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Genetic Determinants of Aspirin Non-Responsiveness: From Mechanisms to Clinical Testing: A Narrative Review

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ABSTRACT

Aspirin is among the most widely used efficient antiplatelet medications in the world for the management and reduction of the risk of heart attacks and strokes in patients with atherothrombosis. Its pharmacodynamics effects are attained by permanently inhibiting the activation of platelet COX enzymes. This inhibition prevents the transformation of arachidonic acid (AA) into thromboxane A2 (TXA2), which effectively reduces platelet activation and aggregation. It is widely recognized that the platelet inhibition reaction to aspirin varies significantly among individuals. Some patients exhibit minimal reduction in platelet activity despite aspirin therapy and are classified as low responders, non-responders, or aspirin-resistant. Various allelic variants of the cyclooxygenase (COX) genes, along with several other genes, are also involved in the pharmacokinetics and pharmacodynamics of aspirin. Research has shown that there is inter-individual variability in aspirin response, ranging from mild to severe reduction, which may be attributable to single-nucleotide polymorphism in genes responsible for aspirin-metabolizing enzymes. This article reviews the mechanism of aspirin action, the mechanism of aspirin resistance involving genetic polymorphisms in different aspirin metabolizing genes and its clinical impacts.

Keywords: *Aspirin resistance, Cyclooxygenase (COX) enzymes, Glycoprotein (GP) genes.*

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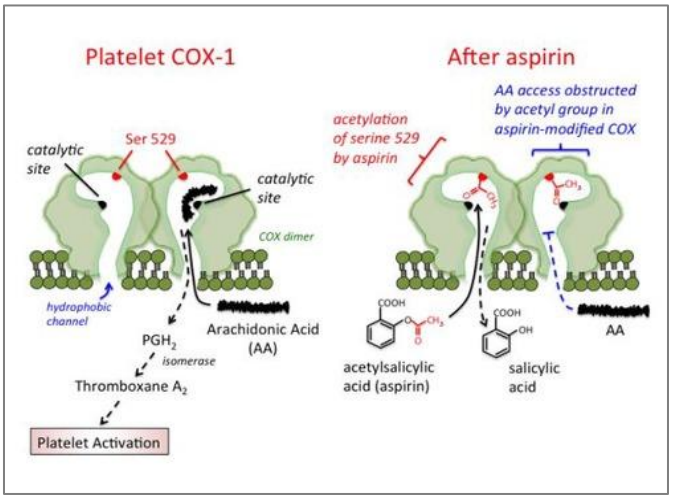
INTRODUCTION

Aspirin is one of the most widely used and effective antiplatelet drugs globally, employed in the management and prevention of heart attacks and strokes in patients with atherothrombosis. Aspirin reduces inflammation through the inhibition of COX enzymes, which control the synthesis of prostaglandins. Cyclooxygenase enzymes exhibit both catalase and oxidase activities and play a vital role in the metabolism of thromboxane A2 (TXA2) and prostaglandins (PGs). Approximately 12% of individuals in Pakistan with coronary artery disease exhibit resistance to the effects of aspirin. This resistance is affected by a combination of clinical, biochemical and genetic factors.[1] Globally, prevalence of aspirin non responsiveness varies from 0% to 57% with an estimated mean prevalence of approximately 24%. In comparison, studies reported in Pakistan, comparatively lower prevalence of around 12% among subjects with coronary artery disease. Such differences may results from the ethnic and genetic variations, particularly polymorphisms in COX-1, COX-2, GPIIIa and platelet receptor genes, as well as differences in aspirin doses and diagnostic techniques used for platelet aggregation testing.[2] Aspirin exerts its antiplatelet effects by preventing platelet aggregation, which is triggered by arachidonic acid (AA). It specifically binds to the COX-1 gene in platelets, inhibiting the conversion of arachidonic acid (AA) from the platelet membrane into Thromboxane A2. TxA2 is a strong prothrombotic lipid mediator that plays a key role in platelet activation and aggregation.[3]

Figure 1: Overview of the inhibition of the Cytochrome c oxidase I (COX-1) by aspirin in platelets[4]



Ischemic heart disease (IHD) stands as the foremost cause of death globally, responsible for taking close to one million lives

annually. In Punjab, Pakistan, the prevalence of cardiovascular diseases was recorded at 17.5% in 2017.[5] The COX-1 enzyme is encoded by the COX-1 gene, which is situated on chromosome nine at the location q32 to q33.3. The COX-1 enzyme is present in most tissues and helps maintain normal levels of prostaglandins under baseline conditions. In a study, the rs1330344 (− 1676T> C) variant of the COX-1 was found to contribute significantly to the risk of ischemic stroke. The COX-2 enzyme, encoded by the COX-2 gene located on chromosomes 1q25.2-q25.3, plays a key role in the release of prostaglandins after an infection. Similarly, PlA1/A2 polymorphism, a substitution of a single allelic variant (T→C) at position 1565 in exon 2 of the glycoprotein IIIa gene, has been linked to aspirin resistance as detected through laboratory testing.[6]

Research has shown that prolonged use of low-dose aspirin reduces the risk of cardiovascular events. Aspirin has been shown to decrease the risk of persistent ischemic stroke by approximately 60% within twelve weeks. However, its effectiveness varies among individuals, as many patients continue to suffer from adverse cardiovascular events even while taking aspirin. This phenomenon is known as aspirin resistance (AR).[7] The COX-1 enzyme, similar to COX-2, plays a vital role in the production of prostaglandins (PGs). The rs1330344 (-1676 T>C) variation in COX-1 has been connected with an increased risk of ischemic stroke. Specifically, the TT genotype of rs1330344 may lower the likelihood of ischemic stroke, including cardioembolic stroke, and small vessel occlusion. In one of the studies, the frequency of the C50T allele in COX-1 was found to be 8.6%, suggesting that this variation may impact the effectiveness of Aspirin. Other common variations in the cyclooxygenase-1 enzyme, including C22T, A842G, G128A, C644A, and C714A, have been linked to ASA response. Additionally, low-dose aspirin works by irreversibly acetylating cyclooxygenase 1, thereby reducing platelet activity and inhibiting the production of thromboxane A2.[8]

