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‘Does personalised medicine exist and can you test it in a clinical trial?’

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Abstract
The idea that different patients will respond differently to the same treatment is not new. The recent advances in genomics and laboratory medicine have led to the hope that it will be possible to maximise the benefit and minimise the harms of each medical therapy by using an individuals' biomarker status to 'personalise' their treatment. The selection of treatment for each individual would then be determined, not just by their disease status (or an estimate of the risk of developing a disease or disease progression), but also by their genetic make-up or by other measurable characteristics, such as the level of a particular biomarker in the blood. This review discusses the extent to which personalised medicine might be applied in stroke, and the implications for global stroke health care.

Introduction
How plausible is this concept? There is a considerable variation in opinion about its likely current and future clinical impact of personalised medicine, ranging from the wildly optimistic[1] to the more cautious.[2, 3] However, whatever the benefits, widespread implementation of personalised medicine will have huge financial, ethical, regulatory and other implications for all stakeholders.[4] If the stroke community 'get it right', wise and wide application of personalised medicine might contribute to reducing the global burden of stroke. If we get it wrong, there is scope for huge waste and greater global stroke health inequality.[5]

Terminology
Personalised medicine[4] means tailoring the management of an individual person to the characteristics of their disease or biology, by the use of diagnostic technology to target treatment or to modify the treatment itself to maximise benefit and minimise harm (e.g. by the use of pharmacogenomics to tailor the dose, choice of agent or other aspect of drug delivery).[4] Stratified medicine operates at the level of groups of people, identified by a particular set of characteristics, and is defined by the US Food and Drug Administration (FDA) as: 'Using a biomarker to match a patient to a cohort that has exhibited a differential response to a treatment.'[6] Precision medicine is an analogous term recently given prominence by President Obama and is 'intended to avoid the implication that medications would be synthesized personally for single patients and to convey a broader concept that would include precisely tailoring therapies to subcategories of disease, often defined by genomics'.[7]

Methods of this review
Aims. The aims of this review were to: a) search the literature for papers relevant to stroke, cerebrovascular disorders and cardiovascular medicine, b) review relevant publications and identify the key clinical and methodological themes and c) summarise implications for current and future clinical practice and research in an attempt to answer the question posed in the title.

Protocol: there is no formal protocol for this narrative review, but where appropriate, I have tried to meet the criteria set out in the PRISMA checklist for systematic reviews.[8]

Search. A structured MEDLINE search of the literature; MEDLINE Search terms: (*Pharmacogenetics/ or *Individualized Medicine/ or personalised medicine.mp. or stratified medicine.mp.or precision medicine.mp or precision medicine.mp. or *Molecular Targeted Therapy/) and (Stroke, Lacunar/ or exp *Stroke/ or stroke.mp or cerebrovascular disorders).

The MEDLINE search yielded 94 potential papers. Material in this report is drawn from a variety of additional sources; I scanned the bibliography of the reports selected for inclusion in this article; the bibliography of the Wikipedia entry on Personalised Medicine;[9] the 4th Edition of the Personalised Medicine Coalition's publication;[10] the BMC Medicine collection of articles on Personalised Medicine[11]; the 2013 report of the FDA 'Paving the way for personalised Medicine'[6]; and searches of my personal files.

Data extraction and risk of bias. Without using formal qualitative research methods, I have extracted some key themes and the papers relevant to that theme. Risk of bias and conflict of interest are difficult to judge objectively, but are mentioned where necessary.

