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Genetic Signatures in the Treatment of Stroke

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Abstract: Stroke is the fourth leading cause of mortality and neurological disability. It is caused by an intricate interplay of environmental and genetic factors. Genes not only influence susceptibility to stroke but have also been found to alter the response to pharmacological agents and may also influence the clinical outcome of the disease. Current treatment strategies for stroke include tissue plasminogen activator, antiplatelet agents and lipid lowering drugs. These act via diverse mechanisms of actions and are centered around the management of modifiable risk factors to prevent the recurrent stroke events. However, a significant number of patients experience poor clinical outcome due to recurrent stroke events and drug induced adverse reactions. Therefore, accurate risk management and targeted prevention strategies remain yet to be explored at the level of individual patients with stroke.

Pharmacogenetic based research studies have identified the relation between genetic factors and inter-individual variability towards drug treatment. Several single nucleotide polymorphisms in genes encoding for metabolizers, transporters and target receptors have been reported to influence the pharmacokinetics and pharmacodynamics of drugs used in the treatment of stroke. Many candidate gene studies have investigated the role of genetic variants in association with altered drug response in stroke treatment. However, these results are limited to clinical trials and should be replicated in Genome Wide Association (GWAS) Studies. In addition to this long term follow up prospective studies would be helpful in predicting drug induced risk/benefit ratio. Pharmacogenetic studies will reveal the correlation between variation in drug responses on the basis of the individual's genomic profile better known as *Personalized or Individualized Medicines*. This will also optimize risk assessment and will stratify the population requiring careful attention before prescribing a particular medicine to achieve maximum therapeutic benefit. Moreover, this will help in designing the novel therapeutic agents with a targeted approach. In this concern, the Genomics and Randomized Trials Network (GARNET) has been created, which is a Pharmacogenomics Consortium aimed to identify genetic variants affecting an individual's response to treatment with the help of advanced technology. This review will address the major issues of therapeutic failures concerned with existing drugs used in the treatment of stroke and the need for exploring new and targeted therapeutic strategies based on pharmacogenetics.

Keywords: Personalized medicine, pharmacogenetics. stroke, single nucleotide polymorphisms, drug induced adverse reactions, therapeutic agents.

INTRODUCTION

Stroke is a complex neurological disease comprising of a heterogeneous group of disorders with multiple risk factors [1]. It is the fourth leading cause of death in the world. According to the World Health Organization (WHO), stroke and cerebrovascular diseases kill approximately 5.7 million people each year. About 80% of strokes are ischemic caused by occlusion of an intracerebral artery and 20% caused by intracerebral hemorrhage. The well-established risk factors for the development of stroke are age, hypertension, diabetes mellitus, obesity, cigarette smoking and cardiovascular diseases. Twin and family studies have reported a significant association for heritability, indicating that the genetic determinants are important in the development of stroke. Genetic causes of stroke, range from classic Mendelian (a single gene leads to disease) to complex (multiple genes contribute to disease in combination with other genetic and/or environmental factors) [1]. Success in stroke genetics with the discovery of mutations in some genes such as phosphodiesterase (PDE4D) and arachidonate 5-lipoxygenase-activating protein (ALOX5AP) has improved our understanding of the potential genetic variants of stroke and its subtypes [2].

Currently, the pharmacological agents or drugs, prescribed as the mainstay in stroke management are lipid lowering agents e.g. statins and recombinant tissue plasminogen activator (rtPA). Antiplatelet agents e.g. aspirin or ecosprin and clopidogril are prescribed as secondary preventive agents to reduce the stroke risk. Other prevention strategies for the individual patients focus on management of conventional risk factors like hypertension, hyperlipidemia and diabetes [2]. The accurate risk management and targeted prevention strategies remain yet to be explored at the level of the individual patient.

Genetic variants not only influence susceptibility to stroke but have also been found to alter the response to pharmacological agents and influence the clinical outcome of the disease. The effect of drugs prescribed to a group of patients with same age and same disease may differ from individual to individual. The Inter-individual variability in drug efficacy and toxicity has been reported to be related to several factors such as age, gender, race, comorbid factors and concomitant medicines. In addition to this inherited difference in the genes that control drug disposition and effects in humans have also been found to affect the drug response. The heterogeneous mechanisms involved in the pathogenesis of stroke and variability in drug response create opportunities for the development of novel and targeted therapeutic agents using pharmacogenetics. The major problems associated with stroke treatment are unpredictability of efficacy, drug resistance, adverse drug reactions and inter-individual variation in the dosage prescribed, which at least in part is the result of genetic variation. It is obvious that genetic variation plays an integral role in the variability of pharmacokinetics and pharmacodynamics of drugs. Single nucleotide polymorphisms (SNPs) are the most frequent form of sequence variations in the human genome affecting the therapeutic response of drugs used in the treatment of stroke. Pharmacogenetics is the science which identifies the influence of genetic variation on the efficacy and tolerability of various therapeutic agents. Genetic variants of genes encoding drug metabolizing enzymes (e.g. Cytochrome P450 family), transporter proteins [ATP caste binding protein (ABCB1), Selective cation transporter (SLCO1B1) and Organic

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anion transporter polypeptide (OATP1B1) and target receptor proteins [Cholesterol Ester transfer protein (CETP), Low density lipoprotein (LDL)] have been reported to influence the functional activity of respective enzymes/proteins, thus significantly altering the drug response.

We are at the dawn of a new era of personalized medicine in cerebrovascular diseases including stroke. Pharmacogenetics promises to personalize the treatment strategies in stroke with maximum therapeutic benefits and minimum side effects [3]. For example, an alpha adducin gene polymorphism in hypertensive patients treated with diuretics has been found to reduce the risk of stroke and cardiovascular mortality [4]. Similarly, it was reported that the specific genotype of the type III collagen alpha I gene was not only associated with stroke reduction but also reduced the risk of bleeding complications in those treated with 2A/3A inhibitor [5]. The major issues of therapeutic failure concerned with existing drugs used in the treatment of stroke and the need for exploring new and targeted therapeutic strategies based on pharmacogenetics have been discussed in this review.

Antiplatelet Agents

Antiplatelet therapy is a cornerstone to prevent cardiovascular events and stroke. Antiplatelet agents act via inhibiting the platelet activation. Aspirin and clopidogrel have emerged as important clinical entities in the treatment of these diseases. Numerous clinical trials have shown that both the drugs are effective in reducing ischemic deaths in myocardial infarction and stroke risk.Variable platelet response, potential resistance to therapy and drug associated adverse reactions (ADRs) are the major causes of therapeutic failure with aspirin and clopidogrel [6]. The mechanisms of resistance remain incompletely defined but an interplay of clinical (failure to prescribe appropriate medication or patient's noncompliance), cellular, biochemical and genetic factors has been found to be responsible for the therapeutic failure [7].