The primary antithrombotic effect of low-dose aspirin (75–125 mg) is achieved through the selective inhibition of the COX-1 enzyme. This action prevents the production of thromboxane A2, which is a crucial mediator in the platelet activation and aggregation, and aspirin therapy effectively inhibits its formation for the whole life span of the platelet, which lasts 7-10 days in humans. The alleles in the PTGS1 and the PTGS2 genes, which encode COX enzymes, have been shown to impact Aspirin resistance (AR).[9] Aspirin resistance in laboratory terms is defined differently depending on the specific tests used to measure residual platelet reactivity and the selection of cutoff values. Although light transmission Aggregometry (LTA) was previously considered the gold standard for assessing antiplatelet drug responsiveness, it has several drawbacks, including a lack of standardization and poor specificity when evaluating the inhibition of PTGS1, PTGS2 and GPIIIa genes. Hence, a new and more definite assay might be more suitable to evaluate the potency of aspirin.[10] This article reviews the mechanism of aspirin action, the mechanism of aspirin resistance involving genetic polymorphisms in different aspirin metabolizing genes and its clinical impacts.

There are two studies which have been performed in Pakistan regarding screening of genes involved in aspirin reduced response as per our information but there are limitations in both studies regarding narrow selection of patients in both studies and reduced sample size in one study which offer hindrance in clear interpretation of study results. There is another study performed in Chinese elderly CAD patients regarding assessment of reduced aspirin response by genetic screening of *COX-I, COX-II* and *GP IIIa* allelic variants which were found to be associated with aspirin resistance. The present study aims to determine genetic screening of *COX I, COX II* and *GP IIIa* allelic variants in diabetic, hypertensive and cardiac patients on aspirin therapy by applying allele specific PCR methodology and validation of this method through Sanger sequencing and finally determination of *COX-I* and *COX-II* protein expression by applying Sandwich ELISA technique.

MECHANISM OF ASPIRIN ACTION

Aspirin is a prodrug belonging to the salicylate group. It remains the golden standard for antiplatelet therapy. Prostaglandin endoperoxide synthase (PGHS) is an enzyme responsible for converting arachidonic acid into prostaglandin H2, which serves as the primary precursor for both prostacyclin and thromboxane A2 (TXA2). Prostacyclin has a role in inhibiting platelet aggregation, reducing inflammation and promoting Vasodilation.[11] Aspirin inhibit the cyclooxygenase (COX) enzyme in the platelet, which has a crucial role in the production of thromboxane A2 (TXA2). TXA2 initiates processes that promote platelet activation and aggregation. Similarly, aspirin exerts its antithrombotic effect by permanently inhibiting the activity of cyclooxygenase 1 and cyclooxygenase 2, also recognized as prostaglandin endoperoxide synthases 1 and 2, respectively.[12] A low dose of aspirin (50-100 mg per day) effectively suppresses TXA2 synthesis by the COX-1 gene. TAX2 is released into the blood and interacts with glycoprotein-coupled receptor (GPIIb/IIIa) on adjacent platelets, activating phospholipase C and promoting platelet aggregation.[13] Aspirin has been demonstrated to lower the risk of major vascular events by as much as 25% in patients at high risk for such incidents.[14] As a non-steroidal anti-inflammatory (NSAID) drug, aspirin effectively blocks this pathway, preventing platelet-mediated clot formation.[15, 16] Aspirin resistance is attributed to various underlying mechanisms. One of the most commonly studied phenomena is the impact of genetic SNPs on aspirin resistance. Previous research studies have acknowledged various allelic variants that may be linked with aspirin resistance, such as multidrug resistance 1 (MDR1), thromboxane A2 (TBXA2R), platelet endothelial aggregation (PEAR1) and PLZ2G7 receptors. These allelic genes are linked to platelet clumping and stimulate the pathway of the cyclooxygenase (COX) enzymes. Nevertheless, a reliable diagnostic tool for identifying individuals with aspirin resistance is still lacking.[17] Due to the key role of cyclooxygenase in the mechanism of drug antiplatelet therapy, particularly rs20417, it has been extensively studied to explore its involvement in aspirin resistance (AR). However, no definitive consensus has been established regarding the association between rs20417 and the risk of aspirin Non-responsiveness. It can result from various aspects. It has been linked to poor patient consent and

inadequate drug quantity in clinical settings. Molecular research study indicates the mechanisms underlying drug resistance include platelet allelic polymorphisms that enhance the turnover rate of platelets, inflammation and other related pathways.[4, 18]

Figure 2: Molecular structure of aspirin.[4]

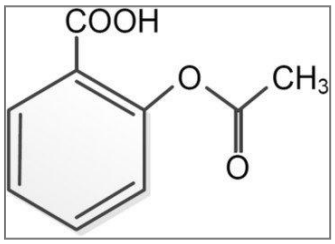
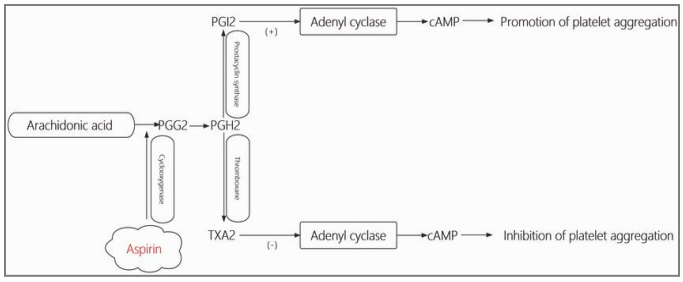


Figure 3: Mechanisms of action of aspirin in terms of reducing platelet aggregation. Prostaglandin G2 (PGG2), prostaglandin H2 (PGH2), Prostaglandin I2 (PG12), Thromboxane A2 (TXA2), and Cyclic Adenosine Monophosphate (cAMP): The colour version of this figure is available at: http://imr.sagepub.com.[4]



