„BABEŞ-BOLYAI" UNIVERSITY
CLUJ NAPOCA

MOLECULAR GENETIC STUDIES OF VARIABILITY IN HUMAN NORMAL AND PATHOLOGICAL CASES (SCHIZOPHRENIA)

Summary of PhD Thesis

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CONTENT

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INTRODUCTION

The most spectacular scientific discoveries in the current era has been made in genetics, the identification of DNA (genetic fingerprint) being one of them.

Tandem repeated DNA sequences, which are spread throughout the human genome, polymorphic in nature, are important genetic markers for genetic mapping studies, for disease diagnosis and human identity testing. Being a type of repeated DNA sequences, STRs (Short tandem repeats) contains 2–6 bp repeat units, and can be amplified using polymerase chain reaction (polymerase chain reaction, PCR).

Single nucleotide polymorphisms in certain genes cause changes in proteins that trigger diseases. Thus, a single nucleotide polymorphism in the gene that encodes methylenetetrahydrofolate reductase (MTHFR) with a central role in folate cycle, causes a phenotype characterized by affecting the nervous and vascular system.

This PhD thesis presents the genotyping methods used in human genetic fingerprinting and DNA sequencing methods to detect single nucleotide polymorphisms involved in schizophrenia. A study of a group of 214 individuals taken from the general population was used to establish a DNA data base. Our data were compared with similar studies carried out on samples from Romania and other countries. After sequencing DNA samples collected from patients and control group, we studied the correlation between the two single nucleotide polymorphisms in methylenetetrahydrofolate reductase gene and schizophrenia.

I. The importance of genetic methods in medicine

Medical Genetics studies the genetic history of family, genetic risk determination, providing genetic advice in preventing and diagnosing genetic diseases. Genetic family history can identify risk of developing mono-and multifactorial diseases.

II. Human prints

Man can create on the spot shape material traces and leave biological evidence.

In the traces category there are: hand prints, footprints, lip prints, toothprints, earprints, nose prints and prints of other parts of the human body, traces of voice, writing and speech, and manner of execution of nodes and links.

Material traces of human nature are called biological evidence, In general, they did not dress well defined, except bones, nails and hair.

III. Human genetic fingerprint

Just as digital fingerprinting, which is used in police laboratories since 1930s, each person has a unique DNA fingerprint. Unlike fingerprint that appears on the top (pads) of the fingers and can be altered by surgery, DNA fingerprint is the same for every cell, tissue and organ of a person and can not be changed by known treatments. Thus, human genetic fingerprint is fast becoming a primary method to identify and distinguish between humans (Church, 2006).

DNA fingerprinting is used for:

1. Developing treatments for inherited diseases - more than 30 genetic diseases can be detected by genotyping (Serre, 2002).
2. Biological samples - genetic fingerprint method allows not only discrimination of a suspect, but also the conviction of another using DNA traces left at the crime scene (Betsch, 1994).
3. Identifying persons - DNA fingerprint is required to identify dead or missing persons (Betsch, 1994) as was the case for bodies after the terrorist attacks of September 11th, 2001 in theUnited States of America.
4. Veterinary Medicine - DNA fingerprint can be used in selective breeding (Epplen, 1999).
5. Population studies - human genetic fingerprint is also used for comparing allele frequencies between populations at a given locus (Edwards, 2008).

IV. Schizophrenia

Schizophrenia is a mental disorder characterized by a disintegration of the process of thinking and of emotional responsiveness. Strictly etymologically, the word translates as "dissociation of mind" (Greek "schizein" means to separate and "fren" means mind) (Carata-Dejoianu, 2008).

One of the most common assumptions in the study of schizophrenia is the "dopamine hypothesis" which suggests that a dysfunction of the dopamine neurotransmitter system leads to

a decrease the concentration of dopamine in cortical regions and an excess of dopamine in the striated areas of the brain (Howes and Kapur, 2009).

Small chromosomal aberrations is a risk in the occurrence of schizophrenia. Such a chromosomal aberration is the 22q11.2 deletion syndrome (22q11.2 DS). This common syndrome of congenital microdeletion gives predisposition for schizophrenia (Karayiorgou et al, 1995; Bassett et al, 2005).

V. Materials and methods

Based on the genetic characteristics of studied populations for forensic analysis, such as compliance with Hardy-Weinberg equilibrium and independence of alleles of different loci has chosen a set of 13 loci (D3S1358, VWA, FGA, D8S1179, D21S51, D5S818, D13S137, D7S820, D16S539, CSF1PO, TPOX, TH01) in order to be genetic markers for forensic analysis and paternity tests.