Outcome criteria for 'personalisation'. My criterion for accepting that a method for personalising treatment is truly 'effective' is that there is clear evidence:

a) that the 'personalising' test provides reliable guidance on prognosis, and
b) that the test performs better than clinical judgement, preferably when added to a validated prognostic score, and preferably also
c) of a significant quantitative marker x treatment effect interaction.[12]

In other words, the test reliably identifies that a patient identified 'positive' by the test will clearly respond better (or clearly worse) than those tested 'negative.' This is referred to as 'effect modification' or treatment interaction. Many factors influence prognosis, but markers that can identify groups where there is a clinically significant degree of effect modification are uncommon.[13, 14]

Breast cancer oestrogen receptor (ER) status as a biomarker of treatment-responsiveness

In 1988, the Early Breast Cancer Trialists Collaboration published a meta-analysis of the anti-oestrogen agent tamoxifen for the long-term treatment of breast cancer.[15] The analysis included data from 28 trials on 16,513 women of whom 4000 had died. About half the women had had ER status (ER rich or ER poor) determined at baseline; those with ER poor status had a worse prognosis, but ER status did not identify a group wholly unresponsive to tamoxifen.[16] While these early studies included very large numbers of women and a large number of deaths, the sample size was not sufficient to confirm that women with breast cancer who were 'ER poor' would not gain sufficient benefit from the drug to justify the significant anti-oestrogen side-effects. However, with the accumulation of more trials and longer term follow-up, an updated meta-analysis in 1998 with data on 37,000 women from 55 trials concluded 'For women with tumours that have been reliably shown to be ER-negative, adjuvant tamoxifen remains a matter for research. However, some years of adjuvant tamoxifen treatment substantially improves the 10-year survival of women with ER-positive tumours… with the proportional reductions in breast cancer recurrence and in mortality appearing to be largely unaffected by other patient characteristics or treatments.'[15] This example shows how difficult it can be, even for a common disease like breast cancer, that has been studied in large-scale long-term randomised trials, to identify and characterise the importance of a biomarker in prognosis (evident in the 1988 paper) and even harder to determine its importance as a valid and reliable marker of treatment response (in this case, it took an additional 10 years of clinical trial data collection to establish that fact). Put simply, to determine reliably that a biomarker x treatment interaction exists requires an enormous amount of data.

Genomic analysis to select patients with cancer or infectious disease

There are now quite a number of examples where genomic testing to personalise treatment have been (or could be) applied. The Personalized Medicine Coalition has published a list of examples[10] and it is striking that many are drawn from cancer or infectious disease. In patients with non-small cell lung cancer (NSLC), clinical trials have established that, among patients with mutations in the epidermal growth factor tyrosine kinase receptor (EGFR-TK), the EGFR-TK inhibitors gefitinib and erlotinib target specific cancer cells and are more effective and less toxic than standard chemotherapy regimens.[17] The value of diagnostic testing depends on the mutation frequency in the population; *EGFR* activating mutations occur in approximately 10% of Caucasian patients with NSCLC and up to 50% of Asian patients.[9] From the perspective of the UK NHS, personalised medicine must also be cost-effective; as it happens, identifying NSLC patients with EGFR mutations to select for treatment has proved cost-effective.[17] In the field of infectious disease, in patients requiring antiretroviral therapy with abacavir, those who carry the *HLA-B*57:01* allele are at high risk of experiencing a severe hypersensitivity reaction to the drug. In predominantly white populations, 94% of patients do not carry the HLA-B*5701 allele and are at low risk for this serious adverse reaction, but among the remaining 6% with the allele, the test has sufficient sensitivity and specificity to justify avoiding the use of abacavir and using alternative antiretroviral agents. Thus pharmacogenetic testing can be used both to maximise benefit and reduce harm from a drug in settings where testing is affordable.

