Impact of pharmacogenetics on aspirin resistance: a systematic review

Impacto da farmacogenética na resistência à aspirina: uma revisão sistemática

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Abstract	 Background Pharmacogenetics promises better control of diseases such as cardio-vascular disease (CVD). Acetylsalicylic acid, aspirin, prevents the formation of an activating agent of platelet aggregation and vasoconstriction, and it is used to prevent CVD. Nevertheless, patients may have treatment failure due to genetic variants that modify the metabolism of the drug causing aspirin resistance (AR). Objectives To realize a systematic literature review to determine the impact of genetic variants on AR.
	Methods Articles published in the MEDLINE/PubMed, Cochrane, Scopus, LILACS, and SCIELO databases were systematically screened. A total of 290 articles were identified and 269 articles were excluded because they did not comply with the previously established inclusion criteria. A total of 20 case-control studies and 1 cohort was included.
Keywords	Results The genetic variants rs1126643 (<i>ITGA2</i>), rs3842787 (<i>PTGS1</i>), rs20417 (<i>PTGS2</i>), and rs5918 (<i>ITGB3</i>) were the most studied. As for relevance, of the 64 genetic variants evaluated by the articles, 14 had statistical significance (<i>p</i> < 0.05; 95% confidence interval [CI]) in at least one article. Among them, the following have had unanimous results: rs1371097 (<i>P2RY1</i>), rs1045642 (<i>MDR1</i>), rs1051931 and rs7756935 (<i>PLA2G7</i>), rs2071746 (<i>HO1</i>), rs1131882 and rs4523 (<i>TBXA2R</i>), rs434473 (<i>ALOX12</i>), rs9315042 (<i>ALOX5AP</i>), and rs662 (<i>PON1</i>), while these differ in real interference in AR: rs5918 (<i>ITGB3</i>), rs2243093 (<i>GP1BA</i>), rs1330344 (<i>PTGS1</i>), and rs20417 (<i>PTGS2</i>). As study limitations, we highlight the nonuniform methodologies of the analyzed articles and population differences.
 Pharmacogenetics Aspirin Genetic Variation 	Conclusion It is noteworthy that pharmacogenetics is an expanding area. Therefore, further studies are needed to better understand the association between genetic variants and AR.

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Resumo	Antecedentes A farmacogenética promete melhorar o controle de doenças como as
	cardiovasculares. O ácido acetilsalicílico, a aspirina, previne a formação de um agente
	ativador da agregação plaquetária e vasoconstrição e é usado na prevenção de tais
	doenças. No entanto, os pacientes podem ter falha no tratamento devido a variantes
	genéticas que modificam o metabolismo da droga causando resistência à aspirina (RA).
	Objetivos Realizar uma revisão sistemática da literatura para determinar o impacto
	das variantes genéticas na resistência à aspirina.

Métodos Artigos publicados nos bancos de dados MEDLINE/PubMed, Cochrane, Scopus, LILACS e SCIELO foram sistematicamente selecionados. Foram identificados 290 artigos e, destes, 269 artigos foram excluídos por não atenderem aos critérios de inclusão previamente estabelecidos. Um total de 20 estudos caso-controles e 1 coorte foi incluído.

Resultados As variantes genéticas rs1126643 (*ITGA2*), rs3842787 (*PTGS1*), rs20417 (*PTGS2*) e rs5918 (*ITGB3*) foram as mais estudadas. Quanto à relevância, das 64 variantes genéticas avaliadas pelos artigos, 14 tiveram significância estatística (p < 0.05; intervalo de confiança [IC] de 95%) em pelo menos um artigo. Entre eles, os seguintes tiveram resultados unânimes: rs1371097 (*P2RY1*), rs1045642 (*MDR1*), rs1051931 e rs7756935 (*PLA2G7*), rs2071746 (*HO1*), rs1131882 e rs4523 (*TBXA2R*), rs434473 (*ALOX12*), rs9315042 (*ALOX5AP*) e rs662 (*PON1*), enquanto estes diferiram na interferência real na RA: rs5918 (*ITGB3*), rs2243093 (*GP1BA*), rs1330344 (*PTGS1*) e rs20417 (*PTGS2*). Como limitações do estudo, destacam-se as metodologias não uniformes dos artigos analisados e as diferenças populacionais.

Palavras-chave

Farmacogenética

► Aspirina

Variação Genética

Conclusão Vale ressaltar que a farmacogenética é uma área em expansão. Portanto, mais estudos são necessários para entender melhor a associação entre variantes genéticas e RA.