STR are DNA regions with short repeat unit of 1-5 nucleotides, the number of repetitions ranging from a few dozen to a hundred bases or even more, although some notable exceptions are repetitions of three nucleotides. Microsatellites are spread more or less randomly in the entire human genome. Total number of repetitions of each microsatellite decreases with increasing size of repetitive unit (Brinkmann, 1998).

SNPs are single nucleotide polymorphisms in DNA sequence that can be found both in coding regions, but especially in the noncoding ones. SNPs are used in molecular diagnostics, forensics, population genetics and evolutionary studies (Linacre et al, 2002).

V.1. Analysis of 15 STR loci in the North-Western Romanian population

In this study we worked on a sample of 214 individuals of different ethnic group, from Satu Mare county (figure 1). The biological sample was 2 ml of whole blood collected on EDTA. It should be noted that in this county is located in the northwest of Romania and on the borders with Hungary and Ukraine. The population is formed by romanians, hungarians, germans, ukrainians and gypsies.

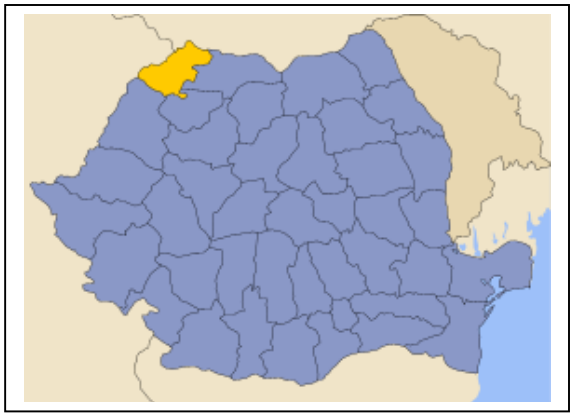

Figure 1. Location of Satu Mare County

The samples for this study were collected with the consent of the persons from whom blood samples were collected.

Samples were collected in 2005-2006.

V.1.1. DNA isolation and purification

The DNA of some of the collected samples were extracted using a conventional method with potassium chloride and proteinase K, and for the remaining samples we used the `Wizard Genomic DNA Purification kit-100 isolation`.

Genomic DNA extraction using `Wizard Genomic DNA Purification kit-100 isolation`

We followed the steps listed by the manufacturer, but to obtain a higher yield we changed centrifugation steps, incubation steps, and the number of blood washing steps.

An important thing for experiments using the DNA is to avoid contamination of DNA samples. Equally important are the quality and quantity of genetic material.

DNA quality can be examined by agarose gel electrophoresis or Spectophotometric method selecting samples with A260/A280 ratio ranging from 1.7 to 1.9. Less values indicate contamination with proteins or organic chemicals.

The amount of DNA can be so critical to the success of sequencing reactions and for human identification (AbiPrism 310 User's Manual, 2001, AbiPrism 310 User's Guide, 2001).

V.1.2. Genotyping method

Genotyping experiments determinate the size and quantity of DNA sample fragments by comparing our data with an obtained data from a molecular weight marker.

We used for genotyping the AmpFlSTR Identifiler kit. Identifiler AmpFlSTR kit is a multiplex technology that amplifies in a single reaction 15 loci and amelogenin locus. There were amplified the 13 loci of the CODIS system and two more loci: D2S1338 and D19S433.

We used Mastercycler (Eppendorf) to amplify the studied loci.

After the polymerase chain reaction, the samples were ready for migration. We also prepared a mixture for allelic marker, but instead DNA we added 1 ml amplified product of Allelic Ladder found in the kit.

We used heat for DNA samples denaturation, then we put the samples on ice. Special tubes were used for genotyping and sequencing which are placed in media with 96 wells, and inserted into the genetic analyzer.

The size of each fragment were determined using electrophoretic migration by comparison with a marker containing fragments of known size. This determination was made by GeneScan v.3.7. (Applied Biosystems) program.

V.1.3. Statistics analysis of obtained data

Statistical analysis was performed to confirm compliance with Hardy-Weinberg equilibrium to validate the STR loci and calculate the statistical parameters of forensic relevance: matching probability, power of discrimination, polymorphism information content, power of exclusion, typical paternity index, heterozygozity anf homozygozity

Statistical tests for Hardy-Weinberg equilibrium were carried out by GenePop program.

