

## R117H MUTATION OF THE GENE ENCODING THE CYSTIC FIBROSIS TRANSMEMBRANE REGULATORY PROTEIN IN PATIENTS WITH CHRONIC RELAPSING PANCREATITIS

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Received 24 Sept 2019, Accepted 07 November 2019

<https://doi.org/10.31688/ABMU.2019.54.4.05>

### ABSTRACT

**Introduction.** Chronic pancreatitis (CP) is a variable part of cystic fibrosis (CF) syndrome caused by mutations in CFTR gene.

**The objective of the study** was to assess the frequency of CFTR gene mutations in patients with chronic relapsing pancreatitis (CRP).

**Material and methods.** The study enrolled 41 patients with CRP and control group (CG), which consisted of 100 healthy people. The R117H mutation of the CFTR gene was confirmed in the Molecular Genetics Laboratory of the Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences of Moldova. As a biological specimen, venous blood was used. The genetic polymorphism was identified through the polymerase chain reaction and analysis of enlarged fragment length and restriction fragment length polymorphism (RFLP), with the use of the respective primers.

**Results.** The study detected the presence of the R117H/CFTR mutation in 31 (75.61%) of CRP patients and in 53 (53%) healthy persons from CG. A more significant difference was demonstrated when evaluating the ratio between homozygous and heterozygous variant of R117H mutation. In the group

### RÉSUMÉ

La mutation R117H du gène codant pour la protéine régulatrice transmembranaire de la fibrose kystique chez des patients atteints de pancréatite chronique récurrente dans la République de Moldova

**Introduction.** La pancréatite chronique (PC) est une partie variable du syndrome de la fibrose kystique, causée par les mutations dans le gène CFTR.

**L'objectif de l'étude** a été l'évaluation de la fréquence de la mutation CFTR chez les patients atteints de pancréatite chronique récurrente (PCR).

**Méthodes.** L'étude comprend 41 patients avec PCR et le lot de contrôle (LC), qui a été composé de 100 personnes en bonne santé. La mutation R 117 H du gène CFTR a été confirmée dans le laboratoire de Génétique Moléculaire de l'Institut de Physiologie et de protection des Plantes de l'Académie des Sciences de Moldavie. Comme épreuve biologique, on a utilisé le sang veineux. Le polymorphisme génétique a été identifié par la réaction de la polymérisation en chaîne et l'analyse polymorphique et de la longueur du fragment de restriction, à l'aide de premiers respectifs.

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of the CRP patients, detected with the respective mutation, 11 (35.48%) had homozygous variant and 20 (64.52%) – heterozygous variant; in CG – in 11 (20.75%) persons the homozygous variant and in 42 (79.25%) – the heterozygous variant of the mutation have been confirmed. According to literature data, a high frequency of R117H mutation in heterozygous variant represents a higher risk of developing pancreatic pathology. The predominant CRP installation was confirmed at a young age of 25-34 years (48.8%).

**Conclusions.** The high frequency of R117H/CFTR mutation in the heterogeneous population of the Republic of Moldova, in combination with other genetic and nongenetic risk factors, represents a high degree of risk for the development of pancreatic disorders.

**Keywords:** chronic pancreatitis, cystic fibrosis, transmembrane conductance mutation R117H/CFTR.

**Résultats.** L'étude a détecté la présence de la mutation R117H chez 31 (75,61%) des patients avec PCR et chez 53 (53%) des personnes saines. Une différence plus significative a été démontrée à l'évaluation du rapport de la variante homozygote et hétérozygote de la mutation R 117 H/CFTR.

Dans le groupe de patients atteints de CRP détectés avec la mutation respective 11 (35,48%) avaient une variante homozygote et 20 (64,52%) une variante de type hétérozygote; chez les personnes LC-11 (20,75%) la variante homozygote a été confirmée et chez 42 (79,25%) – la variante hétérozygote de la mutation. Selon les données de la littérature, la fréquence élevée de la mutation R117H dans la variante hétérozygote représente un risque plus élevé de développer une pathologie pancréatique. L'installation prédominante de CRP a été confirmée chez les 25 à 34 ans (48,8%).

**Conclusions.** La fréquence élevée de la mutation R117H/CFTR dans la population hétérogène de la République de Moldova en combinaison avec d'autres facteurs de risque génétique et non génétique représente un degré élevé de risque pour le développement des affections pancréatiques.