INTRODUCTION

Cardiovascular disease (CVD) is the first cause of mortality worldwide, with all the healthcare systems facing this very challenging issue. The World Health Organization (WHO) estimates that 31% of deaths worldwide are due to CVD, with \sim 17.7 million CVD-related deaths in 2015. Approximately 7.4 million of these deaths were due to heart disease and 6.7 million deaths were due to stroke.¹ Platelet activation plays an important role in the development of CVD. Acetylsalicylic acid (ASA), commonly known as aspirin, is an irreversible inhibitor of platelet cyclooxygenase (COX), which prevents the formation of thromboxane A2 by arachidonic acid and, therefore, prevents the formation of this activating agent of platelet aggregation and vasoconstriction.² Aspirin is a widely used antiplatelet for primary and secondary prevention of CVD, such as stroke and heart attacks.³

Nevertheless, several patients may still experience treatment failure with ASA and an increased risk in recurrent stroke events.⁴ There are several contributing factors for treatment failure including medication adherence, drugdrug interactions, aspirin-independent thromboxane A2 synthesis and also genetic variations.² Even low daily aspirin doses (in the range between 75 and 150 mg) are able to suppress biosynthesis of thromboxane, inhibiting the accumulation of platelets, and reducing the risk of CVD.⁵ However, aspirin does not always prevent the formation of thromboxane A2 due to failure to inhibit platelet COX.⁶ Because of that, all individuals do not respond to antiplatelet therapy in a similar way. In this sense, the genetic mutations have been related with aspirin resistance (AR) and may cause reduction or increase in drug absorption and metabolism, contributing to AR.^{6,7}

Aspirin resistance can be diagnosed by clinical criteria or by laboratory tests. Clinically, the patient has a new episode of CVD, despite the regular use of aspirin. While the failure of aspirin to inhibit a platelet function test can be seen by Platelet Function Analyser (PFA-100) or light transmission aggregometry (LTA), for example.³

The field of pharmacogenetics, which aims to implement specific pharmacological therapies to genetic characteristics with the intention to provide greater efficiency, is a constant target of research.⁸ Therefore, several studies have been published about candidate genes associated with the genetic predisposition of resistance to AAS, such as *COX-2, GPIIIA*, and *P2Y1*.⁹ Resistance to antiplatelet therapy and the indiscriminate use of ASA can increase rates of recurrence and mortality from cardiovascular diseases, such as stroke.¹⁰ Hence, the aim of the present study was to perform a systematic literature review to determine the impact of genetic variants on AR.

METHODS

The present systematic review was established according to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) statement published by Moher et al. (2019). Five following databases were systematically screened: MEDLINE/PubMed,¹¹ Cochrane,¹² Scopus,¹³ LILACS,¹⁴ and SCIELO.¹⁵ The research was restricted to a period of 10 years (December 2009 to December 2019) and the following search terms were applied: *Aspirin* AND *Resistance* AND *Polymorphism* and *Aspirin* AND *Resistance* AND *Genetic variation*.

Eligibility criteria

Only articles published in English were included in this search. Also, only articles describing the relation between AR, proven by laboratory tests or a new case of CVD, and polymorphisms or genetic variations were included in the present systematic review. The final articles included (n = 21) in the present review were 20 case-controls and 1 cohort.

Assessment of risk of bias

The authors, using the combined search terms and based on the inclusion criteria, conducted the primary literature search. In that first moment, titles and abstracts were screened. All reports that appeared in accordance with the inclusion criteria were full-text screened. All studies that did not comply with pre-established eligibility and inclusion requirements were excluded. In a second step, the researchers independently evaluated whether the full-texts previously selected followed the inclusion criteria. In case of disagreement between two authors, a third author was consulted, and a consensus was reached by a meeting between them.

Furthermore, to assess and minimize the presence of potential biases, the Risk of Bias in Systematic Reviews (ROBIS) method was used as a reference.¹⁶

Data extraction and synthesis

In the primary literature search, a total of 290 articles were found: 178 in SCOPUS, 104 in MEDLINE/Pubmed, 5 in Cochrane, 2 articles in LILACS, and 1 in SCIELO. Of those, 19 were duplicated. Hence, 271 articles were screened for reading of title and abstract, 216 of which were excluded for not meeting our inclusion criteria.

In the next step, the authors independently reviewed 65 full-text articles. Then, 44 articles were excluded for not meeting our inclusion criteria. So, in the end, 21 articles were included in the present systematic review (**-Figure 1**).

RESULTS

In the 21 final articles selected, a total of 10,873 patients were analyzed, of which 3,014 were aspirin resistant and 6,882 were aspirin sensitive (some articles brought semiresistance values and were disregarded, and another 2 articles did not classify their patients as sensitive and not sensitive). Of the 21 articles studied, 11 included patients with a cerebrovascular event, totaling 4,835 patients. The



Figure 1 Flowchart of selected articles.

other 10 articles mostly analyzed cardiac outcomes. We also emphasize that the clinical conditions of the evaluated patients were varied among the articles, with some articles evaluating patients with > 1 disease: ischemic stroke (10 articles), coronary artery disease (9), peripheral arterial disease (3), acute vascular event (1), age > 80 years old (1), adults (1), and hypertension (1). Most of the patients in the selected articles are from the Asian continent (9 from China, 4 from India, 2 from Turkey, and 1 from Jordan), and regarding the other works, 3 articles are from the American continent (all from the United States of America), 1 from the European continent (Belgium), and 1 from the African continent (Tunisia).