Applicability of personalised medicine for stroke and cardiovascular disease

In stroke medicine, we have a number of simple tools that effectively stratify patients and identify groups of patients most likely to benefit from treatment: degree of carotid stenosis in patients with carotid territory transient ischaemic attack, risk scores for patients with atrial fibrillation and onset to treatment time in acute ischaemic stroke. However, stroke and cardiovascular medicine are, *a priori,* perhaps less susceptible to genomic approaches, for several reasons, but chiefly that the proportion of common cardiovascular disorders that are attributable to a single gene mutation is small and the already identified genetic common risk variants explain only a small proportion of overall stroke risk.[3] Although the discovery of highly effective biomarker-targeted therapies could be beneficial for a small number of individuals, the population–level impact on reducing the global burden of disease is likely to be small. Secondly, if commonly used therapies are to be targeted on those most likely to benefit and least likely to be harmed, then all patients will need to undergo (potentially expensive) biomarker characterisation before treatment is initiated. If that is the case, the pre-treatment testing must provide a high degree of discrimination and perform significantly better than targeting strategies based on simple clinical risk scores or basic laboratory data.

Stroke and cardiovascular disease

Risk scores to target drug-based Diabetes Mellitus prevention therapies?

Diabetes mellitus is a major risk factor for cardiovascular disease and stroke. Patients with impaired glucose metabolism, at high risk of developing diabetes, have substantial variation in their likelihood of receiving benefit from diabetes prevention treatments.[18] The Diabetes Prevention Program compared the impact of two different lifestyle interventions with lifestyle intervention plus metformin therapy on the development of new onset diabetes mellitus.[18] In a post hoc-analysis, the authors sought to determine whether some participants in the Diabetes Prevention Program were more or less likely to benefit from metformin.[18] They developed a diabetes risk model and found that the benefit of metformin was seen almost entirely in patients in the top quarter of risk of diabetes who averaged a 21.4% three year absolute risk reduction (number needed to treat 4.6), and no benefit was seen in the lowest risk quarter. Of course, decision making must be based on an accurate, simple, reliable and externally validated risk prediction tool. If, this tool were validated and

widely applied, it could target drug therapy on those most likely to benefit, decrease overtreatment, and make prevention of diabetes far more efficient, effective, and patient-centred.

Target folate therapy on hypertensive patients according to MTHFR genotype and baseline folate levels?

Methylenetetrahydrofolate reductase (MTHFR) is the main regulatory enzyme for folate metabolism. Polymorphism of the *MTHFR* gene C677T leads to a reduction in enzyme activity, resulting in decreased folate levels. Low folate levels are a modest risk factor for coronary heart disease (CHD) and stroke.[19] A meta-analysis of observational studies showed that individuals with the *MTHFR* 677 TT genotype had a significantly higher risk of coronary heart disease, particularly in the setting of low folate status.[20] Despite this background, folate supplementation for stroke prevention has –up to now - been controversial, and a series of trials testing the hypothesis did not provide clear evidence of benefit.[21] Recently, the large-scale trial CSPPT study in China (a population without folic acid fortification), aimed to assess the joint effects of baseline folic acid and MTHFR C677T genotype on response to folic acid supplementation added to antihypertensive therapy for primary stroke prevention.[22] All subjects had MTHFR C677T genotype determined before study entry and treatment allocation was stratified by genotype (CC, CT, or TT). The study included over 20,000 subjects with hypertension, and showed clear evidence of a reduction in stroke with folate supplementation. Though there was no significant interaction of treatment effect with baseline folate level, the authors noted – as might be expected - that the greatest reduction in stroke appeared to be greatest in patients in the lowest quarter of baseline folate levels.[22] The results from the joint analyses of *MTHFR* genotype and baseline folate level showed that among participants with the CC or CT genotypes, the highest risk of stroke and the greatest benefit of folic acid therapy were in those with the lowest baseline folate levels. In addition, the data suggested that individuals with the TT genotype may require a higher dosage of folic acid supplementation to overcome biologically insufficient levels.[22] While these data are of biological interest, the practical implications are limited. For a low-cost, non-toxic therapy like oral folate, folate supplementation of relevant foodstuffs for the general population would be more likely to yield greater population health gain than folate therapy targeted on individuals selected by genotype and folic acid level.

Genetically –determined dosing of clopidogrel?