**Mots-clés:** pancréatite chronique, fibrose kystique, conduction transmembranaire, mutation R117H/CFTR.

## INTRODUCTION

Chronic relapsing pancreatitis (CRP) is a disease of recurring acute episodes of severe upper abdominal pain, which are progressive and gradually may become severe and frequent; fibrosis or necrosis and atrophy are the constant chronic changes; pancreatic lithiasis, pseudocysts and cysts are less frequent evidence of chronic damage.

The incidence of chronic pancreatitis (CP) at 100,000 inhabitants is estimated to be about 7-10 persons<sup>1</sup>, the highest being in India – up to 114-200/100,000 inhabitants<sup>2,3</sup>. In the Republic of Moldova, the incidence of pancreatic diseases in 2018 was 233 cases/100.000 inhabitants<sup>4</sup>.

The prevalence of CP/100,000 inhabitants varies between 0.4 and 26.4 in Europe, in Asian countries being higher<sup>2</sup>: in Poland-17<sup>5</sup>, Denmark-10<sup>6</sup>, France-26.47<sup>7</sup>, Japan-36.9<sup>8</sup>, China 13.52<sup>9</sup>, reaching 126 in India<sup>10</sup>. In the Republic of Moldova, the prevalence of pancreatic diseases has increased obviously compared to the prevalence of other gastrointestinal diseases (about 2 times) from 2005 to 2018: from 1069 to 2173/100.000 inhabitants<sup>4</sup>.

Extensive studies in the recent decades have shown that CP is a variable part of cystic fibrosis caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (CFTR). CF is an

autosomal-recessive disorder, which mainly affects Caucasians, with an incidence of about 1: 3000 live births, 1 in 25 people in this population carrying CFTR mutations<sup>11</sup>. In 1989 CFTR was identified as a basic gene, and in 1998 Sharer et al and Cohn et al have shown an association of CFTR mutations with CP<sup>12,13</sup>. The impact of CFTR remains debatable, and the role of gene variants in CP pathogenesis is obviously strong. The variety of pancreatic disorders in this gene mutation differs greatly, from complete loss of pancreatic function to normal pancreatic function. The gene encoding CFTR is located on the long arm of chromosome 7: 7q31, extends approximately 250 kb and contains 27 exons from the base pair 116,907,253 to the base pair 117,095,955<sup>14</sup>. CFTR (also known as ABCC7) encodes a transmembrane protein on the surface of most epithelial cells and functions as an AMF-cyclic channel, which allows certain anions to pass through the cell wall (Cl, HCO<sub>3</sub><sup>-</sup>, etc) due to their electrochemical gradient<sup>15-17</sup>, being at the same time regulator of other ion channels, e.g. the Epithelial sodium channel (ENaC-epithelial sodium channel) and a group of bicarbonate transporters (SLC26)<sup>18</sup>. In the case of mutations in the CFTR, the reduction of the pancreatic fluid flow occurs, with hyper concentration of proteins and blocking of the pancreatic ducts<sup>15,18</sup>.

Currently, more than 2000 mutations of CFTR are known<sup>15,19</sup>, but most of them have a frequency <0.1% and are not associated with CF, and in > 85% of them the mechanism of action is unknown<sup>20</sup>. These mutations can be divided into 6 classes, based on the functional consequences of the polymorphism on the channel function: classes I-III are considered severe, class IV-medium variability, mild -V-VI classes<sup>21,22</sup>.

The R117H/CFTR mutation (c.350G> A) refers to class IV, is present in 0,3% of the Caucasian population and can be manifested through a wide variety of clinical manifestations, depending on the presence of other genetic variations, e.g. the Poly-T tract, which represents a series of thymine bases, located in intron 8 of the CFTR gene (3 forms-5T, 7T, 9T) and the TG tract (3 forms-TG11, TG12, TG13), the combinations of these genes resulting in a wide variety of clinical presentations of CF<sup>23</sup>. One study found the R117H mutation in combination with the IVS8-T5 haplotype in 2.3% of CP patients and 0.7% in CG (OR = 3.49; p = 0.0007), and R117H \* T7 / T9 - in 9 of the 80 cases of severe concurrent mutations of CF and not in a patient with CF of CG; association of R117H / CFTR with N34S / SPINK1 in heterozygous form in 5.5% CP patients (OR = 8.74; p = 0.0002)<sup>24</sup>.