Among the resistance analysis methods, 4 articles used clinical outcome and 17 used platelet aggregation measurement. Among those who performed platelet aggregation measurement, the most common method was LTA (8 articles), followed by PFA-100 system (3), thromboe-lastography platelet mapping assay (TEG) (2), VerifyNow (2), PL-11 platelet analyzer (1), TXB2 elisa kit (1) and urinary 11-dehydro TXB2 (1), with some articles using >1 method.

In **- Table 1**, we detail the following information from the 21 final articles included in the present review: Type of article, country, clinical condition, sample number, number of aspirin resistant patients, number of aspirin sensitive patients, gene, risk allele, protective allele, genetic variant, p-value, Odds Ratio (OR), CI, resistance assessment method, and daily aspirin dose.

In addition, we have highlighted in a separate table the genetic variants with relevant results for AR (**-Table 2**). As for relevance, of the 64 genetic variants evaluated by the articles, 14 had statistical significance (p < 0.05; 95%CI). Among them, the following polymorphisms have had concordant results so far: rs1371097 (*P2RY1*), rs1045642 (*MDR1*), rs1051931 and rs7756935 (*PLA2G7*), rs2071746 (*HO1*), rs1131882 and rs4523 (*TBXA2R*), rs434473 (*ALOX12*), rs9315042 (*ALOX5AP*), and rs662 (*PON1*). In turn, these genetic variants differ in real interference in AR: rs5918 (*ITGB3*), rs2243093 (*GP1BA*), rs1330344 (*PTGS1*), and rs20417 (*PTGS2*).

DISCUSSION

To study the relationship between polymorphisms and AR, it is necessary to consider the resistance analysis mode, which can be performed in two ways: clinical or laboratory. In the first, the patient is considered resistant if there is a negative outcome (death or stroke for example).¹⁷ In the second, several types of tests can be used, such as PFA-100, Verify-Now Aspirin, TEG, PL-11 platelet analyzer, serum and urinary TXB2, LTA, and multiplate analyzer. However, it is important to highlight that the measurement of platelet response to aspirin is highly variable, likely due to differing dependence of the arachidonic acid pathway between techniques. In our research, the most used laboratory method was the LTA, which is considered the gold standard for testing platelet function.¹⁸

The relationship between polymorphisms and AR has been described by Yi et al. This study assessed the interaction with PTGS1 (rs1236913 and rs3842787), PTGS2 (rs689466 and rs20417), TXAS1 (rs194149, rs2267679, and rs41708), P2RY1 (rs701265, rs1439010, and rs1371097), P2RY12 (rs16863323 and rs9859538), and ITGB3 (rs2317676 and rs11871251) gene variants. In the laboratory analysis, only rs1371097 of the P2RY1 gene, comparison CC x TT+CT, obtained statistical relevance (p = 0.01), even after adjusting for other covariates (*p* = 0.002; OR = 2.35; 95%CI: 1.87–6.86). In addition, using the generalized multifactor dimensionality reduction (GMDR) method, the following 3 sets of gene-gene interactions were significantly associated with AR: rs20417-CC/rs1371097TT/rs2317676GG (*p* = 0.004; OR = 2.72; 95%CI: rs20417CC/rs1371097TT/rs2317676GG/AG 1.18 - 6.86; (p = 0.034;OR = 1.91; 95%CI: 1.07-3.84); rs20417-CC/rs1371097CT/rs2317676AG (*p*=0.0025; OR=2.28; 95% CI: 1.13-5.33). These high-risk interactive genotypes were also associated with a bigger chance of early neurological deterioration (p < 0.001; Hazard Ratio [HR] = 2.47; 95%CI: 1.42-7.84).19

Peng et al. (2016) also assessed genes related to thromboxane and others. The analyzed polymorphisms were *ABCB1* (rs1045642), *TBXA2R* (rs1131882), *PLA2G7* (rs1051931 and rs7756935) and *PEAR1* (rs12041331–rs1256888). There was statistical significance for 3 of them: rs1045642 (p = 0.021; OR = 0.421; 95%CI: 0.233–0.759), rs1131882 (p = 0.028; OR = 2.712; 95%CI: 1.080–6.810) and rs1051931–rs7756935 (p = 0.023; OR = 8.233; 95%CI: 1.590–42.638),²⁰ while Wang Z. et al (2013) researched the association with *TBXA2R* (rs4523), *ITGB3* (rs5918), *P2RY1* (rs701265), and *GP1BA* (rs6065) polymorphisms. The only polymorphism significantly associated with AR was rs4523 (p = 0.001; OR = 4.479; 95% CI = 1.811–11.077).²¹