Antiplatelet therapy is a cornerstone of drug treatment for stroke prevention. Bedside testing of blood or genetic biomarkers have been suggested as a way to maximise antiplatelet drug efficiency.[23] Variation in response to clopidogrel is partly determined by genetic factors, and CYP2C19 genotyping has been considered potentially helpful in dose-selection, but a systematic review and meta-analysis found that, in effect-modification studies, CYP2C19 genotype was not associated with modification of the effect of clopidogrel on cardiovascular disease end points or bleeding ($P > 0.05$ for interaction for both).[24]

Genetically –determined dosing of Warfarin?

Oral anticoagulation in patients with atrial fibrillation is an effective form of stroke prevention, but the (partly genetically-determined) inter-individual variation in dosing requirements of warfarin makes it difficult to establish a therapeutic level of anticoagulation rapidly and efficiently.[23] An algorithm for estimating the appropriate warfarin dose that is based on clinical and genetic data has been developed and validated, and provided significantly better predictions of appropriate dose of warfarin than either the clinical

algorithm or a fixed-dose approach.{The International Warfarin Pharmacogenetics Consortium, 2009 12989 /id} However, a formal cost-effectiveness model has shown that, given the additional cost of genotype testing (currently bout US $ 400 per person), this strategy is unlikely to be cost-effective for typical patients with non-valvular AF, but may be cost-effective either in patients at high risk for haemorrhage who are starting warfarin therapy, or if the cost of the genetic test falls substantially.[26]

Brain imaging to personalise therapy in atrial fibrillation (AF)?

A review of the role of MR brain imaging to stratify bleeding risk and stroke risk to optimise treatment of patients with AF concluded that, while MR is promising in this role, large scale trials are needed to determine its effectiveness and cost-effectiveness.[27] Looking to the future, the Atrial Fibrillation competence NETwork/European Heart Rhythm Association (AFNET/EHRA) consensus conference identified several potential methods that might allow more personalized management of AF beyond the traditional risk scores: integrating atrial morphology and damage; genetic predisposition; markers of systemic or local inflammation, and markers of cardiac strain.[28]

Avoiding statins in people at high risk of myopathy?

There is strong evidence that lowering LDL-cholesterol reduces the risk of cardiovascular events, and the Cholesterol Treatment Trialists Collaboration concluded *'In individuals with 5-year risk of major vascular events lower than 10%, each 1 mmol/L reduction in LDL cholesterol produced an absolute reduction in major vascular events of about 11 per 1000 over 5 years. This benefit greatly exceeds any known hazards of statin therapy.'*[29] It has been argued that the population health benefit will be greatest with a policy of 'statins for all over the age of 50.'[30] Personalising such therapy to reduce the harms of treatment, would require population-wide genetic testing of all people aged over 50 to identify the small number of patients with the SLCO1B1 variant who are at high risk of statin induced myopathy;[31] not an attractive or cost-effective prospect!

Clinically-stratified and targeted antithrombotic therapy for acute ischaemic stroke?

Patients with acute ischaemic stroke are at high risk of arterial and venous thromboembolic events that can significantly worsen outcome, yet immediate administration of antiplatelet or anticoagulant agents may, by increasing the risk of haemorrhagic events, negate any benefit.[32, 33] Two recent individual patient data meta-analyses of large-scale trial data have addressed whether stratification by the use of clinical risk scores might lead to better targeting of antithrombotic therapy with either aspirin or heparin[34, 35] in patients with acute ischaemic stroke. Unfortunately, none of the risk scores evaluated had sufficient discriminative power to be clinically useful.[34, 35]

Clinical or imaging targeting of intravenous thrombolysis for acute ischaemic stroke?