V.2. Allelic association of the methylenetetrahydrofolate reductase gene with schizophrenia

There is consensus among researchers that the schizophrenia is a multifactorial disease. Methylenetetrahydrofolate reductase (MTHFR) is the central enzyme in the folate cycle and contributes to the metabolism of the amino acid homocysteine. It catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5- methylenetetrahydrofolate, which generates the active form of folate necessary for remethylation of homocysteine to methionine (Goyette et al, 1993). MTHFR deficiency is associated with hyperhomocysteinemia and homocystinuria with altered folate distribution and a phenotype characterized by affecting the nervous and vascular system (Schwahn and Rozen, 2001; Földinger et al, 1999).

The homozygous form of MTHFR C677T mutation occurs in more than 5% of the adult population and produces thermolabile form which reduces enzyme activity below 30% compared to the normal form (Bjelland et al, 2003).

V.2.1. Sample processing for MTHFR allele analysis

Genomic DNA was isolated using Qiagen kit (DNA Blood Mini Kit QIAmp ®) from 300 ml of blood collected on EDTA.

V.2.2. Amplification of fragments which containe the polymorphisms

To amplify the fragment containing the gene MTHFR C677T polymorphism were used the following primers:

C677T-forward 5'-CAG AAG CAT ATC AGT CAT GAG C-3'

C677T-reverse 5'-CCA AGC AAC GCT GTG CAA GT-3'

The primers flank a fragment of 500pb.

To amplify the fragment containing the gene MTHFR A1298C polymorphism were used the following primers:

A1298C-forward 5'-CAG ACC TTC CTT GCA AAT ATA TCT TTG- 3'

A1298C-reverse 5'-CGG CCC CCA ACA AAG ACC-3'

The primers flank a fragment of 510pb.

V.2.3. Sequencing PCR

To determine the exact nucleotide sequence of the amplified DNA fragments we used Sanger method (dideoxy method). Sequencing PCR was performed with BigDye ® Terminator kit v3.1 Cycle Sequencing Kit (Applied Biosystems) using the purified PCR DNA strands obtained after purification from electroforesis gel.

VI. RESULTS AND DISCUSSIONS

VI.1. Analysis of 15 STR loci in the North-Western Romanian population

To get the best results we performed several dilutions of genomic DNA.

To obtain more precise data, it was necessary to optimize the PCR. There were observed artifacts (false alleles) if quantity of genomic DNA was too large. If the amount of genomic DNA was too small, there was loss of alleles or loci.

The preliminary analysis of data requires some steps to achieve compliance with more precise results. It is indicated to migrate first the Allelic Ladder for observing small changes in the conditions of electrophoresis. The first step is to verify the Allelic Ladder. Following preliminary analysis of the evidence, we can decide whether the DNA concentration was optimal, aiming artifacts (electric arc, "peeks", false alleles). We analyze each locus and we note the alleles, according to the Allelic Ladder migrated under the same conditions and concentration of the obtained fragments After determining alleles, we did statistical evaluation of the results as follows: Hardy-Weinberg equilibrium, the degree of heterozygozity, rare alleles, to produce an overview of existing alleles in the studied population.

Following statistical analysis, we obtained the allele frequencies for studied loci, the number of homozygous and heterozygote and p value for Hardy-Weinberg equilibrium (Table 1). In our population, there are some rare alleles, such as: at D21S11 locus- alleles 33 and 34, at D7S820 locus - allele 13, at D3S1358 locus - allele 13, at TH01 locus - allele 4, at D13S317 locus – allele 15, at D2S1338 locus - alleles 10 and 14, at D19S433 locus – allele 10, at TPOX locus- alleles 7 and 13, at D18S51locus - alleles 13,2 and 14,2, at D5S818 locus - allele 7 and at FGA locus - allele 29. We noticed that the for TH01, D2S1338, TPOX, D18S51 and FGA loci, p-value for Hardy-Weinberg equilibrium is below 0.05 so the population study for these loci did not respect Hardy-Weinberg equilibrium. This may be because many nationalities live and most people come from mixed families in Satu Mare county.

The population study was compared with other populations in the country. Thus, if we take into account the population of Satu Mare and a sample from Bucharest, we see that there are a few significant values ($P < 0.05$) if we apply the test to compare populations (FST) and exact test for population differentiation. These differences arise if we compare with FST the D21S11 and TH01 loci, and at D21S11, TH01 and FGA loci when using the exact test. If we compared in the population study our population sample with a population from Timişoara county, we observe that there are differences for loci D13S17 and TPOX applying FST, respectively D21S11, D13S17 when using the exact test.

Comparing the population from Satu Mare with a Hungarian population from Harghita county, four of the 15 loci differ in both tests compared: D7S820, D13S317, TPOX and D5S818. If we use the same tests for comparison with the Csángó population from Harghita county, we get the following differences for loci: D7S820, D13S317, TPOX and FGA if we apply FST test and D21S11, D7S820, D13S317, TPOX and FGA for exact test.