Another study that assessed the *TBXA2* and glycoprotein genes was done by Gao et al. *GP1BA* (rs6065), *ITGB3* (rs5918), *P2RY1* (rs701265), and *TBXA2R* (rs4523) genetic variations were researched, but only *TBXA2R* (rs4523) polymorphism was related (p = 0.01).²² In addition, Patel et al. also studied the *ITGA2B/ITGB3* polymorphisms. They analyzed the relationship with *CYP2C19* (rs4244285) and *ITGA2B/ITGB3* (rs5918) polymorphisms. However, no association was observed (p = 0.171 and p = 0.960, respectively).²³

Moreover, still in the scope of glycoprotein genes, Derle et al. conducted a study with 208 patients with vascular risk factors. *ITGB3* (rs5918) polymorphism was screened, and the results showed that there was no significant difference in the presence of the C allele between the groups (p = 0.277). In addition, in the relationship between the presence of the C allele and atherothrombotic stroke, no significant difference was found (p = 0.184).³

A study by Wang B et al. also analyzed the rs5918 (*PLA1*/*A*2) polymorphism of the *ITGB3* gene. All 214 patients in the aspirin sensitive group had the *PLA1*/*A1* genotype and no patients with *PLA2*/*A2* were found. However, of the 236 patients in the AR group, 12 had *PLA1*/*A2* heterozygous genotype (p = 0.002), finding a statistically significant differenc.²⁴

Aspirin dose/ day	75mg		300mg												100mg		500mg
Resistance assessment method	Platelet Aggregation	Measurement - LTA	Platelet Aggregation	Measurement - VerifyNow Assay											Platelet Aggregation	Measurement - PL-11 plate- let analyzer	Platelet Aggregation Measurement - VerifyNow P2Y12
D	z	Z												Z	z	Z	
OR	z	z	ĪZ	z	īz	z	Z	z	īz	IN	īz	z	īz	z	z	ĪZ	z
p-value	0.171	0.960	z	z	z	z	z	z	z	īz	z	z	z	0.005	0.210	0.69	0.7
Genetic variation	rs4244285 (CYP2C19*2)	rs5918 (PLA1/A2)	rs10306114 (A842G)	rs3842787 (C221) (C221) (F3388 (F644A) (F5789 (F714A)										rs1126643 (C807T)	rs20417 (G765C)	rs5065 T2238C)	
Risk allele	×	U	U	<u>н</u>	¥	¥	<u>н</u>	¥	<u>н</u>	U	<u>н</u>	ь	<u>н</u>	J	<u>н</u>	υ	U
Protective allele	J	ь	A	U	U	U	U	J	U	Т	U	U	J	A	U	U	F
Gene	CYP2C19	ITGA2B/ ITGB3	PTGS1	PTGS1	PTGS1	PTGS1	ITGA2	ITGA2	ITGA2	ITGB3	GP6	P2RY12	F13A1	PON1	ITGA2	PTGS2	NPPA
Aspirin sensitive	62		123		1		1		1		I		1		54	1	z
Aspirin resistant	2		31												43		z
Sample number*	65		154												67		597
Clinical condition	lschemic stroke		Peripheral artery	disease											Ischemic stroke		Stable CAD patients undergoing elective PCI
Country	India		USA												China		Belgium
Type of article	Case- control		Cohort	Cohort Case-													Case- control
Author (year)	Patel S. et al	(2019) ²³	Yeo et al. (2018) ³⁵												Wang et al.	(2017) ²⁰	Strisciuglio et al. (2017) ³⁶