At present, the most important stratification tool for patient selection is the clinical history (to determine whether time from symptom onset is less than about 4.5 hours).[36] The use of risk scores, either to avoid treatment in people with high a risk of bleeding or select those with the greatest likelihood of benefit, is again beset with the problem that none of the risk scores have the discriminative power to be clinically useful.[37] The role of brain imaging is chiefly to exclude intracranial haemorrhage as the cause of the stroke. There are some imaging features that provide an additional degree of guidance; the pre-treatment scan appearance can predict prognosis,[38] and there is a significant interaction of extensive early ischaemic acute changes with the risk of intracranial bleeding.[39, 40] However, the extent of early ischaemic change did not interact with the effect of treatment on functional outcome.[40] In addition, some pre-stroke scan findings (such as atrophy and old infarcts) a

have clear effect on modifying the response to thrombolysis.[41] The role of advanced brain parenchymal, blood vessel and tissue perfusion imaging to personalise reperfusion and other acute therapies is worthy of a review in its own right.[42] There has been an extensive search for other blood and imaging biomarkers to supplement clinical tools, but none have yet proved robust enough for clinical use.[42] A model based on clinical and genetic factors has shown some promise as a tool to predict symptomatic intracranial haemorrhage and death, but the study begs the question as to whether genotyping could ever be done fast enough to guide thrombolysis.[43]

Scores, brain imaging, or other biomarkers to target stroke rehabilitation?

Rehabilitation is a huge field that is advancing rapidly, so just to give a flavour of the current state of affairs, I will focus on just one topic; interventions targeted at improving upper limb function. To start with, the best predictor of recovery of arm function after stroke is the severity of the initial stroke, and there is – as yet - little evidence that more complex clinical scores are any better.[44] As to therapy for improving arm function, a 2014 Cochrane 'overview of reviews' identified 40 completed reviews (containing 503 studies and 18,078 participants), covering 18 individual interventions.[45] The authors concluded 'there was moderate-quality evidence supporting the benefit of constraint-induced movement therapy (CIMT), mental practice, mirror therapy, interventions for sensory impairment, virtual reality and a relatively high dose of repetitive task practice, suggesting that these may be effective interventions.' However, there was no evidence of a score or biomarker that reliably predicted the response to these therapies. Yet, the development of animal models of post-stroke recovery, advanced neuroimaging and brain stimulation techniques and other advances offer the promise that basic science research will provide better tools to develop, refine and target rehabilitation therapies. For example, structural brain imaging with automated MRI scan analysis[46] and an algorithm combining baseline measures of arm strength and response to transcranial magnetic stimulation can help predict recovery of arm function at 3 months.[47] While predicting prognosis is helpful, identifying markers of response to treatment is more important; structural brain imaging[48] and a functional MRI show promise in selecting 'dose' of robotic arm therapy to promote motor recovery.[49]

Can personalised medicine be tested in trials?

Treatments with very large effects are rare in medicine, most have only moderate effects, and to detect such moderate effects reliably requires trials with very large sample sizes.[13] The best estimate of a relative treatment effect in an individual is the overall effect seen in a large trial or meta-analysis of large trials.[13, 14] Trying to determine whether a patient with a particular characteristic (e.g. having a particular genetic mutation) really will respond much better or much worse (or not at all) to the treatment than the 'average' is difficult. This property of modifying the effect of a treatment is detected by a statistical test for interaction.[50] Interactions can be quantitative (treatment is effective in both subgroups, but clearly more effective in one than the other) or qualitative (treatment is clearly beneficial in one subgroup and clearly harmful in the other). Reliable detection of true subgroup interactions even in very large meta-analyses requires both cautious interpretation and analyses that have substantial statistical power.[13, 14, 51] As Richard Peto puts it, in the context of personalised medicine for breast cancer, 'questions about such interactions are easy to ask, but difficult to answer.'[14] If it has been difficult in cancer medicine, I think the literature reviewed here suggests it will be even harder in stroke medicine.