Of interest is the fact that the clinical evolution of CF can be variable in patients who undergo the same mutations, indicating the influence of environmental factors and possibly other genetic changes. To date, not all the mechanisms underlying the development of CP in CFTR gene mutations are known. The study of pathologies associated with CFTR mutations is limited by the impossibility of completely investigating the genomic sequence of the CFTR gene in large groups of patients, as this gene encodes 1480 amino acids. Different people, but with the same mutation, may have different degrees of impairment, the manifestations of CF being influenced by the interaction of other genetic factors with the environmental ones.

Studies to date indicate that CFTR mutations alone are not sufficient for CP development in most patients and further studies are needed to elucidate the role of these mutations in CP pathogenesis.

**THE OBJECTIVE OF THE STUDY** was to assess the frequency of CFTR gene mutations in patients with chronic relapsing pancreatitis (CRP).

## MATERIAL AND METHODS

A prospective study was conducted to meet the research objectives. The study enrolled 41 patients with CRP, 17 men (41.46%) and 24 women (58.54%), with a mean age of 45.54±1.74 (19-59) years, hospitalized in the Municipal Clinical Hospital „Sf. Arch. Mihail ” from Chisinau, Republic of Moldova, in the

gastroenterology and surgery departments, between May 2009 and July 2012. The control group (CG) consisted of 100 healthy people, 56 men (56%) and 44 women (44%), with a mean age of 23.23±0.49 (19-39) years. The informed consent was obtained from all the patients included in the study.

Clinical-paraclinical changes, specific to CP, were evaluated according to the recommendations of the European Society of Gastroenterology, the International Pancreatology Association and the National Clinical Protocol. The R117H mutation of the CFTR gene was confirmed in the Molecular Genetics Laboratory of the Institute of Genetics, Physiology and Plant Protection of the Academy of Sciences of Moldova. As a biological specimen, venous blood was used, collected in „Eppendorf“ tubes in a volume of 1.5 ml in the presence of 5 µl of EDTA, which, being thus prepared, can be used immediately or can be frozen at -20° C for long-term storage. The genetic polymorphism was identified through the polymerase chain reaction and analysis of enlarged fragment length and restriction fragment length polymorphism (RFLP), with the use of the respective primers: -forward 117H 5'-ACCCGGATAACAAGGAGGAGG-3'-revers 117H 5'-GGCCTGTGCAAGGAAGTATT-3'.

The statistical analysis was performed using EPIINFO, version 6.0, program of the Center of Disease Control and Prevention in Atlanta-CDC (Center of Disease Control and Prevention) and WHO, adapted to the processing of medical statistics.

## RESULTS

The patients included in the study were divided by age: 14 (35.15%) patients aged between 45-54 years, 12 (29.27%) patients aged between 35-44 years, 10 (24.39%) patients aged between 55-59 years, 3 (7.32%) patients aged between 18-24 years, 2 (4.88%) patients aged between 25-34 years (Fig.1). We observed a greater number of patients aged 45-54 years.

The evaluation of the patients according to the age at onset of the disease showed, predominantly, an onset at the young age of 25-34 years (48.78%), (Fig. 2).

The results (Fig. 3) demonstrate the presence of the R117H/CFTR mutation in 31 (75.61%) patients with CRP and in 53 (53%) of those from the CG.

The results regarding the presence of the R117H mutation in the homozygous and heterozygous variant are of interest. A more significant difference was demonstrated when evaluating the ratio between homozygous and heterozygous variant of R117H mutation (Fig. 4).

