Роль и значение ряда полиморфизмов генов у пациентов с аневризмой брюшной аорты

Egan L. Kalmykov1, И. А. Сучков2, Р. Е. Калинин2, О. Нематзода3, Д. С. Додхоев4

1 Clinic for Vascular and Endovascular Surgery, Theodor Fontaine Medical Institute, Brandenburg, Germany;
2 Рязанский государственный медицинский университет имени академика И. П. Павлова, Рязань, Российская Федерация;
3 Республикалинский научный центр сердечно-сосудистой хирургии, Душанбе, Республика Таджикистан;
4 Таджикский государственный медицинский университет имени Абуали ибни Сино, Душанбе, Республика Таджикистан

АННОТАЦИЯ

Введение. До настоящего времени многие факторы, влияющие на риск и течение развития аневризмы брюшной аорты (АБА), являются неизученными. Все большее значение в этиологии и развитии АБА придается наличию некоторых генетических полиморфизмов, роль многих из которых также не изучена.

Цель. Проанализировать наличие ассоциации аневризмы брюшной аорты с рядом полиморфизмов генов (ПГ).

Материалы и методы. Проанализированы ПГ у 20 пациентов с АБА (исследуемая группа, ИГ; 18 мужчин (90%) и 2 женщины (10%), средний возраст — 68,1 ± 7,3 года) и у 5 пациентов без АБА (контрольная группа, КГ; 4 мужчины (80%) и 1 женщина (20%), средний возраст — 64,2 ± 7,2 года). Определялась частота сопутствующих заболеваний и факторов риска АБА. Изучены ПГ: Lys198Asn в гене EDN1; С-786Т в гене NOS3; Leu28Pro в гене APOE; Val174Ala в гене SLC01B1; Thr715Pro в гене SELP; C807T в гене ITGA2; Ser447Ter в гене LpL; Thr174Met в гене AGT; Met235Thr в гене AGT. Статистический анализ проводили с помощью «IBM SPSS Statistics 21», корреляционный анализ проводили по Пирсону. Результаты считаются статистически значимыми при p < 0,05.

Результаты. В ИГ корреляционные связи были выявлены при полиморфизме Ser447Ter в гене LpL: прямые связи с полиморфизмом Lys198Asn (r = 0,63; p < 0,001) в гене EDN1, Leu28Pro (r = 0,70; p < 0,001) в гене APOE и Thr715Pro (r = 0,63; p < 0,001) в гене SELP; обратная связь с полиморфизмом C786T (r = 0,35; p = 0,006) в гене NOS3. Столько же связей у полиморфизма Leu28Pro в гене APOE: наряду с Ser447Ter в гене LpL ещё имеются прямая связь с Lys198Asn (r = 0,70; p < 0,001) в гене EDN1 и Thr715Pro (r = 0,63; p < 0,001) в гене SELP; обратная связь с C786T (r = 0,35; p = 0,006) в гене NOS3. У полиморфизма Thr715Pro в гене SELP также наряду со связями Ser447Ter (r = 0,63; p < 0,001) в гене LpL и Leu28Pro в гене APOE имеется дополнительно прямая связь с Lys198Asn (r = 0,55; p < 0,001) в гене EDN1. У полиморфизма Thr174Met в гене AGT имеется обратная связь с Leu28Pro (r = 0,35; p = 0,006) в гене APOE и обратная связь с Val174Ala в гене LpL (r = 0,40; p = 0,002) в гене SLC01B1. При этом у полиморфизма Met235Thr в гене AGT имеется прямая связь с Val174Ala (r = 0,33; p = 0,011) в гене SLC01B1 и обратная связь с C807T в гене ITGA2.

Заключение. Установлено наличие прямых корреляций некоторых полиморфизмов генов у пациентов с аневризмой брюшной аорты, что указывает на их возможную роль в развитии данной патологии и может являться скрининговым тестом для определения вероятности ее развития.

Ключевые слова: аневризма брюшной аорты; полиморфизмы генов; корреляция полиморфизмов; генетика аневризмы брюшной аорты

The Role and Significance of Polymorphisms of Certain Genes in Patients with Abdominal Aortic Aneurysm

Egan L. Kalmykov1, Igor’ A. Suchkov2, Roman E. Kalinin2, Okildzhon Ne’matzoda3, Dzhamsheed S. Dodkhoyev4

1 Clinic for Vascular and Endovascular Surgery, Theodor Fontaine Medical Institute, Brandenburg, Germany; 2 Ryazan State Medical University, Ryazan, Russian Federation; 3 Republican Scientific Center for Cardiovascular Surgery, Dushanbe, Republic of Tajikistan; 4 Avicenna Tajik Medical University, Dushanbe, Republic of Tajikistan

ABSTRACT

INTRODUCTION: To date, many factors that influence the risk and course of abdominal aortic aneurysm (AAA) are not studied. Increasing significance in the etiology and development of AAA is assigned to the existence of some genetic polymorphisms, the role of many of them is not studied either.