Current implementation in clinical guidelines

While there are many suggestions about risk stratification tools, their implementation in practice is less well studied. A review of clinical practice guidelines for common diseases

(heart disease and stroke prevention, diabetes and breast cancer), sought to identify how evidence on the value of risk stratification tools was incorporated in the guideline.[52] The review included 133 guidelines but found that only a small proportion made risk-stratified treatment recommendations that were well supported by appropriate evidence.[52] It is clear that there will be many ethical, regulatory, financial and societal aspects to be dealt with in research and in clinical implementation of personalised medicine.[4]

Cautions on personalised medicine especially for low- and middle-income countries

Coote reminds us that wide implementation of personalised medicine could distract from low-cost and effective population-wide interventions and so may not be the route to a healthy world.[2] There is a danger that expensive hospital-focused medicine will draw resources away from disease prevention and long-term care for chronic diseases.[5] In low- and middle-income countries, therefore, access to personalised medicine for the wealthy few may have adverse consequences for the care of the many less well-off citizens in the country.

Conclusion

Methods for tailoring therapy for the prevention, acute treatment or rehabilitation of each individual stroke patient are at an early stage of development. In the near future, personalised medicine seems unlikely to make a major contribution to reducing the global burden of stroke and so – as stroke clinicians - we should focus on implementing interventions that are known to be effective (which and that will give us plenty of work to do for now). In the meantime, research teams must continue to study whether, and how to tailor the treatment to the patient more effectively than we do at present.

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Conflict of Interest

None

Author contribution

Peter Sandercock designed and performed the searches, selected the articles for review and wrote the manuscript

Reference List

(1) Dzau VJ, Ginsburg GS, Van NK, Agus D, Goldman D. Aligning incentives to fulfil the promise of personalised medicine. *Lancet* 2015 published on line May 6.

(2) Coote JH, Joyner MJ. Is precision medicine the route to a healthy world? *Lancet* 2015;385:1617.

(3) Markus HS. Stroke genetics: prospects for personalized medicine. *BMC Medicine* 2012;10:113.

(4) Godman B, Finlayson AE, Cheema PK, et al . Personalizing health care: feasibility and future implications. *BMC Med* 2013;11:179.

(5) Patel V. Rethinking personalised medicine. *The Lancet* 2015;385:1826-7.

(6) US Food and Drug Administration. Paving the way for personalised Medicine. 2013 Available at: URL http://www.fda.gov/downloads/ScienceResearch/SpecialTopics/PersonalizedMedicine/UCM372421.pdf (accessed 27/05/2015)

(7) Ashley EA. The precision medicine initiative: A new national effort. *JAMA* 2015 Published online April 30.

(8) Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA Statement for Reporting Systematic Reviews and Meta-Analyses of Studies That Evaluate Health Care Interventions: Explanation and Elaboration. *PLoS Med* 2009;6:e1000100.

(9) Personalised Medicine. *Wikipedia* 2015. Available at: URL: http://en.wikipedia.org/wiki/Personalized_medicine. (accessed 27/05/2015)

(10) The Personalized Medicine Coalition. The Case for Personalized Medicine. 4th. 2014. Washington, Personalized Medicine Coalition.. available at URL http://www.personalizedmedicinecoalition.org/Userfiles/PMC-Corporate/file/pmc_case_for_personalized_medicine.pdf (accessed 27/05/2015)

(11) BMC Medicine. Personalised Medicine Series. *BMC Med* 2015; Available at: URL: http://www.biomedcentral.com/bmcmed/series/personalized_medicine. (accessed 27/05/2015)

(12) Altman DG, Bland JM. Interaction revisited: the difference between two estimates.. *BMJ* 2003;326:219.

(13) Collins R, MacMahon S. Reliable assessment of the effects of treatment on mortality and major morbidity, I: clinical trials. *Lancet* 2001;357:373-80.

(14) Peto R. Current misconception 3: that subgroup-specific trial mortality results often provide a good basis for individualising patient care. *Br J Cancer* 2011;104:1057-8.

(15) Effects of Adjuvant Tamoxifen and of Cytotoxic Therapy on Mortality in Early Breast Cancer. *NEJM* 1988;319:1681-92.