ASPIRIN RESISTANCE

a) Definitions of Aspirin Resistance

It is widely used as an antiplatelet drug to help prevent cardiovascular (CV) and cerebrovascular disease at all stages. However, certain patients continue to experience ischemic events despite taking the standard dose of the drug.[19] Different studies have various definitions of medicine resistance, as the categorization includes complications. Currently, aspirin resistance lacks a clear definition. The resistance is commonly used once a medicine loses its effectiveness due to its inability to reach the target site. In contrast, failure of therapy refers to cases where patients continue to experience recurrent events despite undergoing treatment.[20] The reported incidence of platelet aggregation inhibitors varies significantly across studies due to differences in platelet function measurement methods, variations in study populations, and differing criteria used to define platelet resistance. According to current studies, the reported prevalence of drug resistance in the population varies widely, from as low as 0.4% to as high as 83.3%.[3] Several studies have examined the relationship between antiplatelet resistance (AR) and single-nucleotide polymorphisms (SNPs) in cyclooxygenase and previously cited allelic variants. For instance, SNPs in glycoprotein IIIa, P2Y1, COX-1, COX-2 and P2Y12 receptors have been identified as contributing factors to AR.[21] To control factors influencing drug absorption and metabolism, we excluded participants according to the subsequent criteria (1) People who had taken medications that could alter how drugs are metabolized, i.e. carbonic anhydrase inhibitors and proton pump inhibitors within a month prior to hemorrhage (2) Individuals who had stopped taking drug for more than three days (3) those who had never taken aspirin (4) individuals with renal impairment following admission such as glomerular filtration rate <90 mL/min/1.73 m² and/or serum creatinine >133 μmol/L, chronic kidney dysfunction (stage II) or hepatic dysfunction and (5) those without information from thromboelastography. The majority of patients in the derivation cohort took 75 mg of clopidogrel or 100 mg of aspirin. Only seven subjects used a combination of aspirin with dipyridamole or ticagrelor, and they were classified under the dual antiplatelet therapy group.[22]

Methods for Laboratory Evaluation of Aspirin Response

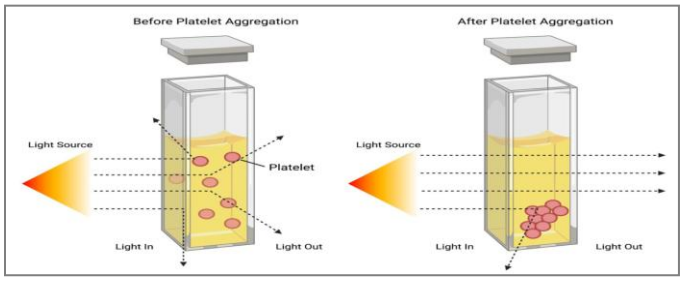
In both clinical and laboratory settings, various methods were performed for laboratory evaluation of aspirin response.

a) Light Transmission Aggregometry (LTA)

LTA is the most commonly used method for evaluating platelet function and is considered the gold standard for testing drug response.[23] Whole blood is drawn from patients and divided into platelet-rich plasma (PRP) and platelet-poor plasma (PPP) based on platelet concentration. Light transmission through both samples is measured by using a photocell. PRP is set at 0% light transmission, while platelet-poor plasma is designated as 100% light transmission.[24] As a platelet agonist is added to platelet-rich plasma, platelet aggregation increases, making the plasma more translucent, allowing more light transmission. LTA measures platelet aggregation by light transmission, providing the total percentage of aggregation in response to specific agonists such as ADP, arachidonic acid and thrombin. In vitro LTA technique enables the platelet activation pathways using various agonists and assessing drug effects on platelet function.[25-27] In patients on anti-platelets, aggregation remains near 0%, whereas in those without therapy, light transmission rises to ~100%.[28, 29]

Multiple studies have identified a diagnostic threshold for aspirin resistance, defining it as ≥20% platelet aggregation in response to AA as measured through light transmission aggregometry.[10] Previous studies have linked ≥20% aggregation to a markedly higher risk of cardiac and thrombotic events.[20, 30] although LTA is the primary method for aspirin sensitivity testing, its results are often inconsistent. In vivo assessment of platelet function is conducted using the prothrombin time test. In contrast, the in vitro evaluation involves measuring thromboxane levels and the aspirin metabolite, thromboxane B2 (TXB2).[31] The widely accepted AR standard requires ADP (10 μmol/L) aggregation to exceed 70% and arachidonic acid (0.5 mg/mL) aggregation to surpass 20% as proposed by.[32] Measured thromboxane formation induced by AA using ELISA and assessed Aspirin's AA-induced antiplatelet effect via the LTA method.[33] Light Transmission aggregometry (LTA) has several limitations as its results can be affected by various genetic factors, i.e., platelet count, hematocrit levels and blood lipid concentration.[24] Light Transmission aggregometry has been considered the gold standard method for assessing drug sensitivity.

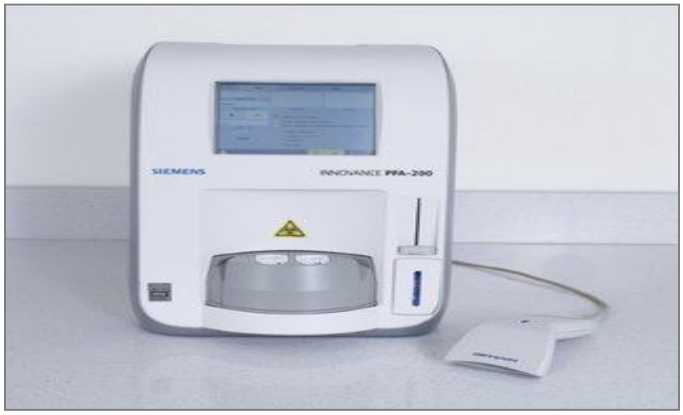
Figure 5: Comparing light transmission before and after platelet aggregation using light transmission Aggregometry. Light rays from the light source are represented by the dotted line.[3]



b) Platelet Function Analyzer 100/200 (PFA 100/200)

The Platelet Function Analyzer is a point-of-care test that measures the platelet function by recording the closure period of a small space mimicking vascular injury. It utilizes a cartridge containing a membrane that is coated with platelet-activating substances such as collagen and epinephrine. The whole blood is drawn via an aperture to assess hemostatic plug formation. The PFA-100/200 measures the function of the platelet by recording closure time as blood flows through a membrane-coated vial. The patients on antiplatelet drug therapy exhibit improved closure times with aspirin affecting collagen/epinephrine cartridges and clopidogrel affecting collagen/ADP cartridges. While light transmission aggregometry (LTA) remains a standard test, its accuracy, reproducibility, and sensitivity limitations highlight the need for a more reliable aspirin sensitivity test.[34] Although the PFA provides quick and convenient results that contrast with other methods, there are concerns regarding its effectiveness in detecting drug resistance in patients. Moreover, a study conducted by Lordkipanidzé et al. (2007), evaluating aspirin resistance in patients with coronary artery disease, found that the platelet function analyzer overestimated resistance compared to Light Transmission Aggregometry (LTA) and showed poor correlation with LTA, regardless of the agonist used.[35] The platelet function analyzer is vulnerable to false positives in the studied individuals due to confounding factors affecting closure time.

