is a critical distinction, as the latter could provide evidence to support a genotype guided antiplatelet strategy, whereas the former would support the wider use of ticagrelor-aspirin,

possibly negating the need for a genotype guided approach. To understand this further, the findings from CHANCE-2 should be viewed in the context of other RCTs, the most pertinent of which is the Platelet Reactivity in Acute Stroke or Transient Ischemic Attack (PRINCE) trial [70].

The PRINCE trial recruited 675 patients with minor stroke (NHSS <3) or high-risk TIA (ABCD2 > 4) from 26 centers across China [70]. The primary outcome was the proportion of patients with high platelet reactivity at 90 days, as defined by a P2Y12 reaction unit of more than 208 measured using the VerifyNow P2Y12 assay. The study also measured the rates of secondary stroke and composite vascular events. Unlike CHANCE-2, the PRINCE trial recruited patients irrespective of CYP2C19 status, but did undertake genotyping for the **2, *3* and **17* alleles in 650 patients. Of the patients included in PRINCE, 57.5% were found to be carriers of *CYP2C19* LoFAs. At the 90 day follow up, high platelet reactivity was observed in 12.5% in the ticagrelor-aspirin group compared with 29.7% in the clopidogrel-aspirin group (HR 0.40, 95% CI 0.28–0.56).

It should be noted that the PRINCE trial was not specifically powered to detect significant differences in clinical outcome. With that caveat, no significant differences were found in the rates of stroke at 90 days, occurring in 6.3% of patients in the ticagrelor-aspirin group and 8.8% of patients in the clopidogrel-aspirin group (HR 0.70, 95% CI 0.40–1.22). However, a pre-specified sub-analysis found that secondary stroke occurred more frequently at 90 days in patients whose stroke subtype was intracranial large artery atherosclerosis (LAA) when treated with ticagrelor-aspirin compared with clopidogrel-aspirin (HR 0.45, 95% CI 0.20–0.98). There was no analysis undertaken to assess whether these differences were still significant when the patient's *CYP2C19* genotype was considered. No significant differences were found for patients who had presented with non-LAA stroke (HR 1.1, 0.46–2.63).

Amongst *CYP2C19* LoFA carriers in the PRINCE trial, those taking clopidogrel and aspirin DAPT, in keeping with the summary outcome for the trial, had increased rates of high platelet reactivity at 90 days in comparison with those taking ticagrelor and aspirin. Notably, no significant difference in platelet reactivity was found between the two treatment groups for those patients who did not carry a *CYP2C19* LoFA (HR 0.59, 0.33–1.03). These data add weight to the hypothesis that at least part of the observed superiority of ticagrelor DAPT regimens is related to the phenomenon of *CYP2C19* LoFA carriers not responding appropriately to clopidogrel. The rates of major bleeding did not vary by treatment group in the PRINCE trial, but 20.5% of patients receiving ticagrelor-aspirin DAPT stopped taking their medicine early due to side effects, mainly dyspnea and epistaxis.

Even after taking into consideration the heterogeneity of study design and clinical outcome measures, there remains consistent evidence from multiple studies within Han-Chinese cohorts that *CYP2C19* LoFA carriers have poorer outcomes when treated with clopidogrel [59,71–73]. This effect is more marked with clopidogrel monotherapy, but many studies, including reanalysis of CHANCE, demonstrate the interaction of *CYP2C19* genotype with outcomes for DAPT [70,82]. However, there remains some uncertainty as to the clinical utility of this data, given the potential superiority of ticagrelor DAPT over clopidogrel DAPT, irrespective of *CYP2C19* genotype. CHANCE-2 demonstrates that ticagrelor-aspirin is a suitable antiplatelet regimen in LoFA carriers. However, even when considering the data from the PRINCE trial, it is uncertain whether this would still be the case in non-carriers. Given the increased rates of side-effects and treatment discontinuation in those taking ticagrelor, it may be that clopidogrel-based regimens remain preferable in *CYP2C19* LoFA non-carriers. Further studies are required to investigate this. Undertaking an adequately powered RCT with genotype guided and non-genotype guided treatment arms following stroke or TIA, similar to the design of Claassens et al performed after primary PCI, would be extremely valuable. Integrated health economic analysis would be required to understand whether a genotype guided antiplatelet strategy is cost effective [74]