(16) Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998;351:1451-67.

(17) National Institute for health and Clinical Excellence (NICE). EGFR-TK mutation testing in adults with locally advanced or metastatic non-small-cell lung cancer. 2013. Available at http://www.nice.org.uk/guidance/dg9 (Accessed 27/05/2015)

(18) Sussman JB, Kent DM, Nelson JP, Hayward RA. Improving diabetes prevention with benefit based tailored treatment: risk based reanalysis of Diabetes Prevention Program. *BMJ* 2015;350;h454.

(19) Homocysteine and risk of ischemic heart disease and stroke: a meta-analysis. *JAMA* 2002;288:2015-22.

(20) Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG. MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. *JAMA* 2002;288:2023-31.

(21) Clarke R, Halsey J, Lewington S, et al, for tBV. Effects of Lowering Homocysteine Levels With B Vitamins on Cardiovascular Disease, Cancer, and Cause-Specific Mortality: Meta-analysis of 8 Randomized Trials Involving 37 485 Individuals. *Arch Intern Med* 2010;170:1622-31.

(22) Huo Y, Li J, Qin X, Huang Y, et al. Efficacy of folic acid therapy in primary prevention of stroke among adults with hypertension in China: the CSPPT randomized clinical trial. *JAMA* 2015;313:1325-35.

(23) Hankey GJ, Eikelboom JW. Antithrombotic drugs for patients with ischaemic stroke and transient ischaemic attack to prevent recurrent major vascular events. *Lancet Neurology* 2010;9:273-84.

(24) Holmes MV, Perel P, Shah T, Hingorani AD, Casas JP. CYP2C19 Genotype, Clopidogrel Metabolism, Platelet Function, and Cardiovascular Events. *JAMA* 2011;306:2704-14.

(25) The International Warfarin Pharmacogenetics Consortium. Estimation of the Warfarin Dose with Clinical and Pharmacogenetic Data. *NEJM* 2009;360:753-64.

(26) Eckman MH, Rosand J, Greenberg SM, Gage BF. Cost-effectiveness of using pharmacogenetic information in warfarin dosing for patients with nonvalvular atrial fibrillation. *Ann Intern Med* 2009;150:73-83.

(27) Haeusler KG, Wilson D, Fiebach JB, Kirchhof P, Werring DJ. Brain MRI to personalise atrial fibrillation therapy: current evidence and perspectives.. *Heart* 2014;100:1408-13.

(28) Kirchhof P, Breithardt G, Aliot E, et al. Personalized management of atrial fibrillation: Proceedings from the fourth Atrial Fibrillation competence NETwork/European Heart Rhythm Association consensus conference. *Europace* 2013;15:1540-56.

(29) Cholesterol Treatment Trialists' (CTT) Collaborators. The effects of lowering LDL cholesterol with statin therapy in people at low risk of vascular disease: meta-analysis of individual data from 27 randomised trials. *Lancet* 2012;380:581-90.

(30) Ebrahim S, Casas JP. Statins for all by the age of 50 years? *Lancet* 2012;380:545-7.

(31) The SEARCH Collaborative Group. SLCO1B1 Variants and Statin-Induced Myopathy -- A Genomewide Study. *NEJM* 2008;359:789-99.

(32) Sandercock PA, Counsell C, Kane EJ. Anticoagulants for acute ischaemic stroke. *Cochrane Database Syst Rev* 2015;3:CD000024.

(33) Sandercock PA, Counsell C, Tseng MC, Cecconi E. Oral antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2014;3:CD000029.

(34) Thompson DD, Murray GD, Candelise L, Chen Z, Sandercock PAG, Whiteley WN. Targeting aspirin in acute disabling ischemic stroke: an individual patient data meta-analysis of three large randomized trials. *Int J Stroke* 2015 May 1;n/a.