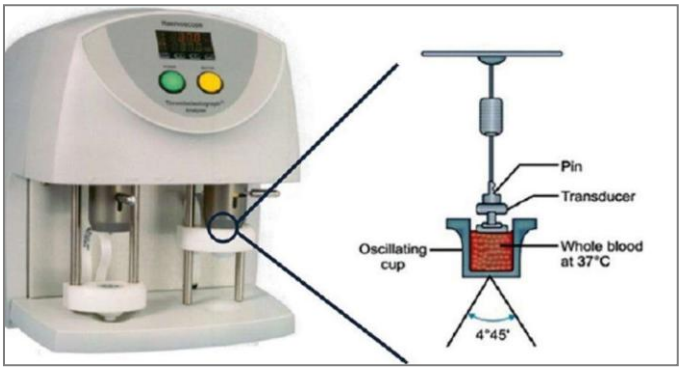
Figure 6: The PFA-200 instrument (Courtesy of Siemens Diagnostics, New York, NY; with permission) (Lordkipanidzé, M., 2013).



c) Thromboelastography (TEG)

Thromboelastography is a noninvasive diagnostic test that provides a quantitative assessment of whole blood's clotting ability. Ex vivo hemostatic assays, such as thromboelastography and rotational thromboelastometry, provide real-time evaluation of viscoelastic clot potency in blood.[36] The principle of this in vitro test measures dynamic changes in blood viscoelasticity during clotting under low shear stress using a thromboelastography. It quantifies clot formation and stability.[37] Both assays use a needle hanging from a torsion wire placed in whole blood, which is incubated in a heated cup and kept in constant oscillation. To initiate hemostatic plug formation, an agonist, i.e., ADP that promotes blood coagulation, such as tissue factor or a platelet activating agent, is introduced. As the plug develops and its strength increases, the rotational torque exerted on the needle rises, which is then transmitted to the wire. The TEG software analyzes this torsion to interpret the kinetics of plug formation and dissolution, ultimately assessing plug strength.[36, 38] most studies utilizing TEG have primarily examined platelet response rather than drug resistance. While some research has explored the effectiveness of TEG in predicting Aspirin resistance in CAD, recent research publications assessing its ability to predict drug resistance in peripheral artery disease patients are lacking.[39] A further study on thromboelastography is needed to evaluate its effectiveness in detecting drug therapy. Different agonists can be utilized in the TEG-ROTEM system, and exploring the arachidonic acid (AA) could be a more effective approach for identifying aspirin resistance.

Figure 6: Thromboelastography (TEG) (Masih, G., & Sukhdev, V., New drlhi-110025).



d) Thromboxane B_2 (TXB_2) assay

Conventional aspirin inhibits TXA2 production by permanently acetylating the cyclooxygenase-1 gene. Once formed, TXA2 is quickly degraded into a stable metabolite, TXB2. Theoretically, aspirin reduces TXA2 levels; patients who are resistant to aspirin would produce more TXB2 than those who are sensitive to the drug.[40] Prior research has indicated that assessing serum TXB2 levels could serve as a reliable approach for identifying aspirin resistance. The quantification of thromboxane B2 (TxB2) in serum, a stable metabolite of TxA2, is the sole assay that evaluates aspirin's impact on COX-1 activity in platelets. Assessing thromboxane B2 levels could serve as a potential biomarker for vascular disease risk in patients undergoing aspirin therapy. A total of 58 articles were ultimately selected.

The findings indicated that elevated TXB2 levels might serve as a risk feature for ischemic stroke or ischemic cardiac disease. However, the evidence remains insufficient to support the use of thromboxane as a clinical marker for ischemic stroke.[41] Further, research is needed to determine a cutoff value for the metabolite used as a diagnostic marker for aspirin resistance in patients.

PHARMACOKINETICS AND PHARMACODYNAMICS

Aspirin resistance can be classified into two types: pharmacokinetic and pharmacodynamics resistance. Pharmacokinetic resistance arises when adequate levels of aspirin fail to reach the bloodstream, preventing the complete inhibition of thromboxane synthesis and platelet aggregation. This may result from factors such as impaired absorption in the gastrointestinal tract, accelerated breakdown of aspirin, or increased platelet turnover, among other causes.[42] On the other hand, Pharmacodynamics resistance occurs when aspirin is present in adequate concentrations in the blood but fails to inhibit COX-1 due to genetic polymorphisms in the COX-1 enzyme or structural modifications in platelets. As a result, platelet aggregation continues despite aspirin therapy. Recent research has identified a COX-1 polymorphism that could potentially heighten the risk of aspirin resistance.[43] Additionally, the anion efflux pump Multidrug Resistance Protein 4 (MRP4) may play a role in decreasing COX-1 inhibition in patients.[44]