AIM: To analyze the existence of association of the abdominal aortic aneurysm with some gene polymorphisms (GPs).

MATERIALS AND METHODS: Gene polymorphisms were analyzed in 20 patients with AAA (study group, SG); 18 men (90%) and 2 women (10%), the mean age 68.1 ± 7.3 years), and in 5 patients without AAA (control group, CG; 4 men (80%) and 1 woman (20%), the mean age 64.2 ± 7.2 years). The frequency of concomitant diseases and risk factors for AAA were determined. The following GPs were studied: Lys198Asn in the EDN1 gene; C-786T in the NOS3 gene; Leu28Pro in the APOE gene; Val174Ala in the SLC01B1 gene; Thr715Pro in the SELP gene; C807T in the ITGA2 gene; Ser447Ter in the LpL gene; Thr174Met in the AGT gene; Met235Thr in the AGT gene. Statistical analysis was performed using IBM SPSS Statistics 21, correlation analysis — according to Pearson. The results were considered statistically significant at p < 0.05.

RESULTS: In the SG, correlation relationships were identified in Ser447Ter polymorphism in the LpL gene: direct relationships with Lys198Asn polymorphism (r = 0.63; р < 0.001) in the EDN1 gene, Leu28Pro (r = 0.70; р < 0.001) in the APOE gene and Thr715Pro (r = 0.63; р < 0.001) in the SELP gene; a reverse relationship with C786T polymorphism (r = -0.35; р = 0.006) in the NOS3 gene. The same amount of relationships were found in Leu28Pro polymorphism in the APOE gene: besides with Ser447Ter in the LpL gene, there is also a direct relationship with Lys198Asn (r = 0.70; р < 0.001) in the EDN1 gene and Thr715Pro (r = 0.63; р < 0.001) in the SELP gene; a reverse relationship with C786T (r = -0.35; р = 0.006) in the NOS3 gene. Thr715Pro polymorphism in the SELP gene, along with relationships with Ser447Ter (r = 0.63; р < 0.001) in the LpL gene and Leu28Pro in the APOE gene, has an additional direct relationship with Lys198Asn (r = 0.55; р < 0.001) in the EDN1 gene. Thr174Met polymorphism in the AGT gene has a reverse relationship with Leu28Pro (r = -0.35; р < 0.006) in the APOE gene and direct relationship with Val174Ala (r = 0.40; р = 0.002) in the SLC01B1 gene. With this, Met235Thr polymorphism in the AGT gene has a direct relationship with Val174Ala (r = 0.33; р = 0.011) in the SLC01B1 gene and reverse relationship with C807T in the ITGA2 gene.

CONCLUSION: The existence of direct correlations of some gene polymorphisms in patients with abdominal aortic aneurysm has been established, which indicates their probable role in the development of this pathology and may be used as a screening test for determination of the likelihood for its development.

Keywords: abdominal aortic aneurysm; gene polymorphism; correlation of polymorphisms; genetics of abdominal aortic aneurysm

INTRODUCTION

Despite a long history of investigation of the etiopathogenesis of the abdominal aortic aneurysm (AAA), many factors that influence the risk and course of the disease, have not been studied up to the present moment. It has been shown in some works that patients with AAA have a number of concomitant diseases mostly associated with disorders of lipid metabolism, vascular endothelial dysfunction, arterial hypertension, diabetes mellitus [1, 2]. However, their role in the pathogenesis of AAA is still being studied, and the results are controversial. Besides, increasing significance in the etiology and pathogenesis of AAA is assigned to some genetic polymorphisms, especially to the risk factors of the development of AAA [3–12]. With this, very few scientific works are devoted to study of gene polymorphisms (GPs) playing a definite role in the development of a number of concomitant pathologies in AAA. In this context, we studied some GPs in patients with AAA in the aspect of their probable influence on the pathogenesis of the disease.

The aim of this study was analyze the existence of association of the abdominal aortic aneurysm with some gene polymorphisms.

MATERIALS AND METHODS

The study was approved by the local Ethics Committee of Pavlov Ryazan State Medical University (Protocol No. 11 of 2021, May 11) and registered on ClinicalTrials.gov platform. All the patients signed a written informed consent to participate in this study.