(35) Whiteley WN, Adams HP, Jr., Bath PM, Berge E, Sandset PM, Dennis M, Murray GD, Wong KS, Sandercock PA. Targeted use of heparin, heparinoids, or low-molecular-weight heparin to improve outcome after acute ischaemic stroke: an individual patient data meta-analysis of randomised controlled trials. *Lancet Neurol* 2013 June;12(6):539-45.

(36) Emberson J, Sandercock P, Hacke W et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 2014;384:1929-35.

(37) Whiteley WN, Thompson D, Murray G, et al. Targeting recombinant tissue-type plasminogen activator in acute ischemic stroke based on risk of intracranial hemorrhage or poor functional outcome: an analysis of the third international stroke trial. *Stroke* 2014;45:1000-6.

(38) Wardlaw JM, West T, Sandercock P, Lewis S, Mielke O, International Stroke Trial Collaborative Group. Visible infarction on computed tomography is an independent predictor of poor functional outcome after stroke, and not of haemorrhagic transformation. Journal of Neurology, Neurosurgery and Psychiatry 2003;74: 452-458.

(39) Larrue V, von Kummer RR, Muller A, Bluhmki E. Risk factors for severe hemorrhagic transformation in ischemic stroke patients treated with recombinant tissue plasminogen activator: a secondary analysis of the European-Australasian Acute Stroke Study (ECASS II). *Stroke* 2001;32:438-41.

(40) Dzialowski I, Hill MD, Coutts SB, et al. Extent of Early Ischemic Changes on Computed Tomography (CT) Before Thrombolysis, Prognostic Value of the Alberta Stroke Program Early CT Score in ECASS II. *Stroke* 2006;37:973-978.

(41) The IST-3 collaborative Group, Wardlaw J. Association between brain imaging signs, early and late outcomes, and response to intravenous alteplase after acute

ischaemic stroke in the third International Stroke Trial (IST-3): secondary analysis of a randomised controlled trial. *Lancet Neurology* 2015;14:485-96.

(42) Saver J, Fisher M. Future directions of Acute Ischaemic Stroke Therapy. *Lancet Neurol* 2015 (In press).

(43) del Rio-Espinola A, Fernandez-Cadenas I, Giralt D, et al. A predictive clinical-genetic model of tissue plasminogen activator response in acute ischemic stroke. *Annals of Neurology* 2012;72:716-29.

(44) Coupar F, Pollock A, Rowe P, Weir C, Langhorne P. Predictors of upper limb recovery after stroke: a systematic review and meta-analysis. *Clin Rehabil* 2012;26:291-313.

(45) Pollock A, Farmer SE, Brady MC, et al. Interventions for improving upper limb function after stroke. *Cochrane Database Syst Rev* 2014;11:CD010820.

(46) Kou N, Park CH, Seghier ML, Leff AP, Ward NS. Can fully automated detection of corticospinal tract damage be used in stroke patients? *Neurology* 2013;80:2242-5.

(47) Stinear CM, Barber PA, Petoe M, Anwar S, Byblow WD. The PREP algorithm predicts potential for upper limb recovery after stroke. *Brain* 2012;135:2527-35.

(48) Riley JD, Le V, Der-Yeghiaian L, et al. Anatomy of stroke injury predicts gains from therapy. *Stroke* 2011;42:421-6.

(49) Takahashi CD, Der-Yeghiaian L, Le V, Motiwala RR, Cramer SC. Robot-based hand motor therapy after stroke. *Brain* 2008;131:425-37.

(50) Altman DG, Bland JM. Interaction revisited: the difference between two estimates.. *BMJ* 2003;326:219.

(51) Schulz KF, Grimes DA. Multiplicity in randomised trials II: subgroup and interim analyses. *Lancet* 2005 May;365:1657-61.

(52) Yu T, Vollenweider D, Varadhan R, Li T, Boyd C, Puhan MA. Support of personalized medicine through risk-stratified treatment recommendations - an environmental scan of clinical practice guidelines. *BMC Medicine* 2013;11:7.