Aspirin

Aspirin is a derivative of salicylic acid and belongs to nonsteroidal antiinflamatory drugs (NSAIDs) category [8]. Aspirin has been reported to reduce the risk of stroke by 13%-25% [9]. Studies have shown that despite the development of many new antiplatelet agents still aspirin is considered golden standard of antiplatelet therapy and reduces the events such as nonfatal myocardial infarction to around 34%, and non-fatal stroke to around 25% [9]. Aspirin irreversibly inhibits cyclooxygenase i.e. COX (COX1 and COX2) enzymes catalyzing the first step of prostanoid synthesis. After a single dose of aspirin, platelet COX activity recovers by 10% per day in parallel with the entry of new platelets into the bloodstream. The usual antiplatelet dose of aspirin varies from 75-325mg/day.

Despite aspirin intake several patients develop adverse reactions of vascular origin, an observation that gave rise to the phenomena of aspirin resistance (AR). AR is a poorly defined term. This term has been used to describe not only the absence of desired pharmacological effects of aspirin on platelets but also poor clinical outcome such as recurrent vascular events in patients on aspirin treatment.

Studies have reported that AR occurs in 5-65% of people with ischemic stroke [10, 11]. We found 48.2% of stroke patients to be AR in a South Indian population from Andhra Pradesh [12].

The mechanisms of AR are multifactorial. It might be on account of inadequate dose of aspirin, reduced absorption, and/or increased metabolism of aspirin, noncompliance, cigarette smoking, diabetes mellitus and drug-drug interaction. Some drugs e.g. ibuprofen may compete with aspirin at COX1 receptor site, and can offset the clinical benefit of aspirin in a number of ways [13]. Poor glucose control and body weight have also been proposed to contribute to aspirin resistance [14]. In addition to this, a number of clinical and miscellaneous factors have been reported to contribute to the development of AR [15].

Apart from these a genetic etiology to AR has also been proposed. Genetic variants of COX-1, COX-2 genes, glycoprotein and several other receptor genes on the surface of platelets have been investigated in association with AR [6, 7]. Several variants of COX genes have been reported to affect the enzyme activity which might lead to the development of AR among patients [16]. Numerous association studies of both conservative and non-conservative variants of the COX-1 gene have been carried out for identification of variable response to aspirin. COX-1 gene polymorphism, C50T (rs3842787) results in a single amino acid change Pro to Leu at 17 position proximal to the signal peptide cleavage region. This variant is common in populations of European descent, but not among the Chinese population [17]. COX-1 haplotype A842G/C50T was significantly reported to be associated with aspirin response as determined by platelet aggregation and serum thromboxane B2 generation [18]. However, in a systemic review by Goodman (2008), no significant association was found between COX-1 polymorphisms (C50T/A842G) and AR [7]. In addition to this, these SNPs have also been reported to be associated with aspirin induced adverse effects such as gastrointestinal bleeding and urticaria. Individuals with the A842G/C50T polymorphism in the COX-1 gene have been found to possess a reduced ability to metabolize arachidonic acid by encoded enzyme. Numerous other single nucleotide polymorphisms in the COX-1 gene have also been evaluated in association with AR ; some showing a positive association whereas others could not establish an association [19].

A large number of variants have been identified in the COX-2 gene. Most of the SNPs were found in the intronic region with unlikely impact on COX-2 enzymatic activity. A limited number of polymorphisms have been found in the promoter region of the COX-2 gene with potential impact on COX-2 expression and activity. A functional G/C polymorphism 765 bp upstream from the transcription site in the COX-2 gene, has been identified. *In vitro* studies demonstrated that C' allele had significantly lower activity in comparison with 'G' allele [20]. We studied the association of COX-2 gene polymorphism -765G/C in stroke patients with aspirin resistance and found that the C allele of COX-2 gene associated significantly with AR in these patients [12].

Aspirin is metabolized by hydroxylation and glucuronidation with the help of UDP-glucuronosyltransferase 1A6 (UGT1A6), cytochrome P4502C9 (CYP2C9), N-acetyl transferase 2 (NAT2) and fatty acid Co IIA ligase (ACSM2) enzymes [21, 22]. Several SNPs in the genes encoding theses enzymes have been reported to modulate the response to aspirin and associate with the development of ADRs (e.g. Urticaria) [21]. So far major polymorphisms which have been reported to affect the biodisposition of aspirin are rs2070959, rs1105879 and rs6759892 in UGT1A6 gene, rs1133607 for the ACSM2 gene, and rs1799853, rs1057910, rs28371686, rs9332131 and rs28371685 of CYP2C9 gene [22]. However, these polymorphisms have not been evaluated for their association with aspirin resistance in stroke.

Genetic variants involved in the transport of aspirin like p-glycoprotein encoded by multidrug resistance gene-1 (MDR1) might be responsible for AR as well as ADRs among ischemic stroke patients. In a study from our laboratory, the role of C3435T polymorphism rs1045642 of multiple drug resistance-1 (MDR-1) gene was investigated with aspirin resistance in ischemic stroke patients. In this study, the risk of aspirin resistance was found to be more in patients bearing 3435TT genotype compared to the ones having CC genotype. However, there are no other studies on MDR1 gene variants in association with AR.

Genetic polymorphisms in the platelet glycoprotein (GPIIb/IIIa) receptors, and collagen receptor and thromboxane receptor genes have been reported to enhance the thrombus formation at the site of

vascular injury even in the presence of aspirin therefore, leading to AR [7, 23]. These observations suggest that the development of AR and ADRs in stroke patients might be on account of variation in COX-1 and COX-2 gene to some extent.

Clopidogrel

Clopidogrel is an oral antiplatelet agent used to reduce the recurrence of atherothrombotic events in patients with coronary artery disease, peripheral vascular disease, and ischemic stroke. The prodrug clopidogrel is metabolized by hepatic cytochrome isozymes (CYP450/CYP) to an active thiol metabolite. Different CYP isoenzymes (CYP3A5, CYP1A2, CYP3A4) are involved in metabolism of clopidogrel, among these CYP2C19 has been found to play a major role [24]. The active metabolite of clopidogrel binds to the P2Y12 receptor via disulfide bridge between the reactive thiol group and cysteine residues (Cys17 and Cys270) present in the extracellular domains of the P2Y12 receptor.

Despite being an effective antiplatelet agent a number of patients experience variability in drug response, drug resistance and associated adverse effects [such as hemorrhage, severe neutropenia, and thrombotic thrombocytopenic purpura (TTP)]. The prevalence of clopidogrel resistance varies from 4%-46%. The clinically poor responsiveness to clopidogrel was assessed in a meta-analysis including 15 studies and 3960 patients. Twenty five percent of the patients were found to be clopidogrel poor responders and the global relative risk (RR) for recurrent stroke events was found to be 3.5 (2.4-5.2, $p< 0.0001$) in this group [25]. The etiology of biologically non-responsiveness to clopidogrel is multifactorial. The factors contributing to the variability in clopidogrel resistance include a high body mass index, diabetes mellitus (insulin resistance), acute coronary syndrome (increased baseline platelet activity) noncompliance with the therapy and drug- drug interactions (e.g. Stations) [6]. In addition to this, genetic variations have also been suggested to play an important role in the development of clopidogrel resistance.