Aspirin dose/ day	200mg	(14 days) and follow-up	with 100mg												100mg						200mg	(14 days) and follow-up with	100mg		100–300mg	1 00mg	100mg		:
Resistance assessment method	Platelet Aggregation Massurement - LTA									Platelet	Aggregation Measurement	- TXB2 ELISA	KIT			Platelet	Aggregation Measure-	ment- LTA		Platelet Aggregation Measurement - PFA-100 system	Platelet Aggregation Measurement - LTA	Platelet Aggregation	Measurement - Multiplate						
Ū	IN	IN	IZ	Z	Z	z	Z	Z	Z	Z	z	Z	Z	z	0.233-0.759	1.080-6.810	1.590-42.638	1.590-42.638	0.260-1.671	0.260-1.671	z	Z	Z	N	Z	z	N	N	Z
OR	IN	IN	IN	z	z	Z	z	IN	IN	z	Z	z	IN	Ī	0.421	2.712	8.233	8.233	0.660	0.660	z	z	IN	R	īz	z	ĪZ	IN	z
p-value	.99**	0.76**		0.26**	0.42**	0.53**	0.72**	0.48**	0.32**	0.01**	0.21**	0.16**	0.24**	0.51**	0.021	0.028	0.023	0.023	0.378	0.378	0.95**	0.78**	0.82**	0.42**	0.277	0.002	0.116	0.003	0.485
Genetic variation	rs1236913	rs3842787	rs689466	rs20417	rs194149	rs2267679	rs41708	rs701265	rs1439010	rs1371097	rs16863323	rs9859538	rs2317676	rs11871251	rs1045642	rs1131882	rs1051931	rs7756935	rs12566888	rs12566888	rs1236913	rs3842787	rs689466	rs20417	rs5918 (PLA1/A2)	rs5918 (PLA1/A2)	rs1126643 (C807T)	rs2243093	rs20417
Risk allele	т	т	ט	υ	A	υ	F	U	U	μ	F	A	0	ى	F	A	υ	A	F	۲	υ	F	υ	υ	U	U	μ	U	υ
Protective allele	С	С	A	U	U	Т	U	A	A	C	C	U	A	A	U	U	A	C	U	ט	т	U	Т	ט	Т	Т	U	Т	U
Gene	PTGS1	PTGS1	PTGS2	PTGS2	TXAS1	TXAS1	TXAS1	P2RY1	P2RY1	P2RY1	P2RY12	P2RY12	ITGB3	ITGB3	ABCB1	TBXA2R	PLA2G7	PLA2G7	PEAR1	PEAR1	PTGS1	PTGS1	PTGS2	PTGS2	ITGB3	ITGB3	ITGA2	GP1BA	PTGS2
Aspirin sensitive	630				1	1	1	1	1	1	1		1	1	33	1			1	1	630	1	1		141	214	492	•	
Aspirin resistant	175														250						175				67	236	92		
Sample number*	850														283					850				208	450	584			
Clinical condition	stroke									lschemic	stroke					lschemic	stroke			Acute vascu- lar event	> 80 years old	Adults							
Country	Chin a									China						China				Turkey	China	Jordan							
Type of article	Case-control									Case-	control					Case-	control			Case- control	Case- control	Case- control							
Author (year)	Yi et al. (2017) ¹⁹									Peng et al.	(2016) ²⁰					Yi et al. (2016)	0			Derle et al. (2016) ³	Wang et al. (2014) ²⁴	Al-Azzam et al. (2013) ²⁷							

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Aspirin dose/ day		75–160mg	75–160mg								100mg				75–325mg	75–325mg	75–100 mg						75–325 mg/dia	100mg		
Resistance assessment method	Analyzer system	Platelet	Platelet Aggregation Measurement - LTA							Platelet Aggregation	Measurement - LTA			Clinical outcome	Clinical outcome	Platelet	Aggregation Measurement	- LTA and TEG	Platelet Map- ping Assay			Clinical outcome	Platelet Aggregation			
a		ĪZ	ĪZ	ĪZ	ĪZ	ĪZ	Z	ĪZ	ĪZ	ĪZ	1.811-11.077	N	0.473-2.934	N	CC: 1.241–8.033 GC: 1.059–2.875	1.884-4.723	1.13-2.92	N	ĪZ	NI	IN	ĪZ	1.142-3.017	IN	N	IN
OR		z	z	īz	z	z	Z	z	z	Z	4.479	īz	1.178	ĪN	CC:OR-3.157 GC: OR-1.745	2.983	1.82	N	z	IN	IN	z	1.85	īz	īz	z
p-value		0.92	0.1	0.92	0.92	-	-	-	0.24	0.04	0.001	z	0.724	ĪZ	CC: $p = 0.016$ GC: $p = 0.02$	<0.001	0.01	0.59	0.66	0.49	1	1	0.012	L	1	0.991
Genetic variation		rs1888943	rs1330344	rs3842787	rs5787	rs5789	rs5794	rs20417	rs5277	rs2071746	rs4523 (T924C)	rs5918 (PLA1/A2)	rs701265 (A1622G)	rs6065 (C1018T)	rs20417 (-765G/C)	rs9315042 (SG13S114T/A)	rs1330344	rs1888943	rs3842787	rs5787	rs5789	rs5794	rs1045642	rs6065 (C1018T)	rs5918 (P1 A1 /A 2)	rs701265 (A1622G)
Risk allele		F	J	г	A	A	A	υ	U	-	U	U	J	⊢	υ	A	J	Т	F	A	A	A	F	ь	U	J
Protective allele		U	A	С	ט	U	U	C	U	A	T	Т	A	U	U	μ	A	С	С	G	С	U	U	C	Т	A
Gene		PTGS1	PTGS1	PTGS1	PTGS1	PTGS1	PTGS1	PTGS2	PTGS2	H01	TBXA2R	ITGB3	P2RY1	GP1BA	PTGS2	ALOX5AP	PTGS1	PTGS1	PTGS1	PTGS1	PTGS1	PTGS1	ABCB1	GP1BA	ITGB3	P2RY1
Aspirin sensitive		231	1			1	1	1	1	1	84 84 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1				233	303	393						222	239		I
Aspirin resistant		36									62				217	307	38						23 338			
Sample number*		431									210				450	610	431						560	262		
Clinical condition		CAD, stroke,	CAD, stroke, and peripher- al artery disease								Patientsun- derwent pri-	mary OPCAB			Ischemic stroke	stroke Ischemic stroke CAD, hyper- tension, pe- ripheral ar- tery disease and stroke						Ischemic stroke Patients un- derwent pri- mary OPCAB				
Country		China	China							China				India	India	China						India	China			
Type of article		Case-	Case- control							Case-				Case- control	Case- control	Case-	control					Case- control	Case- control			
Author (year)		Li et al. (2012)	29 c 1 (2012) c 229 c 1 (2012) c 20 c							Wang et al. C (2013) ²¹ α				Sharma et al. (2013) ³²	Sharma et al. (2013) ¹⁷	Fan et al.	(2012)					Sharma et al. (2012) ³³	Gao et al. (2011) ²²			