Studying the differences between the population of Satu Mare and the Hungarian population in Covasna, significant values were recorded for 5 loci: D7S820, D13S317 and TPOX for FST and D21S11, CSF1PO, D13S317 and TPOX for exact test.

Making a comparison with the general population of Transylvania, differences were obtained for D21S11, D13S317, D16S539, TPOX loci applying FST test and for exact test values we can notice differences at loci D21S11, D13S317, TPOX, D18S51, FGA.

Analyzing the test results accurately, five of the 15 loci tested showed significant values (D21S11, D13S317, TPOX, D18S51, FGA) in the comparison between population in Satu Mare and general population of Moldova. If the two populations were tested with FST, differences occur at D7S820, D13S317, TPOX loci.

Most significant values were obtained in comparison with the general population of Oltenia and Muntenia. For FST, differences are observed at loci: D21S11, D13S317, D16S539, D2S1338, TPOX and D19S51, and for exact test at loci: D13S317, D16S539, TPOX, FGA, D18S51 and D21S11.

Statistical tests showed that the loci D21S11, D7S820, D13S317, TPOX and FGA are the most polymorphic autosomal STR loci specific to this region. Thus, we see that the values for D21S11, D13S317 and TPOX loci are significantly different in the comparison of Satu Mare county population of all other populations studied.

Although the population study is relatively heterogeneous, these loci are specific to the north-western Romania. For this reason, the studied population sample can be used for human identification and differentiation between populations.

By analysis of 15autosomal STR loci we conclude that the genetic distance measured by comparing allele frequencies indicates that the population included in this study shows smaller difference between the population of Romanians in Bucharest and the general population in western Romania (Timişoara county) than to general population from Oltenia and Muntenia and populations from Harghita and Covasna.

Comparing our population with a population of Montenegro, one from Russia, one from Venezuela and one from Tanzania, we can correlate genetic distances measured by comparing the allele frequencies between populations with the geographical distances between populations (Table 2). As might be expected, the studied population and the population from Russia are the

most appropiate. The biggest differences are observed among the studied population and the one taken from Tanzania. Among the studied population and the population from Montenegro, respectively, from Venezuela differences are much smaller. If in case of the countries Russia, Montenegro and Tanzania genetic differences are comparable to the geographical distances between the compared populations, the population from Venezuela (geographically the farthest) show smaller differences than we expected. The greatest variety in terms of frequency of alleles, are observed at loci D7S820, TH01 and TPOX (figures 2,3, and 4), and the more homogeneous distribution of alleles is for locus D5S818 (figure 5).

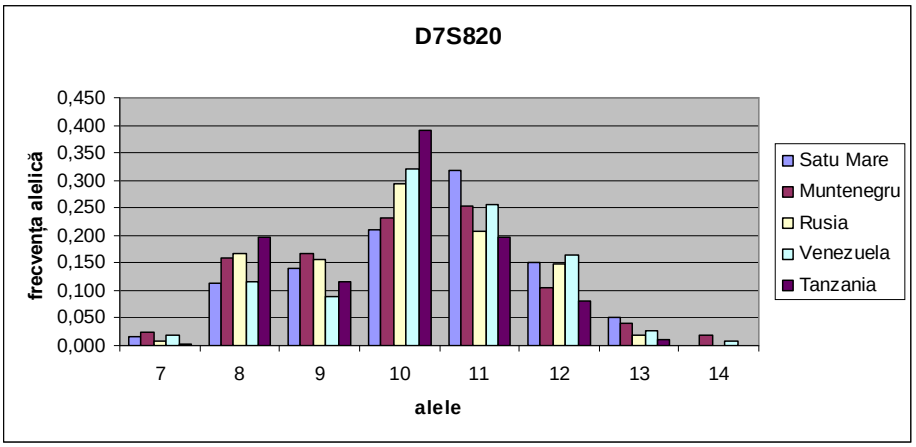

Figure 2. The allele frequency at D7S820 locus for the populations of Satu Mare, Montenegro, Russia, Venezuela and Tanzania