Genetic variants of CYP2C19 gene have been reported to influence the metabolism of clopidogrel thus altering the dose response relationship of the drug. The impact of CYP2C19 genetic variant (CYP2C19*1/*2, rs4244285) on the biological response to clopidogrel was first studied by Brandt *et al*. (2006), followed by several clinical, pharmacokinetic and biochemical studies which further investigated the effect of CYP2C19 gene polymorphism with clopidogrel response [26].

In a retrospective analysis, 74 healthy individuals receiving 300mg clopidogrel loading dose, were genotyped for different CYP genes. It was found that subjects carrying loss of function allele (*2) of CYP2C19 gene had a lower concentration (Cmax) of clopidogrel active metabolite, reduced inhibition of platelet aggregation and were defined as poor metabolizers in comparison with non-carriers [27]. Another study including 24 healthy subjects assessed the effect of CYP2C19 genotype on the plasma concentration of clopidogrel and its antiplatelet effect [28]. It was found that plasma levels of clopidogrel were higher in poor metabolizers than in heterozygous extensive metabolizers or homozygous extensive metabolizers. Poor metabolizers were reported to have a lower antiplatelet effect than heterozygous or homozygous extensive metabolizers.

In a first GWAS ['The Pharmacogenomics of Antiplatelet Intervention' (PAPI study)], it was reported that CYP2C19*2 accounts for 12% inter-individual variability in response to clopidogriel [29]. Another prospective study carried out by Bonello-Palot *et al*. (2009), the predictive factors of clopidogrel nonresponsiveness were high body mass index, diabetes mellitus, acute coronary syndrome and gene variant (*2) of Cytochrome P450 2C19 (CYP2C19) [30]. These results were further supported by a study including 760 cardiovascular patients, where CYP2C19*2 genotype accounted for only 5.2% of the antiplatelet response of clopidogrel assessed with ADP aggregation after a loading dose of 600mg of clopidogrel. This was consistent with the wide variation of clopidogrel responsiveness in both carriers and non-carriers of the CYP2C19*2. A significant proportion of patients without any mutated allele were found to have a high platelet response to clopidogrel (Clopidogrel resistance).

Several studies have also assessed the influence of CYP2C19*2 and other loss of function genetic variants (*3) on clinical events. However, results obtained from these studies are inconsistent. Two meta-analysis have been carried out to study the impact of CYP2C19*2 allele on ADRs in cardiovascular patients. The first meta-analysis gathered the data on more than 8000 patients on clopidogrel treatment (7 studies) and showed that CYP2C19*2 allele was associated with major adverse cardiovascular events (MACE, RR 1.96, 95% CI; 1.14-3-37, p=0. 02) [31]. The second meta-analysis collected the data from 11000 patients (23 studies) and showed that CYP2C19*2 allele independently associated with an increase in risk for MACE in comparison with the non-carriers of this allele (9.7% vs 7.8%, OR 1.29, 95% CI; 1.12-1.49, $p<0.001$) and also with an excess of mortality.

As evident from the above studies, the reduced enzyme activity has been reported to be associated with CYP2C19*2 variant. However, in a recent study involving 1524 patients with cardiovascular disease, an increased activity has been shown to be conferred by other genetic variant such as CYP2C19*17 allele (rs12248560). The increased enzymatic activity conferred by CYP2C19*17 allele was associated with increased response to clopidogrel and bleeding events in comparison with the non-carriers of the allele [32].

In the year 2010, US Food and Drug Administration (FDA) has issued a "boxed warning" on the label of warfarin. This warning addressed the need for pharmacogenomic testing to identify patients with altered clopidogrel metabolism and thus their risk for a suboptimal clinical response to clopidogrel.

Suh *et al*. (2006) reported a functional SNP in another CYP gene, CYP3A5 involved in the metabolism of clopidogrel that distinguishes expressor (*1) and non-expressor (*3) alleles [33]. This polymorphism influences the activity of encoded enzyme and shows racial differences in its frequency [34, 35]. The clopidogrel responsiveness among subjects with CYP3A5 expressor genotypes (*1*3 and *1*1) was higher in comparison with subjects bearing non-expressor (*3*3 genotype). It was also noticed that worse effects and resistance to clopidogrel was higher in patients with non-expressor group. Angiolillo *et al*. (2006), have shown an intronic polymorphism (IVS10+12G>A) in another CYP3A4 gene, which has a positive influence on the variable response of clopidogrel via altering enzyme metabolism [36]. These studies support that insufficient metabolism of clopidogrel might be the cause of non-responsiveness to clopidogrel.

Several variants in the gene encoding for P2Y12 protein which is a target of thienopyridines, have been identified. Fontana *et al*. (2003) reported a specific haplotype that associated with ADP induced platelet aggregation in healthy subjects [37]. However, other studies addressing the issue of a modulation of the biological effect of clopidogrel by variant of P2Y12 gave conflicting results. In addition to this the trials involved in assessing the issue of the clinical impact of polymorphisms of the P2Y12 receptor did not give positive results and therefore the variant in this gene does not seem to have much impact in modulation of clopidogrel response [38].

Another proposed mechanism of clopidogrel non-responsiveness is drug interaction e.g. with statins. Both drugs compete for CYP3A4 enzyme for metabolism. Therefore, the antiplatelet efficacy of clopidogrel is reduced by statins [39]. Genes involved in the absorption and transport of clopidogrel e.g. ATP caste binding protein / Multi Drug Resistance-1 (ABCB1/ MDR1) are the candidate genes which positively influence the variability of clopidogrel response and lead to drug resistance. Simon *et al*. (2009), consecu-

tively enrolled 2208 patients with an acute myocardial infarction on clopidogrel therapy, in a nationwide French registry [40]. They assessed the relation of C3435T polymorphism of ABCB1 gene with risk of death, nonfatal stroke or myocardial infarction during 1 year of follow up. They found that the patients with TT genotype had a higher value of cardiovascular disease events at 1 year than those with ABCB1 'CC'genotype (wild type). Mega *et al*. (2010), reported that this polymorphism was associated significantly with adverse clinical outcome (increased events of cardiovascular death and stroke) ($p<0.0064$) [41]. The patients bearing 'TT' genotype had a higher risk of primary endpoints when compared to the patients having CC and CT genotypes. Another study carried out in German population has indicated that the TT genotype of MDR1 gene leads to diminished concentration of clopidogrel and its active metabolite [42].