Aspirin dose/ day		250mg	Two groups: < 81mg and	Two groups: < 81mg and > 81mg											the difference between the resistant and sensitive groups	was 0.681)			(Continued)			
Resistance assessment method		Platelet Aggregation Measurement - PFA-100 sys- tem and Uri- nary 11- dehydro-TXB2	Clinical Outcome	Clinical Outcome											Platelet Aggregation Measurement - PFA-100 system							
σ	IN	z	Black: 0.71–1.87 White: 0.82–1.07	Black: 0.82–1.46 White: 0.87–1.14	Black: 0.71–1.50 White: 0.85–1.13	Black: 0.66–1.20 White: 0.86–1.15	Black: 0.62–1.14 White: 0.86–1.18	Black: 0.46–2.41 White: 0.83–1.09	Black: 0.80–1.42 White: 0.89–1.16	Black: 0.60–1.54 White: 0.91–1.23	Black: 0.34–1.36 White: 0.88–1.21	Black: 0.94–1.77 White: 0.88–1.29	Black: 0.63–1.51 White: 0.85–1.10	IN	Z							
OR	N	īz	Black: 1.15 White: 0.93	Black: 1.10 White: 0.99	Black: 1.03 White: 0.98	Black: 0.89 White: 0.99	Black: 0.84 White: 1.01	Black: 1.05 White: 0.95	Black: 1.06 White: 1.02	Black: 0.96 White: 1.06	Black: 0.68 White: 1.03	Black: 1.29 White: 1.06	Black: 0.98 White: 0.97	Z								
p-value	0.01	Urinary Tx82: 0.1 PFA-100: 0.43	> 0.05											0.302	0.191	0.644	0.480	0.814				
Genetic variation	rs4523 (T924C)	rs3842787 (C50T)	rs5443 (C825T)	rs1126643 (C807T)	rs5918	rs1613662	rs2243093	rs2768759	rs6583047	rs168753	rs2228262	rs3842787	rs1800544	rs6025 (G1691A)	rs1800595 (A4070G - H1 299R)	rs1799963 (G20210A)	rs5985 (V34L)	rs1800790 (G455A)				
Risk allele	U	μ	т	μ	υ	U	υ	υ	υ	+	U	+	U	×	U	<	+	۷				
Protective allele	Т	U	С	C	Т	×	Т	×	×	×	A	U	U	U	×	U	U	C				
Gene	TBXA2R	PTGS1	GNB3	ITGA2	ITGB3	GP6	GP1BA	PEAR1	VAV3	F2R	THBS1	PTGS1	ADRAZA	F5	F5	F2	F13A1	FGB				
Aspirin sensitive		īZ	2584		1	1	1	1	1	1	1	<u> </u>	<u> </u>	96								
Aspirin resistant		Ē	865											30								
Sample number*		125	3449											126								
Clinical condition		Stable CAD	Coronary stenosis $\geq 75\%$											Stable CAD								
Country		Tunisia	USA											Turkey								
Type of article		Case- control	Case- control	Case- control								Case- control										
Author (year)		Chakroun et al. (2011) ³¹	Voora et al. (2011) ²⁶	Voora et al. (2011) ²⁶									Pamukcu et al. (2010) ²⁵									