4.1. The evidence for CYP2C19 guided antiplatelet therapy in non-East Asian populations

If the data from East Asian cohorts is reasonably compelling, studies in populations of other ancestries are less consistent. The US based cohort studies by Spokoyny et al, discussed above, and Hoh et al did not identify a significant association between *CYP2C19* LoFA carrier status and poorer clinical outcomes following stroke or TIA [56,75]. As already reviewed, the study by Spokoyny et al showed a trend towards an interaction and was likely underpowered to identify a significant association, however this was not the case in Hoh et al. Hoh et al recruited patients with symptomatic intracranial atherosclerotic disease (ICAD) both prospectively and retrospectively, identified by searching an existing electronic patient record (EPR) [44]. Genotyping was performed for the CYP*2C19*2, *3* and **8* alleles. In an unadjusted analysis, *CYP2C19* genotype was not associated with the primary clinical outcome, defined as TIA, IS, MI or death. However, after adjusting for covariates, the data showed that LoFA were associated with lower odds for the primary endpoint, a result which is antithetical to the existing literature and our understanding of *CYP2C19* function in clopidogrel metabolism.

One of the few cohort studies in a predominantly European population to demonstrate an association between *CYP2C19* genotype and poorer outcomes following IS was produced by Tornio et al [76]. This retrospective analysis identified 94 participants from the Tayside region of Scotland who had undergone testing for the *CYP2C19 *2* allele and had an IS treated with clopidogrel. Using a Cox regression model, they found that carriers of the *CYP2C19 *2* allele had an increased risk of further IS compared with non-carriers (HR 2.23, 95% CI 1.17–4.24). A subsequent cohort study, using a similar methodological approach, identified patients who had ICAD, but had not been diagnosed with an IS, meaning they had either had a TIA or were asymptomatic [77]. Univariate Cox regression revealed that *CYP2C19* LoFA carriers had an increased risk of first-time ischemic stroke (HR 2.1, 95% CI 1.1–4.1) if they had previously had a TIA, but not if they were asymptomatic.

Existing data repositories have also been leveraged on a larger scale to investigate the impact of *CYP2C19* genotype

status on clinical outcomes following IS. Pilling et al assessed the records and genetic information of 7,477 UK Biobank (UKB) participants and identified 110 individuals who had been treated with clopidogrel after an incident stroke who also had *CYP2C19* genotype data available [78]. Analysis suggested that *CYP2C19* LoFA carriers were more likely to have an IS whilst taking clopidogrel than non-carriers (HR 1.53, 95% CI 1.04–2.26).

The small number of cohort studies investigating the interaction between *CYP2C19* genotype and clinical outcome following TIA or IS in European populations have produced conflicting results. Interpretation of these studies is limited by small sample sizes, lower frequencies of LoFA carriers, retrospective study designs, and reliance on electronic disease codes to identify eligible participants, rather than in depth phenotyping of the stroke sub-type. Genetic reanalysis of existing RCTs in populations not of East Asian ancestry could provide more robust evidence.

The Secondary Prevention of Small Subcortical Strokes (SPS3) was a randomized, multicenter clinical trial conducted in 82 clinical centers in North America, Latin America, and Spain [79,80]. The trial recruited 3,020 patients with recent symptomatic lacunar infarcts, identified by magnetic resonance imaging, and patients were randomized to either 75 mg clopidogrel plus 325 mg aspirin or 325 mg aspirin monotherapy. The trial found that among patients with recent lacunar stroke, the addition of clopidogrel to aspirin did not significantly reduce the risk of recurrent stroke. The trial was terminated because of lack of efficacy combined with evidence of increased bleeding risk in the DAPT arm.

A genetic sub-analysis of SPS was undertaken with *CYP2C19* data available for 522 patients recruited to the DAPT arm [81]. The overall analysis did not find any significant difference in risk based on *CYP2C19* genotype. However, a sub-analysis of white participants, found that *CYP2C19* LoFA carriers had higher risk for stroke recurrence compared with non-carriers (HR 5.19, 95% CI 1.08–24.90). A similar trend was observed for black participants, though this was non-significant. The group were unable to determine a hazard ratio for the Hispanic population as there were no events in Hispanic LoFA carriers. The uncertainty of these findings may be explained by a low statistical power related to a small sample size.

A more recent genetic sub-study of the POINT Trial, which had a similar design to CHANCE but was performed largely in North America and Europe, found no significant interaction by *CYP2C19* genotype for major ischemia or stroke [83]. The authors suggested that this might be related to an inadequate statistical power which, calculated post-hoc, was estimated to be limited to 50% [83]. However, it should be noted that the point estimates within this sub-analysis trended towards demonstrating that LoFA carriers had greater efficacy from DAPT, counter to what one would expect if there was an association between *CYP2C19* genotype and outcome to be identified

4.2. Understanding the global discrepancy

The evidence in favor *CYP2C19* genotyping to guide antiplatelet therapy following IS or TIA in non-East Asian populations is limited. Although there is some evidence from small cohort and biobank studies, several other well-designed studies have failed to identify an association. Furthermore, retrospective genetic reanalysis of large RCTs have, thus far, not provided compelling evidence. At present, the data do not support the use of *CYP2C19* status to guide antiplatelet therapy in populations outside of East Asia, and the majority of evidence is specifically related to the Han-Chinese population. Within this population there are compelling and consistent data to support *CYP2C19* genotype guided antiplatelet prescribing.