A poor responsiveness to antiplatelet agents is a major problem faced by clinicians and patients. Sometimes dual therapy (aspirin and clopidogrel) is recommended. The Clopidogrel in Unstable Angina to prevent Recurrent Events (CURE) trial reported that dual antiplatelet therapy using aspirin and clopidogrel reduced adverse coronary events by 20% [43].

However, the routine screening for antiplatelet resistance including platelet function test and genotyping is not supported by clinical guidelines till date.

Statins

The clinical use of statins consistently reduces the risk of coronary artery disease and stroke by lowering blood cholesterol through inhibition of the β-hydroxymethyl glutaryl coenzyme A (HMG-CoA) reductase enzyme. The relative risk of stroke is reduced by about 20% for each 1-mmol/lL decrease in low density lipoprotein cholesterol achieved using statins [44]. Several clinical trials have demonstrated the beneficial effects of statins in the primary and secondary prevention of cardiovascular and cerebrovascular diseases. The beneficial effects of statins exist beyond their cholesterol lowering effects, termed as "pleiotropic effects" [45]. These effects involve improvement of endothelial function, stabilization of atherosclerotic plaque, inhibition of cell migration, proliferation and also the reduction of inflammation and oxidative stress.

Although the clinical trials have shown 27% average relative risk reduction of major coronary events, there is large inter-individual variability in response to statins [46, 47]. The genes involved in the pharmacodynamic pathway of statins have been the focus of pharmacogenetic research in individuals with hypercholesterolemia [46, 47]. No doubt the emphasis is on genes involved in the cholesterol pathway, the genes involved with possible pleiotropic effects of statins have also gained a lot of interest. Statins are among the safest drugs in clinical practice, but ADRs like statin induced myotoxicity and rhabdomyolysis, a life-threatening event has been reported to occur in certain individuals.

Despite the well-defined safety profile of statins about 25-50% patients are noncompliant with statin medications even after one year of prescription [48]. One of the statins, cerivistatin has been removed from the market after multiple cases of severe myopathy and rhabdomyolysis and this led to focus scrutiny of all other available agents. Although the mechanisms for these side effects are not clear, certain patient characteristics such as low body mass, hepatic/renal dysfunction, concomitant medications have been identified to be responsible for impairment of statin disposition and metabolism [49]. Beside these factors genetic variation has also been reported to play an important role in the inter-individual variability for statin response [45, 50, 51].

The genetic variants of metabolizing genes have been shown to impair the pharmacokinetic of statins in a significant way. More than 40 SNPs in CYP enzymes have been documented to impair the response to statins in a number of research studies. A study carried out in Chinese hyperlipidemic patients identified the frequency of a common variant (CYP3A4*1G) gene as 2.76%. A gene dose-effect was found with increasing percentage reduction in serum total cholesterol in *1*1, *1/*1G, *1G/*1C with atorvastatin. However, this effect could not be seen in patients on simvastatin [52]. In a study carried out in Chinese hyperlipidemic patients, it was demonstrated that CYP3A4*4 allele results in lipid lowering effects of simvastatin to be increased for total cholesterol and triglycerides but not for LDL in comparison to those with the wild type allele [53].

The polymorphism identified in intron 6 of CYP3A4 gene (C>T, rs35599367), has also been shown play an important role in determining response to statin. Therefore, carriers of this polymorphism required significantly lower doses of statins for optimum control [53].

The CYP3A4 is another gene involved significantly in the metabolism of simvastatin. SNPs in CYP3A5 have been reported to influence enzyme activity. Genetic variant *3 of the CYP3A5 gene has been found to be responsible for the decreased enzyme activity thus leading to increased systemic exposure to simvastatin. The frequency of CYP3A5*3 variant has been reported to be approximately similar among Chinese (76%), Japanese (77%), and Caucasian subjects (85%) [51].

In Caucasian patients from Brazil no association between these alleles and response to simvastatin was found. However, in another study in subjects being treated with various statins, smaller lipid lowering responses in CYP3A5*1 wild type expressor compared to homozygous CYP3A5*3 enzyme non-expressor were observed. All these studies support the hypothesis that CYP3A4 and CYP3A5 related metabolism of statins might influence the clinical efficacy [51].

Drug transporters play an important role in the disposition of numerous drugs especially those which are more hydrophilic [51]. Prvastatin, rosuvastatin, simvastatin are hydrophilic and thus subjected to little metabolism. Therefore, drug transporters have a major effect on their disposition. Other statins or their active or inactive metabolites have also been demonstrated to be substrates for some transporters. Therefore, the drug transporters are likely to influence the pharmacological effects and potentially the adverse effects of some statins. Functional polymorphisms in genes encoding these drug transporters are likely to influence the pharmacokinetic profile as well as the efficacy of the transporter dependent drug. The frequencies of the polymorphisms of the genes for SLCO1B1 influx transporter and ABCB1 efflux transporter have been shown to vary between different ethnic groups and this might account for the interethnic variability in the pharmacokinetic and pharmacodynamic of statins. A detailed account of common polymorphisms in some well documented transporter proteins, which have close relationship with statin disposition has been given by Hu *et al*. [51].

Numerous studies have examined the effects of variants in transporter genes encoding for efflux (SLCO1B1) and influx transporter proteins (MDR1 gene) in association with statin's response. SLCO1B1 encodes for the enzyme organic anion transporter polypeptide1B1 (OATP1B1), which is responsible for the transport of stations to the liver. Almost all of the statins are the substrates of SLCO1B1 to a greater or lesser extent therefore, SLCO1B1 protein mediates hepatic uptake of most of the statins. A number of variants have been reported to influence the transport of statins [51]. The 388A>G (*1b allele), is highly prevalent in East Asian ethnic groups especially Malay population with a high frequency of 87%, but it is less frequent in Caucasians (14-20%) [54]. The frequency of another variant 521T>C (*5) has been reported to be 11-16% in East Asians which is similar to that reported in Caucasians (14-20%). The frequency is very less (1%) among Blacks [55]. The variant 521T>C (*5) of SLCO1B1 has been found to influence the pharmacokinetics of certain statins in various ethnic groups.