Aspirin dose/ day									Not	unitorm			/ intervention:
Resistance assessment method									Platelet	Aggregation Measurement	- TEG Platelet	funddenn	taneous coronar
U									ĪZ				vpass; PCI, percut
OR									IN				ronarv arterv b
p-value	0.656	0.623	0.362	0.421	0.713	1	0.695	0.695	0.043	0.440	0.580	IN	B. off-pump cc
Genetic variation	rs1799889 (4G/5G)	rs5918 (HPA1a/b)	rs1801133 (C677T)	rs1801131 (A1298C)	rs1799752 (ACE I/D)	rs5742904 (R3500Q)	rs429358 (C1 12R)	rs429358 (C1 58A)	rs434473	rs4792147	rs1126667	rs3892408	informed: OPCA
Risk allele	U	υ	<u>н</u>	υ	Del	¥	U	C	ט	¥	A	A	: NI. not
Protective allele	۲	Т	U	A	Ins	J	т	Т	A	C	G	C	areaometry
Gene	SERPINE1	ITGB3	MTHFR	MTHFR	ACE	APOB	APOE	APOE	ALOX12	ALOX15B	ALOX12	ALOX15	mission ac
Aspirin sensitive		I	I		I		I		54				light trans
Aspirin resistant									27				terval: LTA.
Sample number*									81				ufidence int
Clinical condition									Candidates	for interven- tional cardiol-	ogy on aspirin	шегару	disease: Cl. co
Country									NSA				arv arterv
Type of article									Case-	control			CAD. coron
Author (year)									Carroll et al.	(2010)			Abbreviations:

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Table 1 (Continued)

TxB2, thromboxane B2. Notes: *The number of semiresistants is not included. **These p-values are the result of comparing the Aspirin Sensitive group. There is no individual comparison between aspirin resistance X aspirin sensitivity.

Biomarker (Pharmacogene)	Alleles	Refs.
PON1	rs662	35
P2RY1	rs1371097	19
ABCB1	rs1045642	20,33
TBXA2R	rs1131882, rs 4523	20,21
PLA2G7	rs1051931, rs7756935	20
ITGB3	rs5918	24
GP1BA	rs2243093	27
HO1	rs2071746	29
PTGS2	rs20417	17
ALOX5AP	rs9315042	17
PTGS1	rs1330344	29,30
ALOX12	rs434473	34

 Table 2
 Genetic variants with relevant results for aspirin resistance

In the study by Pamukcu et al., 13 polymorphisms of 10 different genes were tested, including *ITGB3*. The genes *F5* (rs6025, rs1800595), *F2* (rs1799963), *F13A1* (rs5985), *FGB* (rs1800790), *SERPINE1* (rs1799889), *ITGB3* (rs5918), *MTHFR* (rs1801133, rs1801131), *ACE* (rs1799752 - Ins/Del), *APOB* (rs5742904), and *APOE* (rs429358 - C112R and C158A) were evaluated. However, there was no significant result for any polymorphism (p > 0.05).²⁵ Furthermore, in the case-control study by Voora et al, 11 polymorphisms of 11 different genes were assessed: *GNB3* (rs5443), *ITGA2* (rs126643), *ITGB3* (rs2243093), *PEAR1* (rs2768759), *VAV3* (rs6583047), *F2R* (rs168753), *THBS1* (rs2228262), *PTGS1* (rs3842787), and *ADRA2A* (rs1800544). When comparing the groups, there was no relationship (p > 0.05).²⁶

Another research that studied some of the same genes was conducted by Al-Azzam et al.: *GP1BA* (rs1126643), *ITGA2* (rs2243093) and *PTGS2* (rs20417). Of these, only the *GP1BA* (rs2243093) gene was related (p = 0.003), analyzing the presence of the C allele.²⁷ Additionally, Wang et al. (2017) conducted a study about the following polymorphisms: *ITGA2* polymorphism gene at rs1126643 and *PTGS2* polymorphism gene at rs20417. The authors found no association: p = 0.21 for rs126643 and p = 0.69 for rs20417.²⁸

Moreover, Yi et al. used Matrix-Assisted Laser Desorption/ Ionization-Time Of Flight (MALDI-TOF) to link *PTGS1* (rs1236913 and rs3842787) and *PTGS2* (rs689466, and rs20417) with AR. The analysis showed that there was no statistical relevance for the relationship. Only when the gene-gene interaction (rs3842787 and rs20417) was evaluated, there was statistical significance: rs3842787/CT + rs20417/CC (p = 0.016; OR = 2.36; 95%CI: 1.12–6.86), rs3842787/TT, CT + rs20417/CC (p = 0.078; OR = 1.36; 95% CI: 0.82–2.01), and rs3842787/CT + rs20417/GC (p = 0.034; OR = 1.78; 95%CI: 1.04–4.58). Highlighting the fact that, for the second combination, there is an invalid CI.¹⁹