DOSAGE OF ASPIRIN

The dosage of aspirin used for both primary and secondary prevention of thrombosis in atherosclerotic cardiovascular disease (ASCVD) varies across countries. It is frequently intended to deliver pain-relieving effects rather than to achieve full acetylation of COX-1, which is necessary for optimal antithrombotic activity. This is typically referred to as low-dose aspirin (75–150 mg), which serves as an effective long-term therapy for preventing platelet aggregation.[45] Dong et al. (2021) analyzed the impact of low (<150 mg) vs. medium (≥150 mg) aspirin doses on cardiac infarction incidence in individuals with unstable angina or AMI over six months, using data from the GUSTO IIb and PURSUIT trials. Their findings indicated that higher aspirin doses were linked to a minor risk of cardiac infection.[46] In this cross-sectional study, 264 individuals without a predictable history of coronary artery disease, who had been taking 80 mg of aspirin daily for a minimum of one month, were enrolled. Aspirin resistance was evaluated by measuring thromboxane B2 levels in urine samples via the ELISA technique. A thromboxane B2 concentration of ≥ 1700 ng/dL in urine was considered indicative of drug resistance. It was found that aspirin non-responsiveness was observed in 9.8% of participants. Age and geographical regions were independently associated with drug non-responsiveness.[11] Haley et al. (2020) determined 12 randomized controlled trials, concluding that the drug's impact on bleeding risk and ASCVD events was not influenced by the dosage, suggesting that the specific dose may not be a determining factor.[47] Xue et al. (2017) examined molecular markers in Chinese patients diagnosed with chronic stable angina following percutaneous coronary intervention. A total of 207 subjects were administered 100 mg of aspirin daily. Platelet inhibition was assessed using LTA, while TXB2 and hs-CRP levels were measured via radioimmunoassay. Genetic mapping was conducted by using the TaqMan probe technology for rs5787→dbSNP: rs5787 and rs5911→dbSNP: rs5911, while the gene sequencing method was used for rs3842788. The findings indicated that the rs5911 A/C, C/C and A/A genotypes and the rs3842788 A/G and G/G genotypes were associated with aspirin non-responsiveness.[48] Arnett et al. (2019) demonstrated that Medical organizations, including the American Cardiac Association and the Society of Cardiovascular Surgery, suggest on a daily basis aspirin intake ranging from 81-325 mg for individuals with peripheral artery disease (PAD) and carotid artery stenting (CAS). This serves as a secondary preventive measure to reduce the risk of serious adverse events such as myocardial infarction, cerebrovascular accident and cardiovascular mortality.[49] Zeng et al. (2024) obtained blood samples from 29 individuals classified as drug non-responders and the 60 ethnically matched responders who had participated in a study investigating the prevalence of drug resistance in Pakistan. This research study was conducted in the Departments of Cardiology and Hematology at Shifa International Hospital, Islamabad. All participants had been taking aspirin (75–300 mg/day) for over seven days and had a confirmed diagnosis of coronary artery disease or conditions such as carotid artery stenosis, diabetes and peripheral blood vessels.[50] Seventy-four patients with a stent implanted who had been taking 100 mg of aspirin daily for five years were included in the research study. Among them, 38 patients who experienced a CE within five years due to drug resistance were assigned to the patient group, while the remaining 36 patients without CE were placed in the control group.[6] Regarding the daily drug dosages (100 or 325 mg) and the length of treatment, 149 mg for an average of 5.3 days, 152 mg for 4.8 days, no discernible differences were found.[16]

GENETIC POLYMORPHISMS IN DIFFERENT ASPIRIN METABOLIZING GENES

Genetic factors might play a crucial role in drug non-responsiveness. A research study conducted on 124 individuals with AMI found a significant correlation between the COX1-A1 mutation and drug resistance. Similarly, a research study involving 450 elderly Chinese patients receiving aspirin treatment, glycoprotein IIIa allelic variants was also associated with aspirin non-responsiveness.[51] Patients who experienced arachidonic acid (AA) induced Thrombus formation were divided into two groups based on their response to antiplatelet drugs, the drug non-responsiveness group consisting of 236 individuals and the drug-sensitive group comprising 214 individuals. The Leu33Pro SNPs in the glycoprotein IIIa allele were analyzed using PCR and RFLP. In the drug non-responsiveness group, 224 participants exhibited the A1/A1 genotype, whereas 12 individuals carried the A1/A2 genotype. The A1/A1 genotype was exclusively observed among all participants in the drug-sensitive group. A statistically significant difference in genotype distribution was observed between the two groups ($p<0.05$). All patients in the drug-sensitive group were homozygous for the PLA1 genotype. In the drug non-responsiveness group, 224 individuals were also

PLA1 allele, while 12 individuals were heterozygous for PLA1/A2, comprising three females and nine males. No individuals in either group were found to be homozygous for the PLA2 genotype. The PLA2 genotype was present in the drug resistance group at a frequency of 5.08% and in the general population at 2.67%. This distribution pattern is comparable to that of Koreans, but very different from that observed in white populations. Among Koreans, the frequency of the phospholipase A1 allele was observed to be 100%, while the PLA2 allele was found in only 1% of the population. Consequently, among 100 Koreans, only one person was identified as a PLA1/PLA2 heterozygote, while no individuals were found to be homozygous for the PLA2 allele. The phospholipase A antigen was primarily expressed in Black, Japanese, and Indian American populations as the phospholipase A1 phenotype.[32, 52]

Aspirin resistance has been associated with the cyclooxygenase one allelic variants rs3842788 (128G>A) and rs1330344 (1676G>A) in cardiovascular disease patients. Nevertheless, there is not enough data to determine if the COX-1 gene's A or G allele contributes to aspirin resistance genetically. We reviewed ten research articles on cyclooxygenase and conducted a meta-analysis to evaluate the association of rs3842788 and rs1330344 allelic variations with aspirin resistance and sensitivity in patients. The findings indicated that no statistically significant difference between the cases and healthy controls for the G+AA and the GG genotypes of the rs3842788. This systematic research study and meta-analysis of the six studies involving 1,940 subjects found no consistent link between the COX-1 mutation (rs3842788) and aspirin resistance.[53] However, our analysis revealed a statistically significant difference among the cases and controls concerning the (GA+GG) and (AA) genotypes of the rs1330344 with odds ratio, confidence interval and p-value. The systematic research review and meta-analysis, which includes five studies with a total of 1,958 subjects, demonstrates a significant correlation between the rs1330344 and drug non-responsiveness. A possible explanation is that the genetic variant rs1330344 is located in the promoter region of the cyclooxygenase one allele, where the GG genotype enhances platelet aggregation by up regulating COX-1 protein expression, leading to aspirin resistance.[54] The study indicates that the G allele has an average frequency of 40% and the distribution frequencies of the allelic variant rs1330344 are identical among the Japanese and Chinese populations. Therefore, rs1330344 may serve as an allelic marker for drug resistance in Asians.[8, 55]