Pasanen *et al*. (2006) reported that subjects with CC genotype of this polymorphism had a 144% or 61% greater area under the curve ($AUC_{0\text{-}48hrs}$) values for atorvastatin and 100% higher values of the drug metabolite (2-hydroxyatorvastatin) in comparison with those bearing TT and TC genotypes respectively [55]. In another study from Finland relatively smaller but statistically significant effect of this polymorphism on rosuvastatin was reported. The $AUC_{0\text{-}48hrs}$ values were found to be 65% higher in those bearing CC genotype compared to the one with TT genotype [51]. However, in another study comparing the single dose pharmacokinetics of rosuvastatin 40mg daily among Chinese, Caucasians, Malay and Asian Indian subjects all living in Singapore, it was found that higher systemic exposure in Asians in comparison to Caucasians was not related to SLCO1B1 521T>C polymorphism, although some of the effects of this polymorphism were observed in these groups [56]. The SLCO1B1*5 polymorphism was found to have a marked influence on the pharmacokinetics of simvastatin with a 120 to 221% higher $AUC_{0\text{-}}$ for active simvastatin acid in carriers of CC genotype compared to those with TC and TT genotypes. Cmax for simvastatin acid was found to be 162 and 200% higher in the CC group than in TC and TT genotype group respectively. However, there was no significant effect on parent simvastatin lactone indicating that simvastatin acid is a specific substrate for SLCO1B1 and not simvastatin lactone.

In a trial, Statin Response Examined by Genetic Haplotype marker study (STRENGTH), the *5 allele (Val174Ala, rs4149056) in SLCOB1 transporter has been found to interfere with the localization of the transporter to the plasma membrane and reduce the statin clearance [57]. This further leads to higher systemic concentration of statins. The *5 allele has been identified in a GWAS as one of the dominant cause of severe statin induced myopathy in patients prescribed with 80mg of simvastatin [58]. In this study elevated levels of simvastatin metabolites were found among patients with targeted SNP in the absence of elevated creatinine kinase levels. The Study of the Effectiveness of Additional Reductions in Cholesterol and Homocysteine (SEARCH), a genome wide association study, has found SLCO1B1*5 to be associated with higher risk of statin induced myopathy in cardiac patients on simvsastatin 40-80 mg daily [58].

Two of the synonymous SNPs (1237C>T in exon 12 and 3435C>T in exon 26) and one non synonymous SNP (2677G>T) in exon 21 have been found in linkage disequilibrium, forming SNP haplotype [51]. This haplotype might be responsible for influencing the pharmacokinetic of statins and is helpful in pharmacogenetic analysis studies. Most of the studies involving 3435C>T polymorphism leading to lesser transport activity of the encoded protein, have provided inconsistent results. The frequency of 3435C>T polymorphism in the ABCB1 gene varies among different ethnic groups.

Fiegenbaum *et al*. (2005) examined the interactions between SNPs 1236C/T, 2677G/A and 3435C/T in the ABCB1 gene in association with the efficacy and safety of simvastatin in the Brazilian population [59]. It was reported that patients bearing ABCB11236T, 2677G and 3435T alleles, had a higher reduction in total and low density lipoprotein (LDL) cholesterol after treatment in comparison to homozygotes with wild type allele and adverse drug reactions.

A significant association of MDR1 and LPL gene variant with a bad outcome in stroke patients on atorvastatin therapy has been found suggesting that individuals bearing [HindIIIHindIII (-/-) genotype of LPL, and CC genotype of MDR1 gene would benefit more from atorvastatin therapy [45].

Vrablik *et al*. (2012), investigated the role of ten variants within the Cadherin EGF LAG seven-pass G-type receptor 2/ Protein Serine Rich Coiled Coil1 (CELSR2/PSRC1), Cartilage intermediate layer protein 2/pre-B-cell leukemia homeobox 4 (CLIP2/PBX4), Apolippoprotein B, E (APOB, APOE/C1/C4), HMGCo reductase, LDL receptor and Proprotein convert asesubtilisin/Kexin type 9 (PCSK9) genes in 895 patients with dyslipidemia treated with equipotent doses of statin, i.e. 90 % on simvastatin or atorvastatin (10 or 20mg/day) and 672 normolipidemic controls in a genome wide study [50]. In this study, carriers of 'G' allele of rs4420638 within the ApoC1/C3 gene cluster were found to have lower response to statin treatment compared to common homozygotes. However, variants (rs599838, rs646776, rs16996148, rs693, rs515135, rs4420638, rs12654264, rs6511720, rs6235, rs11206510) CLIP2/PBX4, PCSK9, LDL receptor, HMG-CoA reductase, CELSR2/PSRC1/SORT and in APOB genes did not modify the therapeutic response of statins [50].

ANTICOAGULANT THERAPY (WARFARIN)

Warfarin is a widely used oral anticoagulant used for the treatment of thromboembolic events and in cardioembolic stroke. It reduces risk of major ischemic vascular events and stroke. The anticoagulant mechanism of action of warfarin starts by inhibiting the enzyme vitamin K epoxide reductase in the liver, which inhibits the clotting factors (VII, IX, X and II). The major complications associated with warfarin therapy are large inter-individual variability in drug response and narrow therapeutic range [60]. Therefore, anticoagulant effect of warfarin is monitored by constant monitoring of international normalized ratio (INR). INR is the function of time required for a patient's blood to coagulate relative to the time it takes for a reference blood sample [61]. The treatment may be ineffective if the INR is low and the increase in INR more than the upper limit of the therapeutic range may increase the risk of bleeding [62]. The drug exhibits multiple food and drug interactions. The required dose of warfarin can be roughly calculated from clinical and demographic factors such as age, body weight, concurrent disease and drug and food interactions [63].

Warfarin is an equal mixture of the enantiomers of S-warfarin and R- warfarin (with S-warfarin being 3-5 times more potent than R-warfarin. S-warfarin is metabolized by cytochrome P450 2C9 enzyme and R warfarin through CYP2C19, CYP1A2 and CYP3A4 [64]. Variation in the CYP2C9 has been reported to influence the dose requirements by influencing pharmacokinetics of warfarin. It has been documented previously that patients homozygous for the wild type allele of CYP2C9*1 metabolize warfarin normally. Two clinically relevant SNPs have been identified in CYP2C9 (*2 and *3) associated with reduced enzymatic activity further leading to reduced drug metabolism and toxic effects. The *2*2 homozygous genotype results in a 12% reduction of CYP2C9 activity and *3*3 has been reported to have 5% reduced enzyme activity as compared to the wild type allele. These SNPs are commonly found in Caucasians. Patients requiring a lower dose of warfarin have a likelihood of having CYP2C9 variant alleles (*2 or *3) and are susceptible to increased risk of major bleeding complications. In another retrospective cohort study of patients receiving long term warfarin therapy for various indications, it was found that among 185 patients 31.4% had at least one variant of CYP2C9 allele and 68.6% had the wild type allele (*1*1 genotype) [65]. Patients with at least one variable allele had an increased risk of above range INRs. The variant group also required more time to reach the stable dose of the drug and were found to have more than twice the risk of serious bleeding events.