Another study that investigated polymorphisms of the *PTGS1* (rs1888943, rs1330344, rs3842787, rs5787, rs5789,

rs5794) and *PTGS2* (rs20417, rs5277) genes was conducted by Li et al.; in addition to these two genes, a genetic variant of the *HO1* gene (rs2071746) was also tested. As a result, only two genetic variations were associated with AR. The rs2071746 polymorphism (*HO1* gene) had statistical significance to genotype TT (p=0.04; OR = 1.40; 95%CI = 0.59– 3.30) and T allele (p=0.04; OR = 1.70; 95%CI = 1.02–2.79), while rs1330344 (*PTGS1* gene) had significant results only when G was the risk allele and analyzed separately (p=0.02; OR = 1.77; 95%CI = 1.07–2.92).²⁹

Still on the *PTGS1* gene, Fan et al. investigated several polymorphisms of the *PTGS1* gene (rs1888943, rs1330344, rs3842787, rs5787, rs5789, and rs5794), but rs1330344 was the only significantly related to AR (p = 0.01; OR = 1.82; 95% CI = 1.13–2.92; allele value) just in LTA+TEG analysis.³⁰ Moreover, another case-control study by Chakroun et al. investigated the relationship between rs3842787 polymorphism of the *PTGS1* gene and AR. Patients with the allele had no statistically significant difference using CEPI-CT (p = 0.1) and uTxB2 (p = 0.43).³¹

Sharma et al. evaluated 3 polymorphisms of 3 different genes, *PTGS2* (rs20417), *ALOX5AP* (rs9315042) and *ABCB1* (rs1045642), to assess their role in AR. The research was performed in 3 different studies and all studies obtained statistical relevance for the CC allele of rs20417 (p = 0.016; OR = 3.157; 95%CI: 1.241–8.033), the GC allele of rs20417 (p < 0.001; OR = 2.983; 95%CI: 1,884–4,723) and for the rs9315042 variant (p < 0.001; OR = 2.983; 95%CI: 1.884–4,723). For the variant rs1045642, 2 comparisons were made, one comparing cases and controls, for the TT x CC alleles (p < 0.001; OR = 2.27; 95%CI: 1.64–3.168), and for the TT x CT + CC alleles (p < 0.001; OR = 1.72; 95%CI: 1.335–2.239) and other comparing AR and sensitive participants (p = 0.012; OR = 1.85; 95%CI: 1.142–3.017).^{17,32,33}

Another study that tested the *ALOX* gene was done by Carroll et al. The study tested 4 genetic variants: rs434473 and rs1126667 of the *ALOX12* gene, rs4792147 of the *ALOX15B* gene and rs3892408 of the *ALOX15* gene. Only the rs434473 polymorphism obtained a significant *p*-value (p = 0.043).³⁴

Furthermore, Yeo et al. analyzed some variants of *PTGS1* (rs10306114, rs3842787, rs5788, and rs5789), *ITGA2* (rs1126643, rs1062535, and rs1126643), *ITGB3* (rs5918), *GP6* (rs1613662), *P2RY12* (rs1065776), and *F13A1* (rs5985) genes, but only rs662 (*A576G*) of *PON1* gene was significantly relevant (p = 0.005) to AR.³⁵

Lastly, a study by Strisciuglio et al. included 450 noncarriers of the T2238C polymorphism (rs5065, *NPPA* gene) and 147 carriers. The authors concluded that there was no statistical difference when comparing the groups, neither in overall CAD patients (p = 0.7) nor in the diabetic group (p = 0.6).³⁶

As limitations of the present study, we highlight the nonuniform methodologies of the analyzed articles, as well as population differences. These divergences made it difficult to compare the results of the articles. Among the studies, there was a great difference among the clinical conditions, as well as in the way of analysis of the resistance and in the dosage of aspirin. Unfortunately, meta-analysis was not performed due to such high clinical and methodological heterogeneity of the findings.

Despite the heterogeneity of the findings in terms of methodology and results, it is clear that some polymorphisms are more studied than others. Among them, rs1126643 (*ITGA2*), rs3842787 (*PTGS1*), rs20417 (*PTGS2*), and rs 5918 (*ITGB3*) were the most studied.

In conclusion, pharmacogenetics is an expanding area that promises a therapy aimed at the individualities of each patient, personalized medicine, for better control of diseases, including cardiovascular diseases, such as stroke.

Finally, further studies are needed to better understand the association between genetic variants and AR and, therefore, the practical application of the findings.

Authors' Contributions

All authors contributed to data collection, information organization and article writing. All authors approved the final version.

Conflict of Interest

The authors have no conflict of interests to declare.

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