Genetic polymorphism in the platelet receptor gene is closely linked to platelet activation, adhesion, aggregation and the development of thrombosis disorders.[56] Wang et al. investigated the association between long non-coding (lncRNA H19) and aspirin resistance (AR) in ischemic stroke patients. They analyzed plasma H19, urine 11-dehydrogenation thromboxane B2, and the 8-iso-prostaglandin F2α stages in 150 acute ischemic stroke patients. The findings indicated a significant elevation in plasma H19 levels among patients with aspirin non-responsiveness. H19 levels were positively correlated with urine 11dhTXB2/creatinine and 8-iso-PGF2α, suggesting that H19 might play a role in acute rejection (AR) by enhancing the production of 8-iso-PGF2α.[57] Xue et al. demonstrated allelic mutations in Chinese patients with chronic stable angina pectoris following PCI. A total of 207 participants were enrolled to receive a daily dose of 100 mg of the drug for the evaluation of its antiplatelet effect. Platelet inhibition was assessed using LTA, while thromboxane B2 (TXB2) and high-sensitivity C-reactive protein levels were quantified via radioimmunoassay. Genotyping of the rs5787 and rs5911 polymorphisms was performed using TaqMan probe-based techniques, whereas rs3842788 was analyzed through direct gene sequencing. The study identified significant associations between aspirin resistance (AR) and specific genetic variants, particularly the rs5911 (A/C, C/C, A/A) and rs3842788 (A/G, G/G) genotypes. Elevated levels of TXB2 and hs-CRP were observed in the Aspirin non-responsiveness group, with rs3842788 A/G carriers exhibiting notably higher plasma TXB2 concentrations. These results indicate that rs5911 and rs3842788 may serve as potential genetic markers for aspirin resistance in Chinese patients with stable angina pectoris.[48]

Liu et al. investigated the prevalence of drug resistance in the 469 elderly individuals with cardiovascular disease and metabolic syndrome who were receiving a daily aspirin dose of 75–100 mg, similar to that used in the current study. Using the LTA diagnostic technique, they found that 8.1% of the individuals exhibited drug resistance, with metabolic syndrome identified as a significant risk factor. Various reports highlight different factors associated with aspirin resistance. The most commonly reported factors include noncompliance, underlying clinical conditions of patients, drug interactions and genetic polymorphisms in key genes, i.e., COX-1 and COX-2.[58]

A meta-analysis of four studies involving patients with hemorrhagic stroke found no significant link between the PIA2 rs5918(C) polymorphism and the condition, as shown by results from both fixed-effect and random-effect models. However, the statistical power of the analysis was below 30%, signifying a limited capacity to identify a true effect. In contrast, a comprehensive meta-analysis of the 28 research studies focusing on ischemic stroke identified a significant relationship between the PIA2 rs5918(C) allelic variant and increased risk of ischemic stroke in both analytical models, supported by a statistical power exceeding 80%. These findings suggest that the PIA2 allele may contribute to the susceptibility of ischemic stroke. In contrast, the lack of association with the hemorrhagic stroke is likely attributable to the small number of included studies and insufficient statistical power.[59]

The single-nucleotide polymorphism (SNP) rs5918 is relatively rare and occurs at varying frequencies across different populations, as reported by the Genome Aggregation Database.[60] A large-scale platform comprising data from over 800 exomes, the genomes across diverse human sequencing research studies reported that the minor allelic variant frequency (MAF) of the rs5918 polymorphism is highest in European populations, reaching approximately 0.15. In contrast, East Asian populations show a markedly lower MAF of 0.004. This significant difference explains the absence of the PIA2 allele carriers in the research study conducted by Saad et al.[61] They conducted their research on cases from Taiwan, where the genetic variations were uncommon. As a result, their research study would have needed an impractically greater

sample size to validate the hypothesis. Another research, that of Liu et al.[62] A study conducted in China with a sample of 285 patients reported a minor allele frequency (MAF) of approximately 0.09. This study, notably, exhibited the lowest statistical power among all those incorporated into our meta-analysis. The most frequently studied allelic variant, rs5918 PlA1/A2, is located in the IIIa subunit and involves a leucine-to-proline substitution, where PlA1 represents leucine and PlA2 corresponds to the proline variant. This genetic variation has been widely examined across various studies focusing on cardiac and cerebral ischemia, as well as other pathological conditions.[59] GP IIb/IIIa variants may have an impact on both ischemic and hemorrhagic strokes, particularly in younger individuals. The threat was found to be high among the Caucasian females under 45 years old, though it did not achieve definitive statistical significance.[63]

A study was conducted in 97 patients who had been diagnosed with acute ischemic stroke. PCR pyrosequencing technology was used to analyze the GPIa genetic variant at the 807C>T (rs1126643) locus and the PTGS2 SNPs at the -765G>C (rs20417) locus. Patients were divided into two groups. 1) The aspirin sensitivity (AS) group, 2) The drug resistance (AR) group based on platelet aggregation rates. The purpose of the research study was to investigate and evaluate the relationship between aspirin resistance and these gene polymorphisms. The frequency of the T allele of the GPIa gene at the 807C>T locus, as well as the distribution of genotypes (CC, CT, TT, CT + TT, and CC), showed significant differences between female patients in the aspirin-sensitive (AS) and aspirin-resistant (AR) groups ($P < 0.05$). After controlling for pertinent variables, logistic regression analysis revealed a significant correlation between AR and the (CT+TT) genotype at the 807C>T locus. The distribution of genotypes and allele frequencies at the -765G>C site of the COX-2 gene showed no significant difference between the two groups ($P > 0.05$). Among Chinese Han females, aspirin resistance (AR) was linked to the GPIa allelic variant at the 807C>T site, with the T allele being associated with a higher risk of AR. In contrast, no clear association was observed between AR and the COX-2 genetic mutation at the -765G>C site.[13]

Noor et al., highlighted in this study, 14.0% (n=54) of patients with ischemic heart disease exhibited resistance to aspirin, while 86.0% (n=330) responded to the medication. Genotyping of the COX2 SNP rs20417 revealed that 55.46% (n=213) of patients had the GG homozygous genotype, 34.11% (n=131) were heterozygous (CG), and 10.41% (n=40) carried the CC homozygous genotype. However, statistical analysis indicated no statistically significant association between any allele of the assessed SNP and drug non-responsiveness ($p>0.05$).[64] Similarly, Yi et al. conducted a study on 634 ischemic stroke patients who were taking Aspirin. Their genotypic analysis included rs20417 (G765C); however, there was no significant difference in the frequency of the rs20417 (COX2) between aspirin responders and non-responders [21].