GPs were analyzed in 20 patients with AAA (study group) and in 5 patients without AAA (control group). Of the total number of patients with AAA (study group) there were 18 (90%) of men, 2 (10%) women. The control group included 4 (80%) men and 1 (20%) woman. The mean age was 68.1 ± 7.3 and 64.2 ± 7.2 years in the study group and the control group, respectively.

In the study group there were 17 (85%) smokers, the main concomitant diseases were:
- coronary heart disease (CHD) in 11 (55%) patients;
- diabetes mellitus in 1 (5%) patient;
- carotid artery atherosclerosis/stroke in 4 (20%) patients;
- peripheral artery diseases in 9 (45%) patients;
- arterial hypertension (AH) in 18 (90%) patients;
- aneurysms of other locations in 6 (30%) patients;
- chronic obstructive pulmonary disease in 1 (5%) patient;
- arrhythmia in 4 (20%) patients.

In the control group of 5 volunteers, there was only one case of AH, no other concomitant diseases were identified.

The genetic status of the patients was studied by a molecular genetic method. Blood was taken from the peripheral vein. The genomic DNA was isolated from the whole blood leukocytes using ‘DNA-ekspresskrov’ reagent (Litekh, Russian Federation) and was analyzed. With the sample of isolated DNA two amplification reactions were performed with two pairs of allele-specific primers, and three conclusions were made: homozygosity for allele1, heterozygosity, homozygosity for allele 2. The choice of genes was based on the integral approach used in the analysis of the etiology and pathogenesis of AAA [3–14].

Polymorphisms of the following genes were analyzed:
- lysine198asparagine (Lys198Asn) in the endothelin-1 (EDN1) gene;
- Ser-786Ter in the nitric oxide synthase 3 (NOS3) gene;
- leucine28proline (Leu28Pro) in the apolipoprotein E (APOE) gene;
- valin174alanin (Val174 Ala) in the gene of solute carrier organic anion transporter family member 1B1 (SLC01B1);
- tryptophan715 proline (Thr715Pro) in the P-selectin (SELP) gene;
- C807T in the integrin alpha-2 (ITGA2) gene;
- serin 447 termination codon (serin447Ter) in the lipoprotein lipase (LpL) gene;
- tryptophan174methionine (Thr174Met) in the angiotensin 1 (AGT) gene;
- Met235Thr in the AGT gene.

Statistical analysis was performed on a PC using IBM SPSS Statistics 21 (IBM Corp., 1989–2012, USA). In the work, qualitative parameters (risk factors and alleles) are presented as fractions. Qualitative parameters were compared using the chi square ($\chi^2$) test for arbitrary tables. Method of logistic regression (the results are given in the form of odds ratio (OR) with confidence interval (CI)), and Pearson correlation analysis (the result is presented as correlation coefficient, r) were used. The differences between the groups were considered statistically significant at $p < 0.05$.

**RESULTS**

Comparison of the frequency of occurrence of homo- and heterozygotes in the study groups are given in Table 1.

Table 1. Frequency of Homozygous and Heterozygous Alleles in the Study and Control Groups

<table>
<thead>
<tr>
<th>Polymorphism in Gene</th>
<th>Group</th>
<th>Homozygote for Allele 1, % (n)</th>
<th>Heterozygote, % (n)</th>
<th>Homozygote for Allele 2, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys198Asn in the EDN1 gene</td>
<td>Study group, n = 20</td>
<td>80 (16)</td>
<td>15 (3)</td>
<td>5 (1)</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>60 (3)</td>
<td>40 (2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = 1.71$)</td>
<td></td>
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</tr>
<tr>
<td>C-786T in the NOS3 gene</td>
<td>Study group, n = 20</td>
<td>10 (2)</td>
<td>55 (11)</td>
<td>35 (7)</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>0</td>
<td>100 (5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = 3.52$)</td>
<td></td>
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<tr>
<td>Leu28Pro in the APOE gene</td>
<td>Study group, n = 20</td>
<td>100 (20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>100 (5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = NaN$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Val174Ala in the SLC01B1 gene</td>
<td>Study group, n = 20</td>
<td>40 (8)</td>
<td>60 (12)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>60 (3)</td>
<td>40 (2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = NaN$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr715Pro in the SELP gene</td>
<td>Study group, n = 20</td>
<td>75 (15)</td>
<td>20 (4)</td>
<td>5 (1)</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>60 (3)</td>
<td>40 (2)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = 1.04$)</td>
<td></td>
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<tr>
<td>C807T in the ITGA2 gene</td>
<td>Study group, n = 20</td>
<td>25 (5)</td>
<td>55 (11)</td>
<td>20 (4)</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>40 (2)</td>
<td>60 (3)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = 1.34$)</td>
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</tr>
<tr>
<td>Ser447Ter in the LpL gene</td>
<td>Study group, n = 20</td>
<td>80 (16)</td>
<td>20 (4)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>100 (5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = NaN$)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thr174Met in the AGT gene</td>
<td>Study group, n = 20</td>
<td>10 (2)</td>
<td>90 (18)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>0</td>
<td>100 (5)</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = NaN$)</td>
<td></td>
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</tr>
<tr>
<td>Met235Thr in the AGT gene</td>
<td>Study group, n = 20</td>
<td>40 (8)</td>
<td>40 (8)</td>
<td>20 (4)</td>
</tr>
<tr>
<td></td>
<td>Control group, n = 5</td>
<td>60 (3)</td>
<td>20 (1)</td>
<td>20 (1)</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>$&gt; 0.05$ (df = 2; $\chi^2 = 0.81$)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Interesting data were obtained in the correlation analysis (Figures 1, 2).

