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AGT A-20C (rs5050) gene polymorphism and ulcer occurrence in patients treated with low-dose aspirin: a case-control study

Polimorfismul AGT A-20C și ulcerele gastro-duodenale la pacienții sub tratament cu aspirină în doze antiagregante: studiu caz-control

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Abstract

Genetic factors may play a role in prediction of gastrointestinal side effects of aspirin, one of the most used drugs worldwide. We aim to determine a possible correlation between AGT A-20C (rs5050) gene polymorphism and gastro-duodenal ulcer in patients taking low-dose aspirin, adjusted for clinical and histological characteristics.

***Results**. We enrolled 211 patients stratified according to AGT A-20C genotype: 122 AA, 83 AC and 6 CC patients. There were no significant differences regarding demographical and clinical parameters, except for the frequency of ulcers (4%, 8.4% respective 50%, p=0.03), endoscopic bleeding signs (12.3%, 14.5% respective 50%, p=0.0001) and the frequency of gastritis in biopsy (63.9%, 54.2% respective 16.7%, p=0.03) in genotype groups. When we compared ulcer and non-ulcer group, variant homozygous CC genotype carried an increased risk for ulcer (OR:9.66, 95% CI: 1.46-63.7, p=0.04) than AA group, as well as variant C allele compared with normal A allele (OR: 2.12, 95% CI: 1.07-4.63, p=0.04). On multivariate analysis, variant homozygous CC genotype AGT A-20C showed an OR: 12.32 (95% CI:1.40 -108.13, p=0.02) for ulcer, while H. pylori infection (OR:2.40, 95% CI:1.18 -6.54, p=0.04) and concurrent use of non-steroidal anti-inflammatory drugs (NSAIDs) (OR:1.31, 95% CI:1.07 - 2.27, p=0.05) remained predictors for ulcer in aspirin consumers.*

***Conclusions**. Variant C allele and variant homozygous CC genotype AGT A-20C, infection with H. pylori and NSAIDs co-treatment are risk factors for gastro-duodenal ulcer in low-dose aspirin consumers. The variant homozygous CC genotype AGT A-20C patients treated with LDA are more prone to have reactive gastropathy and bleeding ulcers in a population with a high prevalence of H. pylori infection.*

***Keywords*:** *AGT A-20C (rs5050) gene polymorphism;low-dose aspirin;gastro-duodenal ulcer;H. pylori;reactive gastropathy*

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Abstract

Factorii genetici pot juca un rol important in evaluarea riscului efectelor secundare gastrointestinale ale aspirinei, unul din cele mai folosite medicamente în intreaga lume. Obiectivul studiului a fost identificarea unei posibile corelații între polimorfismul genic al AGT A-20C (rs5050) și ulcerele gastro-duodenale la pacienții tratați cu aspirină în doze antiagregante

***Rezultate**. Cei 211 pacienți introduși în studiu au constituit conform genotipului AGT A-20C trei grupe: 122 pacienți AA, 83 pacienți AC și 6 CC. Nu au existat diferențe statistic semnificative între cele trei grupe în ceea ce privește caracateristicile clinico-demografice, cu excepția frecvenței ulcerelor (4%, 8.4% respectiv 50%, p=0.03), a stigmatelor endoscopice de sângerare (12.3%, 14.5% respectiv 50%, p=0.0001) și a frecvenței inflamației gastrice în biopsii (63.9%, 54.2% respectiv 16.7%, p=0.03). Comparând pacienții cu ulcer cu cei fără ulcer, genotipul homozigot mutant CC a fost asociat cu un risc crescut de ulcer (OR:9.66, 95%CI: 1.46-63.7, p=0.04) fața de grupul cu genotip AA, iar alela mutantă C s-a corelat cu un număr mai mare de ulcere decât alela normală A (OR: 2.12, 95%CI: 1.07-4.63, p=0.04). Genotipul homozigot mutant CC pentru polimorfismul AGT A-20C a prezentat un OR=12.32 (95%CI:1.40 -108.13, p=0.02) pentru apariția ulcerelor, în timp ce infecția cu H. pylori (OR:2.40, 95%CI:1.18 -6.54, p=0.04) și consumul de anti-inflamatoare non-steroidiene au rămas predictori pentru ulcer la pacienții tratați cu aspirină și după studiul în regresie logistică multiplă .*

***Concluzii**. Alela mutantă C și genotipul homozigot mutant CC pentru AGT A-20C, infecția cu H. pylori și co-tratamentul cu antiinflamatoare non-steroidiene sunt factori de risc pentru ulcer la pacienții tratați cu aspirină în doze antiagregante. Genotipul homozigot mutant CC pentru polimorfismul genic AGT A-20C este asociat mai frecvent cu gastropatia reactivă și risc de sângerare la pacienții tratați cu aspirină.*