Whether *CYP2C19* genotyping becomes part of routine clinical practice in China will depend on whether clopidogrel remains part of standard antiplatelet therapy following IS or TIA. This will be determined, in part, by the outcome of studies which investigate whether non-carriers of *CYP2C19* LoFA have superior outcomes with ticagrelor based DAPT regimens. If trials show that they do not, and clopidogrel based DAPT has equivalent outcomes with a potentially better safety profile, then there would be a compelling argument to integrate *CYP2C19* testing into existing stroke pathways.

Beyond China, the literature does not currently support *CYP2C19* genotype guided antiplatelet therapy following IS or TIA in other populations. The reasons behind this discrepancy are likely to be multifactorial. Some of the drivers of this inconsistency may be methodological in nature. The rate of *CYP2C19* LoFA carriage is far lower in populations outside of East Asia and the absolute rate of stroke is also lower. This can be seen by comparing the event rates for the aspirin treatment arms in the CHANCE and POINT trials, two methodologically similar studies. In patients taking aspirin, stroke occurred in 11.7% and 6.3% of participants in the CHANCE and POINT trials, respectively. The low incidence of *CYP2C19* LoFAs and low event rates mean that many non-East Asian studies have been statistically underpowered.

Increasing sample size might mean that in studies where there is already a strong trend towards significance, such as SPS3, are able to demonstrate statistical significance. However, this is highly unlikely to be the only driver behind the discrepancy. There was no trend towards significance in the POINT trial and it is unlikely that increasing sample size further would have altered this. A further explanation may be related to other differences between the two populations. Rates of tobacco use remain extremely high in China in comparison to other populations, with habitual smoking behavior reported in 72.28% of male stroke survivors [66]. In the POINT and TARDIS trials the comparable figures were 20.6% and 26% respectively.

There is evidence to suggest that cigarette smoking is associated with improved responsiveness to clopidogrel, resulting in decreased clopidogrel on-treatment platelet reactivity. This is known as the 'smokers paradox,' the concept that active smokers treated with clopidogrel have more major adverse cardiovascular event (MACE) reduction compared with non-smokers treated with clopidogrel [84]. There is conjecture regarding the mechanism which underpins this phenomena, but one hypothesis is that smoking can induce enzymes of the P450 cytochrome system meaning that smokers are exposed higher levels of clopidogrel's active metabolite, relative to non-smokers [85,86,87,88]. A reanalysis of the

CHANCE trial found that, compared to patients who never smoked, current smokers derived a greater benefit in stroke prevention at 90 days from clopidogrel [89].

The extremely high rates of smoking in China may also contribute, alongside other environmental and genetic factors, to differences in the distribution of stroke sub-types in China compared with other populations. There is some evidence that there may be higher rates of small vessel lacunar infarcts in the Han-Chinese population compared to individuals of European ancestry [90,91]. It is possible that disparities in stroke sub-types could be one of the drivers behind the variable impact of *CYP2C19* genotype in different populations, although this remains speculative and further evidence is required to test this.

In conclusion, there is good evidence that individuals of Han-Chinese ancestry who carry *CYP2C19* LoFAs have worse outcomes after stroke or TIA when treated with clopidogrel. The evidence is less certain in other populations and the cause of this divergence is uncertain, but unlikely to be related to one factor alone. Given evidence from cardiovascular studies, it is highly unlikely that *CYP2C19* will play no role in IS or TIA outcomes for clopidogrel treated patients [34]. However, more evidence is needed to understand the magnitude of this impact and to assess whether there are certain stroke cohorts where *CYP2C19* genotyping is of particular importance.

5. Expert opinion

Genetics is not a panacea. Decisions around medical treatment should not be made based on genetic information alone. The evidence reviewed here clearly demonstrates that an individual's *CYP2C19* genotype is not deterministic. A poor metabolizer is not destined to have a recurrent stroke if treated with clopidogrel, just as a normal metabolizer is not destined to live recurrence free with clopidogrel. Rather than viewing genetic data as conferring a binary outcome, it is more appropriate to conceptualize a genomic variant as conferring an increased or decreased risk of an event occurring in the context of different treatments. As with any other variable in medicine, this risk does not occur in isolation and must be viewed in the context of other aspects of the patient's clinical history and results. In particular, this may include awareness of a patient's high on-treatment platelet reactivity which, whilst being strongly associated with adverse clinical outcomes, is imperfectly correlated with *CYP2C19* genotype [92,93,94].