From the data presented in Figures 1 and 2 it follows that significant correlations of GPs coincided in the study and control groups for the interrelation of Ser447Ter in the LpL gene and Leu28Pro (r = 0.70; p < 0.001) in the APOE gene.

In the study group of patients, the correlation relationships were identified with Ser447Ter polymorphism in the LpL gene:
- direct correlation with Lys198Asn (r = 0.63; p < 0.001) in the EDN1 gene, Leu28Pro (r = 0.70; p < 0.001) in the APOE gene and Thr715Pro (r = 0.63; p < 0.001) in the SELP gene;
- inverse correlation with C807T (r = -0.35; p = 0.006) in the NOS3 gene.

Similar results were obtained for Leu28Pro polymorphism in the APOE gene:
- direct correlation with Ser447Ter in the LpL gene, Lys198Asn (r = 0.70; p < 0.001) in the EDN1 gene and Thr715Pro (r = 0.63; p < 0.001) in the SELP gene;
- inverse correlation with C786T (r = -0.35; p = 0.006) in the NOS3 gene.

For Thr714Met polymorphism in the AGT gene there were obtained:
- direct correlation with Val174Ala (r = 0.40; p = 0.002)

Note: OR — odds ratio CI — confidence interval. Influence of the parameters was determined by OR calculated by logistic regression method, and the dependence between parameters — by Pearson correlation analysis.
Fig. 1. Results of Pearson correlation analysis of gene polymorphisms in patients with abdominal aortic aneurysm (study group).  
*Note:* the first number — correlation coefficient (r), the second number — statistical significance (p).

Fig. 2. Results of Pearson correlation analysis of gene polymorphisms in patients without abdominal aortic aneurysm (control group).  
*Note:* the first number — correlation coefficient (r), the second number — statistical significance (p).

in the *SLC01B1* gene;  
- **inverse correlation** with Leu28Pro (r = -0.35; p = 0.006) in the *APOE* gene.

To note, polymorphism Met235Thr in the *AGT* gene is:  
- **in direct correlation** with Val174Ala (r = 0.33; p = 0.011) in the *SLC01B1* gene;  
- **in inverse correlation** with C807T in the *ITGA2* gene.  

In the control group, the following correlation relationships were identified:  
- C807T polymorphism in the *ITGA2* gene directly correlates with Lys198Asn (r = 0.70; p = 0.004) in the *EDN1* gene and Val174Ala in the *SLC01B1* gene.  
- Thr174Met polymorphism in the *AGT* gene directly correlates with Ser447Ter in the *LpL* gene and Leu28Pro (r = 1.00; p < 0.001) in the *APOE* gene.

**DISCUSSION**

The analysis of the presented data revealed statistically significant differences in the dominance of homozygous and heterozygous alleles in the main and control groups, which probably influences the occurrence of AAA. There was also found the absence of polymorphism for the 2nd allele in the control group except for the *AGT* gene (Met235Thr polymorphism). In the meantime, there is little information in the literature about dominance of alleles, their role in the development of AAA and related risk factors.
the authors made a suggestion about the association of race and in the population of East Asia. Nevertheless, risk of ischemic stroke, especially of atherosclerotic stroke polymorphism was associated with a considerably lower in the dependence of Ser447Ter in the gene between the study and control groups coincided in therapy including ACE inhibitors.

the patients [2], and only a part of them received combined AH and CHD in patients with AAA reached 80% and 77%, published by us, is was established that the frequency of Met235Thr genotype of angiotensinogen [8]. This aspect associated with the use of ACE inhibitor, did not depend on Thr235 and Thr174 was associated with 10% increase in the level of angiotensin in plasma in both genders compared to homozygosity for Met235 and Thr174 [6].