***Cuvinte cheie:** Polimorfismul AGT-20C (rs5050), aspirina în doze antiagregante, ulcere gastro-duodenale, H. pylori, gastropatie reactivă*

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Introduction

The number of patients using non-steroidal anti-inflammatory (NSAIDs) and antithrombotic (anticoagulants and antiplatelet) drugs is continuously increasing worldwide, and their gastrointestinal (GI) side effects tend to limit the use [1]. The most important causes of peptic ulcer disease are *Helicobacter pylori (H. pylori)* infection and NSAIDs treatments, including aspirin in low-doses. Most studies suggest a decreasing trend in the prevalence of ulcer disease in Western world, due to the efforts for eradication of *H. pylori* infection and use of proton pomp inhibitors (PPI). On the other hand, drug-related ulcers are more prevalent than they were decades ago [2]. The increasing number of *H. pylori* positive elderly patients that need concomitant gastrotoxic therapies sustain the importance for defining risk factors regarding GI complications. Studies conducted on populations with different characteristics (*H. pylori* prevalence and phenotype of gastritis), as well as a different genetic profile are thought to be causes for the controversial results in this respect [3, 4]. The most important risk factors for GI events are slightly different in low-dose aspirin and in NSAIDs consumers in Western and Asian populations. Previous history of ulcer, concomitant treatment with anticoagulants, advanced age and comorbidities seem to increase the risk for GI complications in NSAIDs consumers, while *H. pylori* infection, history of ulcer and concurrent gastrotoxic therapies are thought to carry the most important risk for hemorrhage in low-dose aspirin consumers (LDA) [1, 5].

Genetic factors might play a role in prediction of gastrointestinal side effects of NSAIDs/aspirin. A limited number of studies demonstrated an association between some genetic

polymorphisms and NSAIDs related ulcers [4]. Cytochrome *P450 2C9* (CYP2C9) variants were reported to increase the risk for ulcer bleeding in NSAIDs consumers in several studies [6]. In a Japanese study, a polymorphism of the gene encoding cyclooxygenase (*COX-1*-1676 T allele) was associated with ulcer in NSAIDs consumers [7], while another study reported the carriage of interleukin-1β *(IL*-1b-511T) T allele to be associated with an increased risk for ulcer in aspirin consumers [8]. Another Japanese study has recently demonstrated an increased risk for bleeding in LDA consumers harboring the angiotensinogen variant genotype (*AGT*-20 CC) [9].

RAS (renin–angiotensin system) components seem to play an important role in homeostasis of many tissues in the human body. The current concept of RAS evolved to local autonomous functions, from the classical systemic effect involving conversion of angiotensinogen (AGT) to angiotensin II (Ang II) facilitated by renin and angiotensin converting enzyme (ACE) [10]. Recent studies demonstrated the presence of RAS component in gastric mucosa to mediate cell proliferation, inflammation and fibrosis, promoting wound healing response, microvascular permeability, fluid and electrolyte transport as well as antral endocrine functions [10, 11]. The question remains, however if genetic molecular predictors can be used for estimating the risk of GI events in a specific population.

Objective

Our study aims to determine a possible correlation between *AGT* A-20C gene polymorphisms and gastro-duodenal mucosal lesions in patients treated with low-dose aspirin, adjusted for their clinical and histological characteristics.

Material and methods

The studied group consisted of 211 patients investigated by endoscopy, all of them being successfully genotyped (124 females and 87 males). We included patients with daily low-dose aspirin treatment (LDA 75-125 mg/day), referred to endoscopy for digestive symptoms or anemia and patients without symptoms, evaluated for bleeding risk before major cardiac surgery. Patients were enrolled after a written consent was obtained. The study was approved by the Ethics Committee of the University of Medicine and Pharmacy Tîrgu Mureş, Romania.

Clinical data were collected using a structured interview and medical records. We registered the digestive symptoms (abdominal pain, heartburn, nausea, vomiting, and melena) and history of peptic ulcer. We investigated concomitant use of other gastrotoxic drugs or anticoagulants: non-steroidal anti-inflammatory drugs (NSAIDs, regular adult doses for at least one week before endoscopy), acenocumarolum (at least one month prior to endoscopy, doses for therapeutic INR) and low-weight molecular heparin (LWMH, regular doses at least one week). Gastroprotective treatments with proton pomp inhibitors (PPI) or H2 receptor blockers prior to endoscopy were recorded.