Ikonnikova et al. elaborated that a total of 296 patients were diagnosed with non-cardioembolic ischemic stroke, with 127 (43%) classified into the AR group and 169 (57%) into the AS group. The distribution of genotypes for sixteen SNPs was analyzed across these groups. The CC genotype of the rs1330344 in COX allelic variants was observed frequently in the Aspirin non-responsiveness group compared to the Aspirin sensitive group; however, the difference was not found to be statistically significant. Similarly, individuals with the CC genotype of the prostaglandin synthase 1 rs1330344 showed a 55.4% upper average arachidonic acid-induced platelet aggregation, compared to those carrying the (TT) or (CT) genotypes, with a p-value = 0.026. In contrast, carriers of the TT or CT allelic mutations of rs4523 TBXA2R exhibited a 14.8% higher mean ADP-induced aggregation than those with the CC genotype ($p = 0.031$). Furthermore, individuals with the AA or GA genotypes or allelic variants of the ITGA2 rs1062535 had an 11.6% lower mean ADP-induced aggregation compared to GG homozygotes ($p = 0.051$). However, all p-values were greater than 0.05, signifying a lack of statistical significance.[65] Out of the 16 single-nucleotide polymorphisms, four allelic variants, PTGS1 (rs1330344), ADRA2A (rs4311994), TBXA2R (rs4523), and PEAR1 (rs12041331), demonstrated a statistically significant association with the antiplatelet drug in the overall study population. Notably, the C and CC genotype of the prostaglandin synthase one allele (rs1330344), which encodes the COX-1 enzyme, were associated with aspirin resistance and increased arachidonic acid (AA)-induced platelet aggregation. Similar findings were reported by Li et al.[8]

The cyclooxygenase one and cyclooxygenase two alleles have distinct mechanisms of action of aspirin, and genetic variations in either gene can contribute to drug resistance. The cyclooxygenase 1 (CO-1) enzyme is located on the long arm of chromosome 9, specifically in the 32–33.3 regions. It spans approximately 22 kilobases and consists of 10 introns and 11 exons. The most frequently observed genetic sites in the 5' untranslated region (UTR) of the COX-1 allelic variant include A1201G, G-1498A, A-842G, 1676T>C, T1794C, G-10006A, A-918G, and A-807G.[66] The 842A–G genetic variants were found in 60% of the drug non-responsiveness patients, whereas only 17% of the non-responsiveness individuals exhibited these changes. This indicates a strong association between the A-842G allele mutation and aspirin resistance.[16]

Platelet aggregation relies on the interaction between the glycoprotein Ia/IIa (GPIa/IIa) complex and collagen.[67] The nucleotide sequence at position 807 of the glycoprotein Ia allele is strongly associated with the quantity of glycoprotein Ia/IIa molecules. Individuals with the TT and TC genotypes exhibit an extremely higher number of glycoprotein Ia/IIa molecules compared to those with the CC genotype. This leads to enhanced collagen binding, which improves the platelet activation and aggregation, and a greater likelihood of aspirin resistance.[62]

A meta-analysis incorporating 18 studies on rs20417 revealed a significant association with an elevated risk of AR. Specifically, the C allele compared to the G allele showed an increased risk. The combined GC+CC genotypes versus the GG genotype also indicated a higher risk. Subgroup analyses demonstrated that these associations were more pronounced among Chinese participants and individuals with ischemic stroke.[18] Another study conducted on Chinese individuals with

cardiovascular disease found that aspirin resistance (AR), assessed through light transmission Aggregometry (LTA) and thromboelastography platelet mapping assay (TEG) using arachidonic acid (AA) as a stimulus, was associated with a higher risk of combined cardiovascular events. Furthermore, the presence of the G-allele variant in PTGS1 rs1330344 (-1676 A/G) was statistically significantly linked to an increased risk of Aspirin non-responsiveness [68]. Another research study found that the C-allele in rs1330344 was more prevalent among patients, while the T-allele and CC genotype were linked to a higher risk of aspirin resistance (AR).[6]

Table 1: Genetic polymorphisms in different aspirin metabolizing genes

Author(s) Year	Population Studied	Gene Studied	Polymorphism (SNP ID)	Method Used	Genotype Frequencies	OR (95% CI)	p-value	Main Findings
Han, 2016.[51]	450 elderly Chinese	GP IIIa	Leu33Pro (PlA1/PlA2)	PCR-RFLP	A1/A1= 224 A1/A2= 12 (AR) A1/A1= 214 (AS)	2.23(1.12-4.44)	0.022	Significant association between GP IIIa polymorphism and aspirin resistance
Li et al., 2024.[55]	Asians	PTGS2	Rs20417	Newcastle Ottawa Scale were used to assessing the quality of included studies	GC/CC VS GG = 42% TC/TC VS CC = 48%	0.57(0.44-0.73)	<0.05	Meta-analysis indicates that PTGS2 could be genetic biomarker for AR.
Xue et al., 2017.[48]	207 Chinese	COX-1, GP Ib	rs3842788, rs5911	TaqMan probe, sequencing	AG VS GG AC + CC VS AA	8.358(2.470-28.286) 5.546(1.812-11.404)	0.001	rs5911 and rs3842788 are associated with Aspirin resistance (AR)
Yi et al., 2017.[54, 69]	China	P2Y1	rs1371097	Logistic Regression	C>T	0.78(0.74-0.81)	0.02	Findings suggest that rs1371097 genotypes contribute to variability in aspirin response among AIS subjects.
Wang et al., 2018.[13]	China	GPIa, COX-2	rs1126643, rs20417	PCR Pyro sequencing	CT+TT at 807C>T locus was significantly with the AR.	4.856(1.020-23.108)	0.047 0.05	GPIa 807C>T associated with AR in females; COX-2 -765G>C not significant difference
Liu et al., 2020.[53]	Chinese	COX-1	rs3842788	Meta-analysis of 6 studies (n=1940)	GA+AA VS GG	1.22 (0.85–1.75)	0.29	Non-statistically significant association with Aspirin resistance (AR)
Ikonnikova et al., 2022.[65]	296 Stroke Patients Russia	Multiple COX-1	rs1330344, rs4523, rs1062535	Genotyping + Aggregometry	40.5% CC VS TT+CT, 14.8% TT+CT genotypes **of** rs4523 TBXA2R VS CC genotype, 11.6% AA+GA genotypes **of** ITGA2 rs1062535 VS GG homozygotes	2.48(0.93-6.60)	0.062 0.031 0.051	CC genotype of rs1330344 linked to increased aggregation
Noor et al., 2024.[64]	384 Pakistani Ischemic Heart Disease	COX-2	rs20417	PCR, RFLP	GG homozygous genotype 55.46%(n=213), CG Heterozygous genotype 34.11%(n=131), CC Homozygous genotype 10.41%(n=40).	Not reported	>0.05	No statistically significant association at SNP rs20417.