Despite efforts from within the pharmacogenetic community, there remains a lack of consensus, and even some conjecture, in relation to prescribing recommendations for clopidogrel based on *CYP2C19* status. In 2022, CPIC published their updated guidelines for clopidogrel prescribing based on *CYP2C19* genotype [30]. Recommendations for cardiovascular indications were clear, concise, and given with a 'strong' classification; clopidogrel should be avoided in intermediate or poor CYP2C19 metabolizers after ACS or PCI. For neurovascular indications, such as IS or TIA, the recommendation was much less definitive. The consensus panel gave a recommendation, with only moderate strength, that in poor or intermediate metabolizers alternative antiplatelets should be considered. Meanwhile the Dutch Pharmacogenetic Working Group (DPWG), another institution who curate pharmacogenetic evidence, have recommend that poor metabolizers should avoid clopidogrel after an IS or TIA [95]. In intermediate metabolizers, a recommendation was made to either avoid clopidogrel or double the dose. The rationale for this recommendation is unclear, though the discrepancy between the two guidelines is likely to be a further symptom of the variable and somewhat contradictory literature identified in this review.

The data reviewed here show that *CYP2C19* LoFA carriers of Han-Chinese ancestry are more likely to have further vascular events following a TIA or IS when treated with clopidogrel. In that population *CYP2C19* genotype interacts with clinical outcome in a relatively strong manner, which has been detected in many cohort studies and retrospective analyses of RCTs. It is quite possible that over the next few years, *CYP2C19* genotyping will become part of routine stroke care in China. The one barrier to this is whether clopidogrel remains part of routine antiplatelet therapy. It is unclear if *CYP2C19* LoFA non-carriers also have superior outcomes when taking ticagrelor based DAPT. If this were the case, then clopidogrel containing regimens may become redundant, negating the need for *CYP2C19* genotyping. However, at present, there is strong evidence to suggest that *CYP2C19* LoFA carriers of Han-Chinese descent should not be treated with clopidogrel following IS or TIA.

The evidence to support *CYP2C19* genotype guided antiplatelet therapy is far less clear in other populations. The reasons for this disparity have been discussed at length, but it is very unlikely to be related to one reason alone. Based on evidence from cardiology cohorts and the data from China, it would be highly surprising if *CYP2C19* genotype was not having any impact in cohorts not of Han-Chinese ancestry. However, due to yet unascertained environmental and genetic factors, the relative risk conferred by *CYP2C19* status may be smaller in these other populations. As such, larger trials may be required to accurately measure the impact of *CYP2C19* genotype on IS and TIA outcomes.

Given the need for further evidence in other populations, an important consideration is whether there would be the appetite to explore this research question further. If extremely large cohorts are required to find an effect, it would be reasonable to question whether the approach would be cost effective. If the number needed to genotype (NNG) to prevent a secondary stroke was extremely high, then genotype guided prescribing may not be a practicable part of stroke management. There is no definitive answer to this question at present, however the pharmacogenetic testing paradigm is likely to change significantly in coming years which may facilitate research and address the cost-effectiveness problem.

Over the past decade, many healthcare systems have integrated pharmacogenetic data into their routine clinical practice [44,96,97]. There are several models of delivery, but the most advanced is where pharmacogenetic data is pre-emptively incorporated into an electronic patient record (EPR). This is typically undertaken via a gene panel test, which includes a range of clinically relevant variants across multiple genes. The benefit of this is that if someone were to present with a stroke there is pharmacogenetic data to optimize the entirety of an individual's prescription, not just clopidogrel. There are peer-reviewed pharmacogenetic guidelines to guide the prescription of many medicines

related to stroke care including statins, proton-pump inhibitors, warfarin, and antidepressants [98,99,100,101].

Pharmacogenetics prescribing systems are highly likely to become more widespread over the next 5 to 10 years. These systems alter the health-economic argument for *CYP2C19* genotyping as, rather than a single gene test, a cheaper gene panel can be performed, meaning the utility of the test is not related to clopidogrel alone but to the patient's entire prescription history. Furthermore, having these data available within an individual's medical records could facilitate research, which is urgently needed to quantify the impact of *CYP2C19* genotype on clinical outcomes following IS and TIA in patients not of Han-Chinese ancestry.

Declaration of interest

PM Bath is Stroke Association Professor of Stroke Medicine and an Emeritus NIHR Senior Investigator; he was chief investigator of the TARDIS trial and a member of the CHANCE-2 trial steering committees. He has received honoraria from DiaMedica, Moleac and Phagenesis as a member of advisory boards for activity unrelated to the topic of this review. CJ Smith was an independent member of the TARDIS trial steering committee. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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