In the group of the CRP patients, detected with the respective mutation, 11 (35.48%) had homozygous

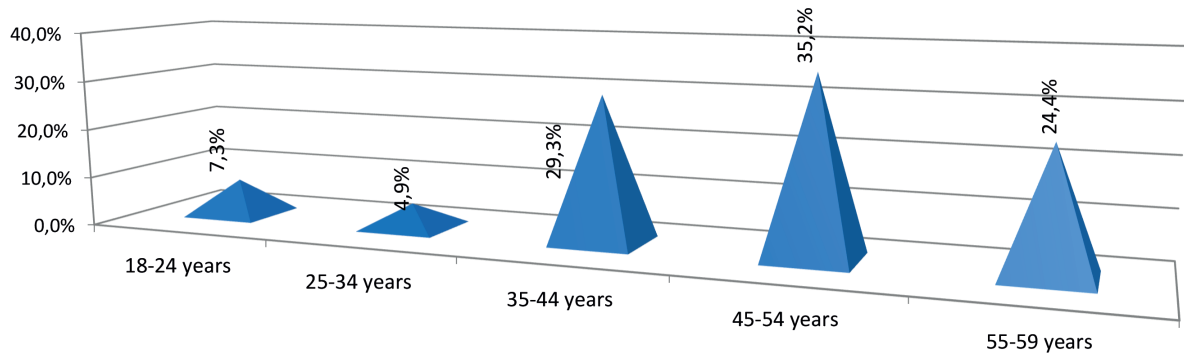


Fig. 1. Distribution of CRP patients by age

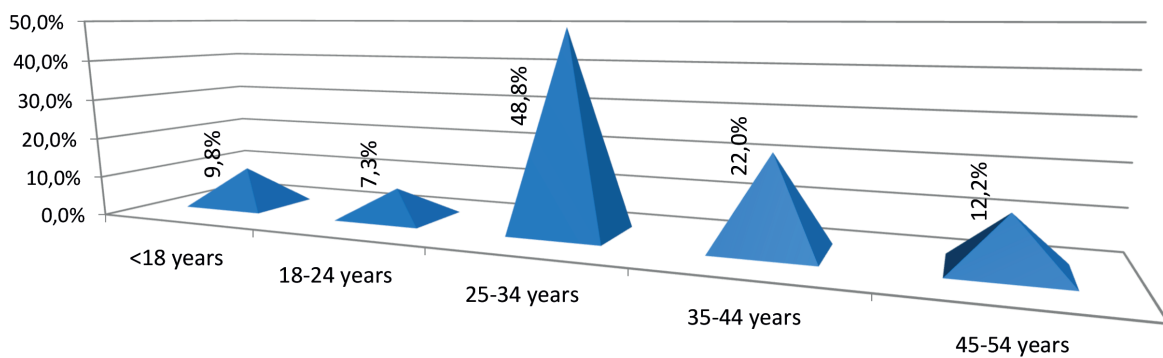


Fig. 2. Distribution of patients with CRP according to the age at onset of the disease

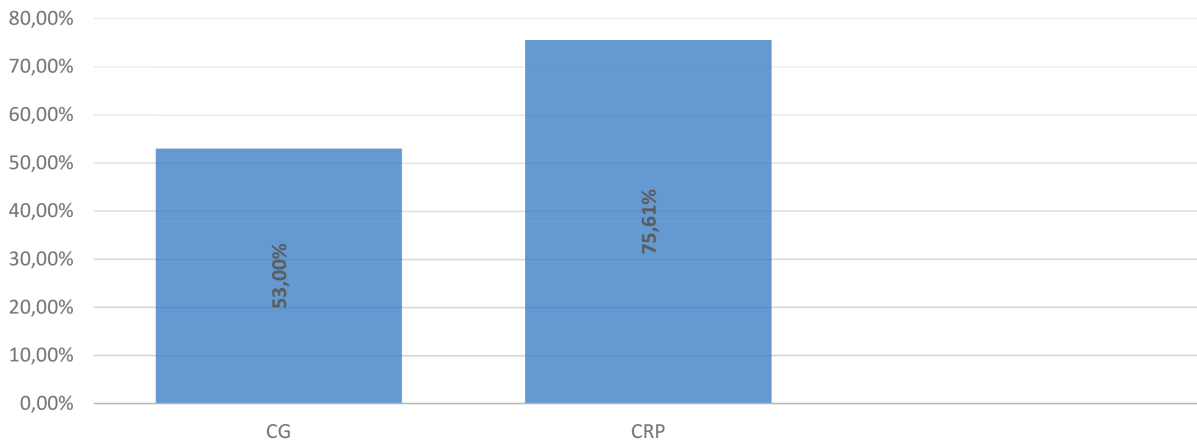


Fig.3. Frequency of R117H mutation (CFTR) in CRP and control group patients.