J. A. Staessen, et al. showed in their work that, compared to MM homozygotes, TT homozygotes and M heterozygotes had excessive risk for AH in 31% and 11% of cases, respectively [7]. As noted in the work of J. C. Bis, et al., in patients with AH receiving pharmacological therapy, angiotensinogen genotype modified relationship of angiotensin converting enzyme (ACE) inhibitors with the development of stroke, and the risk of stroke associated with use of ACE inhibitor in participants with ThrThr genotype (OR 0.37; 95% CI 1.02–1.84) and moderate elevation of arterial pressure (OR 40; 95% CI 1.10–1.77). Here, the authors did not reveal any statistically significant correlation between the elevated arterial pressure and genotype in men or between genotype and systolic arterial pressure, diastolic arterial pressure or pulse pressure in both genders. Homozygosity both for Thr235 and Thr174 was associated with 10% increase in the level of angiotensin in plasma in both genders compared to homozygosity for Met235 and Thr174 [6].

Lys198Asn polymorphism of the EDN1 gene with increased risk of ischemic stroke [9]. Correlation of these parameters in our study in the patients with AAA may evidence increased risk of cardiovascular complications, but requires further study. To add, in patients of the main group atherosclerosis of brachiocephalic arteries/stroke was found in 20% of cases.

An important direct correlation of Leu28Pro in the APOE gene and Thr715Pro in the SELP gene was obtained. Here it is necessary to mention the results of meta-analysis by G. Herrera–Maya, et al. [10], which provide empirical evidence that genetic polymorphisms of SELP may promote development of CHD, in particular, myocardial infarction. Thus, genetic polymorphisms of SELP may be potential and practical biomarkers for early diagnosis of CHD and myocardial infarction. In this connection it should be noted that in the group with the obtained correlations, CHD was diagnosed in 11 (55%) cases.

The data obtained by us demonstrate that along with Ser447Ter in the Lpl gene there is also a direct correlation with Lys198Asn in the EDN1 gene and Thr715Pro in the SELP gene, which, according to some studies, is associated with diabetes mellitus and development of stroke [10, 11]. At the same time, there exist rather controversial data on the influence of diabetes on the pathogenesis of AAA [2].

Thr174Met polymorphism in the AGT gene directly correlates with Val174Ala in the SLC01B1 gene. According to A. Kalliokoski, et al., genetic variability of genes can lead to the interindividual differences in the pharmacokinetics. In particular, single-nucleotide polymorphism (c.521T > C, p.Val174Ala) in the SLC01B1 gene encoding the organic anion transporting polypeptide, 1B1 (OATP1B1), reduces the ability of OATP1B1 to transport the active simvastatin acid to the liver leading to increase in its concentration in plasma, which, in turn, increases the risk of development of simvastatin-induced myopathy. Besides, it is shown in the same review that SLC01B1 polymorphism also affects pharmacokinetics of many statins and of repaglinide anti-diabetic drug, that are used in treatment of atherosclerosis and diabetes in patients with AAA to reduce the risk of cardiovascular complications. With that, Met235Thr polymorphism in the AGT gene has a direct relationship with Val174Ala in the SLC01B1 gene [12].

**CONCLUSION**

Based on the results of our study, statistically significant differences in the dominance of homozygous and heterozygous alleles in the main and control groups were established. The existence of direct correlations of some polymorphisms of a number of genes in patients with abdominal aortic aneurysm has been established, which shows their probable role in the development of this pathology and may be a screening test for determination of the probability for its development.
**ADDITIONAL INFORMATION**

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**Contribution of the authors:** E. L. Kalmykov — study concept and design, statistical analysis, data analysis and interpretation, text writing; I. A. Suchkov — the concept and design of the study, checking the critical intellectual content of the work, final approval for publication of the manuscript; R. E. Kalinin — concept and design of the study, verification of the critical intellectual content of the work, final approval for publication of the manuscript; G. Ne’matzoda — analysis and interpretation of data; O. S. Dodkhoyev — statistical analysis, analysis and interpretation of data. All authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

**REFERENCES**


**REFERENCES**