Exclusion criteria were patients who admitted they failed to take the daily dose of LDA treatment, patients with gastric surgery, gastro-esophageal cancer, variceal bleeding, and patients in whom a complete set of gastric biopsies was not obtained.

Endoscopic evaluation

A single endoscopist examined all the patients, blinded to drug exposure and symptoms. We assessed gastro-duodenal lesions using a modified Lanza score [12], one of the most frequently cited in literature. For no mucosal lesions a 0 score was given, while for one erosion (mucosal defect smaller than 5 mm) or petechia (hemorrhagic area without mucosal defect) a score of 1 was attributed. We considered score 2 when 2–10 erosions or submucosal hemorrhages

were counted, score 3 for more than 10 erosions or submucosal hemorrhages and score 4 when an ulcer was present (defect larger than 5 mm).

Histology

For routine histologic evaluation we obtained at least four biopsy specimens (two from antrum and two from corpus, namely from lesser and greater curvature). A single pathologist blinded to clinical patients' profile examined them. Biopsy specimens were fixed in formalin, embedded in paraffin and examined with hematoxylin-eosin, PAS-alcian blue and Giemsa staining. *H. pylori* infection was considered negative if *H. pylori* were absent from all biopsy sites and positive if at least one histology test was positive. The degree of mucosal chronic inflammation, activity, *H. pylori* infection, glandular atrophy and intestinal metaplasia were classified according to the Updated Sydney System. We also evaluated dysplasia according to the modified Vienna classification, but patients with dysplasia or neoplasia were excluded. Patients without important inflammation, but with prominent foveolar hyperplasia, fibromuscular replacement of lamina propria, and congestion of superficial mucosal capillaries were diagnosed with reactive gastropathy.

Genotyping

Genomic DNA was extracted from 150 µl of EDTA venous blood samples using the Quick-gDNAMiniPrep kit (from ZymoResearch, USA). The *AGT* A-20C gene polymorphism was investigated by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique using the primers (from Eurogentec) and restriction enzyme (FastDigest EcoO109I from Thermoscientific) as previously described [9]. The PCR conditions were similar to those described by Zhang et al. [13].

All collected data were recorded in a specially designed database.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 17, Chicago, IL, USA). To assess the associations between genotype distribution and other categorical variables, as well as peptic ulcer distribution and other categorical variables we used contingency tables and the Chi-square test. We calculated the OR (odds ratio) to demonstrate the probability or susceptibility to ulcer peptic of the given polymorphism. Multiple regression logistic analysis was applied to studied groups, considering peptic ulcer as the dependent variable and other parameters (*H. Pylori* infection, concomitant consumption of gastrotoxic drugs, age) as independent variables. We interpreted all the tests against a p=0.05 significance threshold and statistical significance was considered for p values below the significance threshold.

Results

Clinical, histological and endoscopic characteristics of patients, divided into three groups according to *AGT* A-20C genotypes (normal or wild-type homozygous AA, heterozygous AC and variant homozygous CC) are shown in Table I. No significant statistical differences were noticed in genotype groups except for ulcers (p=0.03) and endoscopic bleeding signs (p=0.0001) which were more frequent in CC variant homozygous genotype group. The frequency of gastritis was higher in AA normal homozygous group compared with the other groups.

We investigated the role of the most important known risk factors for GI lesion occurrence in patients treated with LDA using univariate analysis (Table II). We studied the possible role of premalignant histological changes (gastric atrophy and intestinal metaplasia) in ulcer occurrence, in patients harboring the variant CC genotype and also in those carrying the variant

Table I. Comparison of demographic, clinical and histologic findings in patients stratified after the *AGT* A-20C genotypes