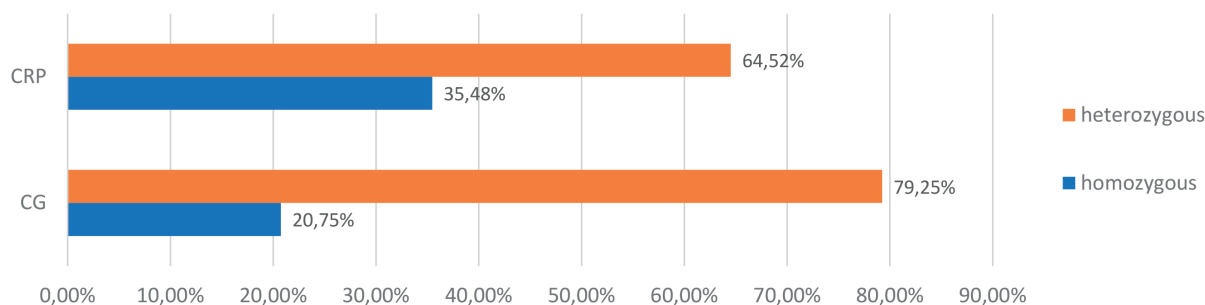
variant and 20 (64.52%) – heterozygous variant; in CG-in 11 (20.75%) persons the homozygous variant and in 42 (79.25%) – the heterozygous variant of the mutation have been confirmed.

## DISCUSSION

The data obtained in our study, compared to the data from the literature, are of interest due to the fact that the presence of the R117H / CFTR mutation

has been confirmed in a big number of persons, both patients with CRP and healthy persons from CG.

It is assumed that patients with isolated CP, in the absence of other clinical manifestations of CF, could carry lighter mutations, V-VI class, in at least one allele. At complete DNA sequencing, 60% of CP patients were diagnosed with CFTR mutations<sup>25,26</sup>. The risk of CP development among heterozygous carriers of CFTR mutations, irrespective of the severity of the mutation, is about 3-4 times higher than in the



**Fig. 4.** Frequency of R117H / CFTR mutations in homozygous and heterozygous variant in CRP patients and control group.

normal population, and for heterozygotes with severe mutations it is 100 times higher; about 8% of the population with CFTR light mutations carries an increased risk for CP. Most patients who develop genetically determined pancreatitis are carriers of several gene variants or support epistatic interaction between several genes. Significant epistasis has been demonstrated between CFTR and SPINK1 mutations<sup>27</sup>; one study<sup>26</sup> has elucidated that SPINK1 mutations create conditions for heterozygous CFTR mutations to affect the pancreas. The combination of 2 CFTR mutations and the N34S/SPINK1 mutation increases the risk for pancreatitis by 900 times<sup>28</sup>.

Of interest is the fact that the clinical evolution of CF can be variable in patients who carry the same mutations, indicating the influence of environmental factors and possibly other genetic and nongenetic (endogenous and environmental) factors.

Other genetic risk factors (R122C/PRSS1, N34S/SPINK1) and nongenetic ones (alcohol, smoking, nutrition factors, pancreatic medication, dyslipidemias, biliary, duodenal and pancreatic duct pathology) were evaluated in the clinical-genetic study conducted in the Republic of Moldova, which have been found to occur quite frequently in patients with CRP. And most non-genetic risk factors were more frequently detected in patients with CP with genetic mutations, possibly favoring the development and evolution of pancreatic disease.

According to literature data, patients with hereditary predisposition develop disorders of the pancreas at a younger age, a fact confirmed by our study - in 48.8% of the patients clinical signs of pancreatitis appeared at the age of 25-34 years.

Increased frequency of R117H/CFTR mutation both in patients with CRP and in the healthy persons from the Republic of Moldova requires the attention of the doctors in order to exclude or diminish the other modifiable risk factors for CP.

## CONCLUSIONS

The performed genetic study revealed a rather high frequency of the R117H mutant of the CFTR gene, especially in the patients with chronic relapsing pancreatitis vs control group, in the heterozygous variant. The high frequency of R117H/CFTR mutation in the heterogeneous population of the Republic of Moldova, in combination with other genetic and nongenetic risk factors, represents a high degree of risk for the development of pancreatic disorders.

## Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

“The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

„No funding for this study“

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