ASPIRIN RESISTANCE IN PAKISTAN SCENARIO

A study was carried out in Pakistan involving a total of 384 patients with ischemic heart disease, comprising 272 males and 112 females, were included in the study. All participants have been on Aspirin therapy for a minimum of seven days. Platelet aggregation was assessed using a Light Transmission Aggregatometer (LTA), with arachidonic acid as an agonist. DNA was extracted using the Invitrogen kit method (Thermofisher). Subsequently, PCR, RFLP, and gel electrophoresis were employed to detect single-nucleotide polymorphism (SNPs) in the Pakistani population, i.e., rs1330344 in the cyclooxygenase-1 allele and rs20417 in the

cyclooxygenase-2 (COX-2) gene. The current research failed to find an association between the COX-1 rs1330344 variant and ischemic heart disease.[64]

Another study in Pakistani patients with coronary artery disease has also been reported, involving a total of 89 patients, out of which 29 were non-responders to aspirin and 60 were aspirin responders. The study investigated the role of SNPs in the COX-1, GPIIIa, GPIa, and P2RY1 genes as potential causes of aspirin resistance in the population. Genotyping was performed for four single-nucleotide polymorphisms: cyclooxygenase 1 (*COX-1*), C50T (rs3842787), P2RY1 C893T (rs1065776), Glycoprotein Ia (GPIa), C807T (rs1126643), and glycoprotein IIIa (GPIIIa), PIA1/A2 (rs5918). However, no significant differences were established in allelic variant or genotype frequencies between AS responders and the non-responders, suggesting that other genetic factors may contribute to aspirin resistance in this population. However, a very small sample size and just one category of patients, i.e., coronary artery disease (CAD), are major limitations exhibiting hindrance in clear result interpretation.[70] A study conducted in the Chinese population indicated that variations in the cyclooxygenase 1 (*COX-1*) and cyclooxygenase 2 (*COX-2*) genes could be linked to a reduced effectiveness of antiplatelet therapy and an improved risk of Aspirin Resistance.

Additionally, the other objective was to primarily investigate genetic polymorphism of the glycoprotein IIIa (*GP IIIa)* gene, which was found to be associated with (ASR), in the elderly Chinese population. However, further validation will require large-scale cohort studies in the future.[50] Two studies have been performed in Pakistan regarding the screening of genes involved in aspirin reduced response, as per our information, but there are limitations in both studies regarding the narrow selection of patients and reduced sample size in one study, which hinders the clear interpretation of study results. These findings imply that aspirin resistance (AR) in Pakistan may be influenced more strongly by non-genetic factors such diabetes, hypertension, obesity, medication adherence, drug interactions and lifestyle-related variables. In order to better predict AR in the local population, future screening methods in Pakistan should concentrate on finding alternative SNPs in genes linked to platelet activation, aspirin metabolism and inflammatory pathways.

CONCLUSION

In recent years, the use of aspirin has significantly increased due to its well-documented therapeutic benefits in patients with ACS, IHD, CHD, Cardiovascular Disease (CVD) and those undergoing Percutaneous Coronary Interventions. Despite its widespread use, growing concern has emerged over the variability in individual responses to aspirin. This variation in drug response is largely attributed to genetic polymorphisms in several genes, including GPIIIa, P2Y1, COX-1, COX-2, and P2Y12. These genetic variations can lead to reduced aspirin efficacy, potentially resulting in recurrent thrombotic events and other serious cardiovascular complications. So, it is the need of the hour to screen these mutations in patients to avoid the abovementioned complications and guide them for the better selection of antiplatelet therapy. Two studies have been performed in Pakistan regarding the screening of genes involved in aspirin reduced response, as per our information. However, there are limitations in both studies regarding the narrow selection of patients and the reduced sample size in one study, which hinders the clear interpretation of study results. The present study determine genetic screening of *COX I, COX II* and *GP IIIa* allelic variants in diabetic, hypertensive and cardiac patients on aspirin therapy by applying allele-specific PCR methodology and validation of this method through Sanger sequencing and finally determination of *COX-I* and *COX-II* protein expression by applying the Sandwich ELISA technique. Future studies should investigate large-scale, well-designed prospective studies using a standardized definition of aspirin resistance and further explore other genetic and non-genetic factors that could better account for the variability of aspirin response.

LIMITATIONS

There are a number of methodological and clinical limitations to the current evidence on aspirin resistance. The statistical power of most studies is limited by a small sample size and the results are less applicable to various populations. Furthermore, the definition and measurement of aspirin resistance is very heterogeneous with different laboratory assays and cut-off values used among studies, reflecting this. Moreover, there are few large, well-designed randomized controlled trials of genotype-guided antiplatelet therapy that limit the possibility of applying genetic insights into clinical practice.

SUGGESTIONS / RECOMMENDATIONS

In the Pakistan scenario, there is a dire need to conduct a study regarding loss-of-function mutation screening of COX-I, COX-II and GP IIIa genes to rule out the status of Aspirin response variability in cardiac, hypertensive and diabetic patients on Aspirin therapy.

CONFLICT OF INTEREST / DISCLOSURE

The authors disclose no conflicts of interest

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