VKORC1 is the main rate limiting step in the biosynthesis of vitamin K-dependent proteins. Studies have implicated that polymorphism in a VKORC1 receptor gene is associated with a need for a lower dose of warfarin [66]. The VKORC1 genotype alone has been reported to explain nearly 40% of the variability in response to warfarin [62, 67]. In a study including 297 patients starting warfarin therapy, CYP2C9 genotypes (*1*2*3) and VKORC1 haplotype (designated as A and non-A) were assessed along with bleeding time, INR and clinical characteristics [68]. It was found that pa-

tients with A/A haplotype, had a decreased time to the first INR within the therapeutic range as compared to patients with non A/non-A haplotypes of VKORC1. In this study, both the CYP2C9 genotype and VKORC1haplotype were found to have a significant influence on the required dose of warfarin after first two weeks of therapy. Several other studies have also identified the role of VKORC1 in dose finding, dose maintenance and bleeding risk associated with Warfarin/acenocoumarol drugs [66, 69, 70, 71, 72]. At present, diet (type of food), concomitant medication and other associated comorbidities are considered to be responsible for warfarin non-responsiveness. Numerous multicenter randomized trials are still underway with unpublished results to clarify the clinical translation of pharmacogenomics of warfarin.

Understanding the consequences of an individual's status of metabolizing genes, transporter genes and frequency of variants in a given subset of patients belonging to different ethinic groups, could have tremendous effect about making the best decision using pharmacogenomic testing clinically. Few genetic variants associated with warfarin have been summed up in Table **1**. FDA has approved pharmacogenetic test for warfarin in order to avoid adverse drug effects [73].

Recombinant Tissue Plasminogen Activator (rtPA)

Thrombolytic therapy is the only effective treatment for ischemic stroke since it quickly restores blood flow after the occurrence of an acute occlusion. Recombinant tissue plasminogen activator (rtPA), is the only licensed pharmacological agent for thrombolysis (within 3-4.5 hours from onset). rtPA is a drug manufactured using recombinant DNA technology and is responsible for the association of plasminogen at intravascular levels and catalyzation of its conversation into plasmin. Plasmin is the main protein involved clot lysis. The first placebo controlled clinical trial on tPA was conducted in the late 1980s, to treat acute ischemic stroke [74].

Despite the benefits of thrombolysis for stroke patients only 14.3% of them are currently treated with rtPA. This is not only due to the fact that many patients arrive beyond the recommended time window for ischemic stroke treatment but also because of the adverse effects associated with rtPA administration. There is considerable mortality associated with thrombolytic therapy mainly due to bleeding complications. Moreover up to 40% of patients treated with thrombolytic drugs do not achieve optimal tissue perfusion.

Many efforts are being made to identify the genetic biomarkers that could predict the response of an individual patient to thrombolytic therapy in stroke patients. Broderick *et al*. evaluated the association of the ApoEphenotype and rtPA efficacy and found that it was greater in acute stroke patients with an ApoE2 phenotype. However, the outcome of placebo-treated patients with or without ApoE2 phenotype did not differ [75]. On the contrary, a Spanish group could not establish any association with ApoE genotype and hemorrhagic risk and recanalisation rate after treatment with rtPA [76]. Fernandez-Cadenas *et al*. (2006) reported that ACE DD genotype (I/D polymorphism in intron 16) influences the tPA induced brain vessel reopening following ischemic stroke [76].

In another study V34L *factor* XII polymorphism was identified as a predictor of clinical outcome with rtPA treatment. Good outcome was found to be associated with VV genotype and low fibrinogen levels. A higher risk of therapeutic ineffectiveness of therapy and mortality was found among individuals bearing LL genotype and with higher levels of fibrinogen [77].

Fernandez-Cadenas and colleagues have studied the influence of two genes encoding for fibrinolysis inhibitors, thrombin-activatable fibrinolysis inhibitor (TAFI), and plasminogen activator inhibitor-1 (PAI-1) genes and found TAFI gene Thr325Ile polymorphism to be responsible for the prediction of the absence of recanalisation with t-PA infusion [78]. PAI-1 4G/5G polymorphism alone was not found to influence recanalisation rate in patients. However, these two polymorphisms together were found to double the risk of negative response to therapy [78].

In a study the association of 263 SNPs in different candidate genes and recanalization rate in tPA treated patients was explored. It was found that cluster of differentiation 40 (CD40) 1C>T and matrix Gla protein (MGP 7A>G) polymorphism associated with reocclusion. However, the MGP associated with neurological worsening. It was suggested that this may be due to the role of CD40 in thrombosis and inflammation, while MGP gene might have a protective role in atherosclerosis [79, 80]. Genetic variants associated with the variability of rtPA have been summed up in Table **2**.

OTHER DRUGS USED TO PREVENT CONVENTIONAL RISK FACTORS OF STROKE

Antihypertensive Drugs

Hypertension is the major risk factor for stroke and the risk increases with every rise in systolic blood pressure [81]. In stroke patients, approximately 9 out of 10 have hypertension. Despite the availability of several antihypertensive drugs which include beta blockers, thiazide diuretics, angiotensin converting enzyme inhibitors angiotensin receptor blockers and calcium channel blockers, less than 35% of hypertensive patients are able to achieve their targeted systolic and diastolic blood pressure with these drugs [82]. A study including 10, 017 patients, has reported around 30% of hypertensives take one antihypertensive drug, 40% take two antihypertensives and 30% take three or more antihypertensives, still they do not achieve the desired therapeutic levels [83]. The prevalence of resistance to antihypertensive medications has been reported in large studies involving clinical outcome. In a study, Antihypertensive and Lipid-Lowering Treatment to Prevent Heart At-

Table 1. Genetic variants associated with warfarin response.

Drug	Effect	Gene Variant	Reference
Phenprocoumon	TT carriers achieved INR of 2-3 after a mean time of 3.2 days CT carriers after 4.4 days and CC after 6.5 days	VKORC1 C283+837C/T (rs2359612)	Arnold *et al*., 2009 [69]
Warfarin	Studied in association with Warfarin effective dose	VKORC11639G/A and Gamma glutamyl carboxylase (GGCX),	Kimura *et al*., 2007 [70]
Warfarin	Effective dose of Warfarin	Calumenin (CALU) 3730G/A	Kimura *et al*., 2007 [70]
Warfarin	Effective dose of Warfarin	CYP2C9 42613A/C	Kimura *et al*., 2007 [70]

Table 2. Genetic variants associated with the variability of rtPA.