	AA N=122 (57.8%)	AC N=83 (39.4%)	CC N=6 (2.8%)	P value
Mean age	62.31 ±12.93	61.47±13.78	60.83±14.13	ns
Sex	52 (42.6)	35 (42.5)	0	0.28
Ulcer history	23 (18.9)	11 (13.3)	0	0.31
Drug consumption				
Anticoagulants	21 (17.2)	12 (14.5)	1 (16.7)	0.87
Clopidogrel	19 (15.6)	9 (10.8)	0	0.38
NSAIDs	15 (12.3)	11 (13.3)	0	0.75
PPI	80 (65.6)	53 (63.9)	2 (33.3)	0.28
Endoscopic findings				
Lanza score 4 (ulcer)	6 (4.9)	7 (8.4)	3 (50)	0.03
Bleeding signs	15 (12.3)	12 (14.5)	3 (50)	0.0001
Histologic findings				
Reactive gastropathy	40 (32.8)	28 (33.7)	3 (50)	0.81
Gastritis	78 (63.9)	45 (54.2)	1 (16.7)	0.03
Intestinal metaplasia/gastric atrophy	46 (37.7)	21 (25.3)	1 (16.7)	0.1
***H. pylori* infection**	34 (27.8)	25 (30.1)	0	0.22
Comorbidities				
Hypertension	98 (80.3)	66 (79.5)	4 (66.7)	0.74
Heart failure	65 (53.3)	45 (54.2)	3 (50.0)	0.97
Cerebrovascular disease	5 (4.1)	4 (4.8)	0	0.84
Diabetes mellitus	25 (20.5)	19 (22.9)	1 (16.7)	0.89
Renal disease	19 (15.6)	13 (15.7)	0	0.68
Liver disease	40 (32.8)	22 (26.5)	3 (50)	0.35
Respiratory disease	24 (19.7)	14 (16.9)	1 (16.7)	0.88
Symptoms				
Epigastric pain	61 (50.0)	45 (54.2)	4 (66.7)	0.69
Heartburn	34 (27.9)	24 (28.9)	2 (33.3)	0.96
Regurgitation	7 (5.7)	2 (2.4)	0	0.46
Nausea/vomiting	19 (15.6)	10 (12.0)	0	0.49
Bloating	28 (23.0)	18 (21.7)	2 (33.3)	0.81
Melena	6 (4.9)	5 (6.0)	0	0.21
Social behaviors				
Alcohol consumption*	16 (13.1)	8 (9.6)	1 (16.7)	0.18
Smoking**	10 (8.2)	4 (4.8)	0	0.18

*more than 2 units/day, 1 unit=10 ml pure alcohol

**>5 cigarettes/day

NSAIDs=non-steroidal anti-inflammatory drugs, PPI=proton pomp inhibitors

Table II. Univariate analysis of risk factors for ulcer in LDA consumers

	Ulcer group N=15		Control group N=196		OR	CI95%	P value
	n	%	n	%			
Male gender	6	40.0	81	41.3	1.00	1.00-1.00	0.95
Age>70	5	33.3	54	27.5	1.31	0.42-4.02	0.76
History of ulcer	4	26.6	30	15.3	2.01	0.60-6.74	0.27
Anticoagulants	5	33.3	29	14.7	2.87	0.91-9.03	0.07
Clopidogrel	3	20.0	25	12.8	1.71	0.45-6.48	0.42
NSAIDs	6	40.0	20	10.2	5.86	1.89-18.19	0.004
H. pylori	8	53.3	51	26.0	3.24	1.12-9.41	0.03
Intestinal metaplasia/gastric atrophy	4	26.7	64	32.7	0.75	0.23-2.44	0.75
***AGT* A-20C genotype**							
AA	6	40.0	116	59.18	-	-	-
AC	7	46.6	76	38.7	5.42	0.84-35.1	0.11
CC	2	13.3	4	2.04	9.66	1.46-63.7	0.04
A allele	19		308		2.12	1.07-4.63	0.04
C allele	11		84				

NSAIDs=non-steroidal anti-inflammatory drugs

C allele. We compared patients with ulcer on endoscopy (n=15 patients) to patients without ulcer (n=196 patients) using univariate analysis (Table II).

Infection with *H. pylori* and concomitant use of NSAIDs were correlated with ulcers in aspirin consumers (p=0.03, respective p=0.004). Variant homozygous CC genotype was found to be a risk factor for gastro-duodenal lesions (p=0.04), while variant C allele of the *AGT* A-20C polymorphism seemed to carry an increased risk for mucosal damage compared with A allele, in our patients (p=0.04) (Table II).

Adjusted for genotype in a multiple regression model including histological factors, our study demonstrated an increased risk for ulcer in patients with variant CC genotype of the *AGT* A-20C (p=0.02) as well as *H. pylori* (p=0.04) infection and concurrent use of NSAIDs (p=0.02) (Table III).

Discussions

It has been previously demonstrated that the C allele of the *AGT* A-20C gene polymorphism (rs5050) is associated with an increased AGT production according to genotype (AA < AC < CC) and its plasmatic concentration is progressively higher [14]. Recent studies in this respect started with the observation that co-treatments with ARB are correlated with a decreased number of ulcer in patients treated with LDA [15]. Researches demonstrating the protective effect of ARB treatments for stress-induced gastric injury were previously published [16, 17]. AngII is a stress hormone that regulates vasoconstriction in resistant arteries, which can determine an impairment of gastric blood flow in stress conditions. On the other hand, the presence of higher expression of angiotensin type 1 receptors (AT1R) in *H. pylori* infected compared to

Table III. Model of multivariate analysis of risk factors for gastro-duodenal ulcer in patients treated with low-dose aspirin, including genotype.