Gene Variant	Effect	Reference
ApoE (E2, E3, E4)	ApoE2 genotype related togood outcomes at 3 months period after treatment with rtpa	Borderick *et al*., 2001 [75]
ApoE (E2, E3, E4)	No association	Fernandez-Cadenas *et al*., 2006 [76]
CD 40 gene (rs 1883832)	Reoccclusion	Del Rio Espinola *et al*., 2010 [80]
ACEI/D	Recanalization rate	Fernandez-Cadenas *et al*., 2006 [76]
PAI4G/5G	No association	Fernandez-Cadenas *et al*., 2007 [78]
Factor XIIIV34L	Higher risk of death	Gonzalez-Conejero *et al*., 2005 [77]

tack Trial (ALLHAT), [including a large number of diverse group of subjects (>33, 000): 47% female, 35% African American, 19% Hispanic, and 36% with diabetes], blood pressure remained uncontrolled among 34% of subjects on an average of two medications after approximately 5 years of follow-up [84]. Around 50% of the subjects needed three or more drugs to achieve optimum reduction in blood pressure. According to an analysis of National Health and Nutrition Examination Survey (NHANES), only 53% treated with antihypertensive drugs achieve a blood pressure of 140/90mm of Hg [3, 85, 86]. In the Framingham Heart Study it was found that only 48% of participants on antihypertensive medication achieved blood pressure <140/90 mm of Hg and less than 40% of elderly participants (>75 years of age) achieved the target blood pressure [87].

The pharmacogenetics of antihypertensive therapy seeks to find genetic prediction of response to drugs that lower blood pressure and also to translate this knowledge into clinical practice.

Beta Blockers (β-Blockers)

β-blockers are competitive antagonists of beta adrenergic receptors. These are mainly used as first line drugs along with thiazide diuretics in the treatment of hypertension. Although β-blockers are among the most widely prescribed antihypertensive drug classes for the management of hypertension and cardiovascular diseases, about 60% hypertensive patients do not achieve therapeutic benefit [88]. The major problems associated with β-blockers treatment are variable responses among patients, adverse drug events, and drug resistance [88].

SNPs in various genes involved in adrenergic/sympathetic and renin-angiotensin-aldosterone systems (RAAS) have been identified to be associated with variability in blood pressure lowering response to β-Blockers. Existing data suggests that polymorphisms in 1-adrenoreceptor β1 (ADRB1) gene, Ser49Gly and Arg389Gly influence the blood pressure responses to β-blocker therapy [89]. The patients bearing homozygous allele for ADRB1 Arg389Arg have been shown to achieve a greater reduction in systolic blood pressure and diastolic blood pressure in Caucasians and Chinese hypertensive subjects [90]. In addition to this ADRB1 Ser49 Arg389/Ser49Arg389 haplotype was found to be a predictor of a good response of systolic blood pressure to metoprolol, suggesting its role in anti-hypertensive therapy across different racial groups [89]. In healthy volunteers with an exercise induced increase in heart rate, Arg389Arg genotype carriers were also found to show a significantly higher reduction in systolic blood pressure in comparison with the individuals bearing Gly389Gly genotype after 1 day of metoprolol treatment [91]. However, plasma metoprolol concentrations were not found to be significantly different between Arg389Arg and Gly389Gly genotypes after 3 hours of metoprolol treatment. This suggested that differences in response were not due to variability in metoprolol pharmacokinetics.

ALLHAT study (the sub study known as GenHAT) enrolled 38, 000 hypertensive patients randomly receiving chlorthiazide, amlodipine, lisinopril and doxazosin. In this trial it was found that patients bearing 'CC'genotype for atrial natriuretic polypeptide (NPPA) gene experienced more favorable cardiovascular disease outcomes as compared to 'TT'genotype. GenHAT has also showed that subjects with 455 allele of beta fibrinogen gene had a higher risk of stroke when treated with lisinopril as compared to amlodipine [92].

Thiazide Diuretics

Thiazide diuretics are prescribed in consensus guidelines as first-line therapy along with β -blockers, for managing hypertension [93]. Two studies have investigated several polymorphisms in genes, which are ostensibly involved in the salt-sensitive form of hypertension [94]. Genetic variations in ADD1 gene have also been documented to account for 50% of variability in blood pressure in hypertensive rats [95].

Randomized clinical trials such as INVEST and ALLHAT examined Gly460Trp in the ADD1 gene in relation with sodium sensitivity and diuretic efficacy [96]. The result did not evidence any interaction between the diuretic and the genotype [97]. On the contrary another population based case control study on the same polymorphism found that diuretic protected the bearer of ADD1 460Trp carriers from combined nonfatal myocardial infarction / nonfatal stroke outcome.

In a study including 585 subjects, the association between diuretic response and genetic polymorphisms in genes encoding for renal sodium transport systems, (WNK lysine deficient protein kinase 1 (WNK1), ADRB2 and epithelial sodium channel-subunit (*SCNN1G*) genes, was reported to be a predictive factor of interindividual variability in response to hydrochlorothiazide [98].

G protein beta 3 subunit (GNB3) gene encodes for the beta subunit of guanine nucleotide binding promoter G (I) /G (S) / G (T) associated with signal transduction across cell membrane, has been reported to influence blood pressure lowering response in hypertensive patients on diuretics. Homozygous carriers of GNB3 825TT (rs5443) were found to show a greater decline in blood pressure in comparison with homozygous CC patients in White Caucasian as well as African Americans [99].

Renin Angiotensin System Antagonists (RAAS)

Renin Angiotensin System (RAAS) plays a key role in the development and progression of cardiovascular diseases by promoting

vasoconstriction, sodium reabsorption, cardiac remodeling and many other potentially detrimental effects. Therefore, RAAS system antagonists are prescribed for the treatment of hypertension.

Angiotensin-converting-enzyme (ACE) inhibitors and angiotensin II type 1-receptor (AT1R) blockers are usually recommended for managing of hypertension and heart failure [93]. However, substantial variability in individual responses to these agents has been reported. Fewer than 50% of hypertensive patients achieve adequate blood pressure control with ACE inhibitor monotherapy [100]. Ethnic variation also influences the individual's response to antihypertensive drugs. Blacks with hypertension, have lower plasma renin activity and are less likely hypertensives as compared to whites achieve adequate blood pressure levels when treated with ACE inhibitors [100].

Variations in the genes encoding for ACE, angiotensinogen, and AT1R have been found to be associated with RAS activity supporting that there might be interindividual variability in response to RAAS antagonists [101, 102]. The ACE gene insertion/deletion (I/D) has been the most extensively studied polymorphism, which results in the presence or absence of a 287-base-pair fragment in intron 16 of the ACE gene. The ACE 'D' allele has been shown to be associated with higher plasma and tissue angiotensin II levels and higher expression of AT1R as compared to the 'I' allele [101, 102, 103].

In a study among Japanese hypertensive patients treated with imidapril 5 mg/day for six weeks, the role of ACE I/D polymorphism on diastolic blood pressure was examined. Diastolic blood pressure tended to decrease more for the individuals with ACE I/I genotype in comparison with other ACE genotypes [104]. The I/I genotype was found to be predictive of higher diastolic blood pressure decline with the angiotensin1receptor blocker (AT1R) irbesartan [105].

Variation in AT1R gene has been reported to mediate many of the detrimental effects of angiotensin II, including vasoconstriction, cardiac remodeling, and aldosterone secretion. Studies have reported the association of 1166C allele of AT1R gene polymorphism with increased arterial responsiveness to angiotensin II in ischemic heart disease and increased aortic stiffness in hypertensive patients [106]. During ACE inhibitor therapy, reductions in aortic stiffness was reported to be three times more among carriers in comparison to homozygotes for 1166A allele [107].

In another study including 66 healthy White volunteers on a single dose of losartan, the AT1R gene polymorphism was reported to be related with significantly higher reductions in mean arterial pressure in carriers of 1166C allele when compared to homozygotes [34].

In addition to alteration in drug efficacy ACE gene polymorphism has been related with adverse effects induced by ACE inhibitors [108]. Decline in renal function and cough susceptibility was reported to be higher in patients with ACEI/I genotype [109].

Most of the data on RAAS gene polymorphism influencing antihypertensive drug treatment is inconsistent and conflicting. The data from various studies and clinical trials suggest that pharmacogenetics has the potential to streamline the treatment of hypertension, for which polytherapy is often devised in an effort to improve symptoms and to manage the condition [94]. The pharmacogenetics of antihypertensives has already been reviewed thoroughly by Khullar and Sharma, [2012], Padamnabhan *et al*. [2010] and Turner *et al*. [2001] [83, 99, 110].

PDE4D GENE AND STROKE TREATMENT

PDE4D is a gene spanning >1.5 Mb on chromosomal region 5q12 and has 24 exons [111]. It is involved in the hydrolysis of cyclic adenosine monophosphate (cAMP) and therefore, plays a crucial role in the control of intracellular cAMP concentrations [112]. A number of variants in the PDE4D gene have been identified to be associated with the risk of stroke through whole-genome linkage screen in an Icelandic population by deCODE group [111]. PDE4D is a potential candidate gene for stroke risk because its enzyme activity regulates the various mechanisms including inflammation, plaque stability, angiogenesis, and susceptibility to stroke [113]. Within this gene haplotypes or specific sets of genetic markers were identified corresponding with either significantly increased or decreased risk of stroke. First the association was identified using microsatellite markers and then the microsatellite data were supplemented with a denser set of SNPs. Two hundred and sixty PDE4D, SNPs were examined. Of these 6 were significantly associated with stroke after adjustment for multiple comparisons [111]. Two SNPs SNP41 (rs12153798) and SNP45 (rs12188950), one microsatellite marker (AC008818-8) and the haplotype constructed by SNP45 and this satellite marker were reported to show a significant association with the stroke. Further, PDE4D gene was found to be highly associated with cardiogenic and carotid stroke [111]. A protective haplotype which included one microsatellite marker and one SNP was also described [111].

More than a dozen follow up studies were taken up to study the SNPs across the PDE4D gene for association with the disease. The studies were taken up in cohorts of different ethnicity. Few follow-up studies have shown positive while as others have reported negative associations [114-124]. The role of PDE4D in association of stroke has been reviewed thoroughly by Munshi and Kaul [1]. A meta-analysis carried out by Yoon *et al*. (2011) including 6 SNPs (SNP26, SNP45, SNP56, SNP83, SNP87 and SNP89) showed the most significant association of SNP56 (rs702553) with ischemic stroke [125]. Studies carried out in our laboratory showed the association of SNPs 83, 56 and 41 with ischemic stroke in the South Indian population from Andhra Pradesh [126, 127]. In a meta-analysis carried out by Liu *et al***.** (2013), the SNP 83 in the PDE4D gene was found to be significantly associated with susceptibility to ischemic stroke especially with large artery atherosclerosis [128]. A study from North India also found an association of SNP83 and SNP87 with ischemic stroke and extracranial large artery atherosclerosis respectively [129].

The anti-inflammatory drug rolipram, is a specific inhibitor of the PDE4 subfamily of PDEs, which represent approximately 70-80% of the PDEs in neural tissue [130, 131]. A study carried out by Li *et al*. (2011) in male Wistar rats has shown that ischemia induced neuron loss in hippocampal CA1, is blocked by rolipram [132]. Cerebral ischemia also led to increase in activity of PDE, primarily PDE4, in the hippocampus which also was antagonized by rolipram. The results of this study suggest that rolipram prevents cerebral ischemia induced memory deficits via inhibition of increased PDE4 activity and attenuation of hippocampal, neuronal damages induced by ischemia. Therefore, PDE4 may be a target for treatment of cognitive disorders associated with cerebral ischemia [132].

The identification of PDE4D gene by Gretarsdottir *et al*. (2003), has set a new stage for research of stroke genetics that would eventually lead to better understanding and treatment of the disease [111]. Once drugs are available to interfere with PDE4D pathways, the variants within this gene might be useful to identify the patients with high risk of disease. This would benefit the concerned physician or neurologist to rationalize the treatment of stroke on an individual basis. With the advances of high throughput technology, the enormous amount of data on gene drug-interaction have also been generated, but the translation of pharmacogenomics in clinical practice is in its infancy. PDE4D gene polymorphism needs to be investigated thoroughly, not only in relation to susceptibility of stroke and its subtypes but also with regard to responsiveness to treatment and the gene-environment interactions in the development of stroke. There seems to be more than one way in

which stroke genetics may alter our current management and treatment of stroke.

CONCLUSION

Pharmacogenetics holds the promise of helping to achieve the goal of personalized medicine to maximize drug efficacy and minimize drug toxicity. The convergence of advances in pharmacogenetics along with the rapid development in human genomics has resulted in the evolution of pharmacogenetics into pharmacogenomics and also lead to the enthusiasm for translation of this science into clinical practice. Genetic variations not only influence the susceptibility to stroke but also alter the response to pharmacotherapeutic agents. However, genomic variants are yet to be confirmed by the identification of functional variants influencing the response of drugs in stroke patients on a large scale. Characterizing the genomic profiling of stroke patients would permit not only more targeted therapeutic approaches in primary and secondary prevention of ischemic stroke, but also would be helpful in reducing drug associated ADRs. In this concern many ongoing clinical trials hold great promise for future refinement of translating knowledge of Pharmacogenomics into stroke treatment. However, at present little information is available for developing drugs targeted at the genetic influences [3]. The genetic information has the potential to go a long way in improving therapeutic outcome.

The identification of candidate genes like PDE4D, associated with stroke gives some hope that genetic studies might have a direct impact on the treatment of the disease. The phosphodiesterase enzymes are well-characterized proteins and thus amenable to the design of pharmaceutical modulation. Therefore, isoform specific, clinically relevant modulators could be designed and tested. These proteins also point to specific pathways that could be targeted in stroke.

Although the pharmacogenetic testing is not routinely performed at present, this science will eventually become part of standard patient management in selecting and monitoring the therapeutic strategy of stroke.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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