Variable	Odds Ratio	95% CI	P
Age	3.10	0.95 to 10.12	0.06
History of ulcer	0.87	0.17 to 4.28	0.87
NSAIDs	1.31	1.07 to 2.27	0.05
Anticoagulants	1.49	0.38 to 5.82	0.56
H. pylori infection	2.40	1.18 to 6.54	0.04
CC genotype of AGT A-20C polymorphism	12.32	1.40 to 108.13	0.02

non-infected patients suggests that RAS components play a role in gastric inflammation [11, 18]. Moreover, AngII seems to aggravate ulceration in animal models [19].

The variant homozygous CC genotype of the *AGT* A-20C gene polymorphism was proved to increase the risk for peptic ulcer bleeding (OR 4.94, 95% CI 1.21–20.2) among Japanese patients treated with aspirin [9]. In our study, variant homozygous CC genotype was associated with ulcer, even after adjustment with the most important known risk factor for GI events. We did not intend to study bleeding risk in the present research, but among our patients with bleeding signs on endoscopy variant homozygous CC genotype was more frequent than in patients with no bleeding suspicion. The predictive value of OR in the present study was not as high as in the Japanese study [9] and therefore should be improved by further research, using an increased number of cases.

Surprisingly, none of the patients with variant homozygous CC genotype of the *AGT* A-20C polymorphism was *H. pylori* positive even when they had a higher number of ulcers than other genotype subgroups; additionally, they presented a more frequent reactive gastropathy in histological samples. On the other hand, wild-type homozygous AA genotype of *AGT* A-20C was statistically significant associated with *H. pylori* gastritis (active or inactive). These observations can suggest the value of variant homozygous CC genotype of the *AGT* A-20C gene polymorphism as an independent risk factor for GI damage in LDA consumers.

The correlation between effects of *H. pylori* infection and aspirin in the upper digestive tract is still a matter of debate due to the controversial study results [4]. Our study proved the role of infection in mucosal damage in aspirin consumers. In our study, more than 50% of patients with ulcer were *H. pylori* positive, a percentage higher than in the majority of similarly published papers [20]. Concurrent use of NSAIDs, frequently with no medical prescriptions (data not showed), was a significant risk factor for ulcer, in our study. Combined therapy with LDA and non-aspirin NSAIDs in aging patients seems to play an important role in GI bleeding prevalence worldwide [22]. The predictive value of other factors, like history of peptic ulcer or dual antiplatelet therapy was not so high, probably due to the lower number of cases.

To the best of our knowledge, the present study is the first one to investigate the role of possible genetic molecular factors for ulcer occurrence in our population, correlated with clinical and histological characteristics. Our observation regarding the correlation between gastric histological changes (gastric inflammation and reactive gastropathy) and homozygous AA as well as CC genotypes of the *AGT* A-20C gene polymorphism in LDA consumers is the first one in literature. The reduced number of patients

treated with ARB and ACE inhibitors did not allow us to study the protective GI effect of these drugs. Our study investigated the value of clinical and histological risk factors usually studied in populations with different characteristics to ours, namely in Western or Asian populations, which can be of a lesser value for our population. Our results can offer directions for further studies more prone to develop preventive strategies for GI bleeding in LDA consumers.

One of the limitations of our study was the reduced number of cases that could not allow us to draw a firm conclusion regarding ulcer occurrence in a specific genotype. The moderate number of samples did not enable us to clarify the role of studied risk factors for GI bleeding, a more important clinical end-point. The possible bias of recruited patients was not completely ruled out, as our study was a case-control one. The compliance of patients for low-dose aspirin therapy in secondary cardiovascular prevention may be on debate, but the use of an interview and medical records of the patients diminished this risk. A further work, with a larger number of included patients may overrule the limitations of the present study.

Conclusions

Variant C allele and variant homozygous CC genotype of the *AGT* A-20C gene polymorphism, concomitant with *H. pylori* infection and NSAIDs co-treatment are risk factors for gastro-duodenal ulcer occurrence in low-dose aspirin consumers. The homozygous AA genotype patients treated with LDA are more prone to have *H. pylori* gastritis in biopsy samples, while those with CC genotype of the *AGT* A-20C gene polymorphism to have reactive gastropathy and bleeding ulcers in a population with a high prevalence of *H. pylori* infection.

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