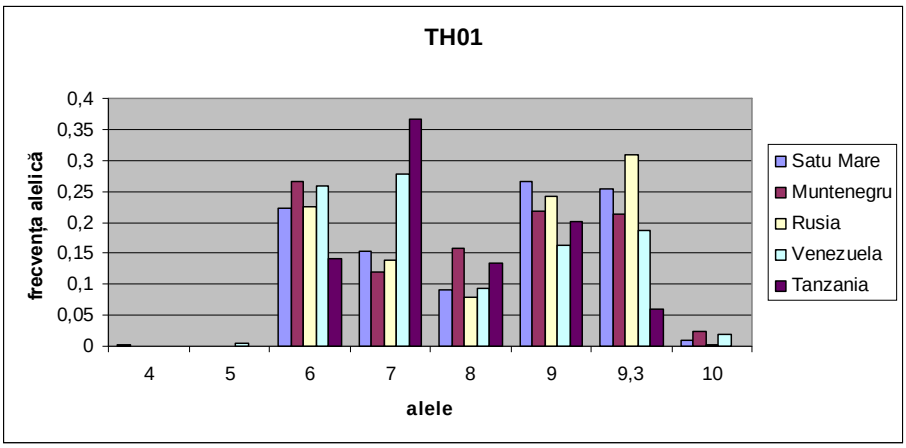

Figure 3. The allele frequency at TH01 locus for the populations of Satu Mare, Montenegro, Russia, Venezuela and Tanzania

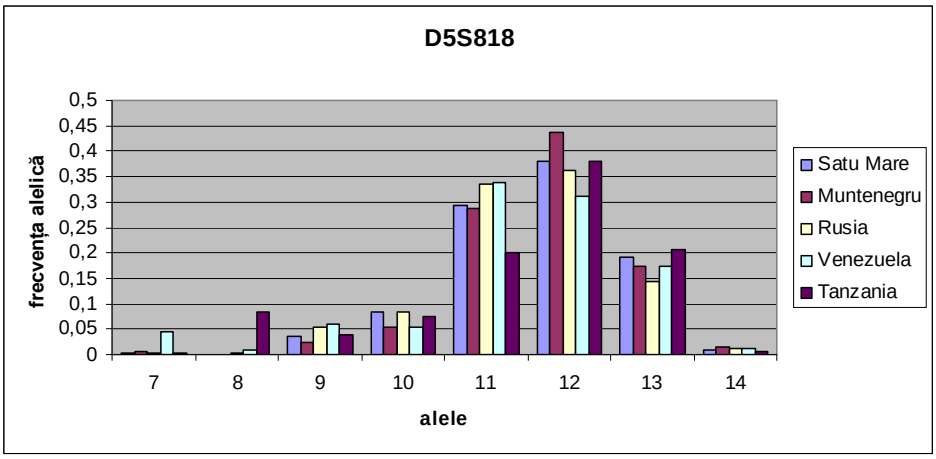

Figure 4. The allele frequency at TPOX locus for the populations of Satu Mare, Montenegro, Russia, Venezuela and Tanzania

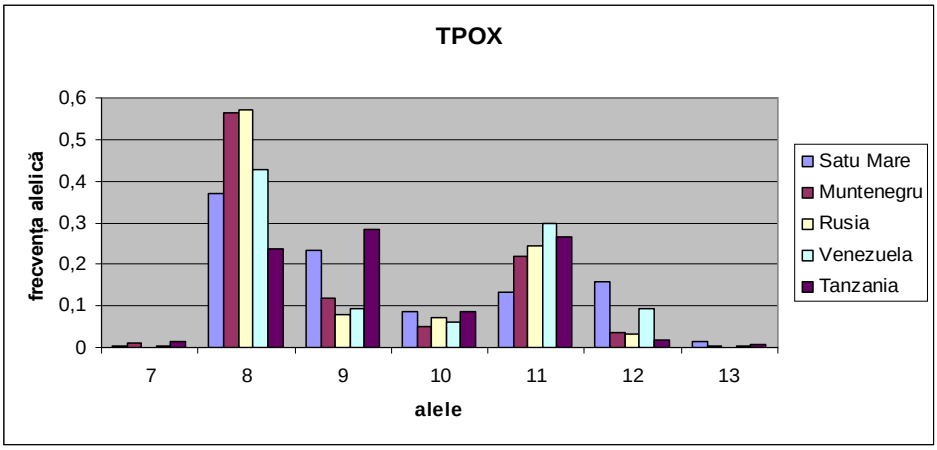

Figure 5. The allele frequency at D5S818 locus for the populations of Satu Mare, Montenegro, Russia, Venezuela and Tanzania

VI.2. Allelic association of the methylenetetrahydrofolate reductase gene with schizophrenia

In this study we analyzed an experimental and a control group for two single nucleotide polymorphisms in the *MTHFR* gene to observe the degree of correlation between certain genotypic frequencies and schizoid type events.

Amplicons were purified from 1% agarose gel. The marker was pQE60 digested with *DraI* (the well 1) (Figures 6 and 7).

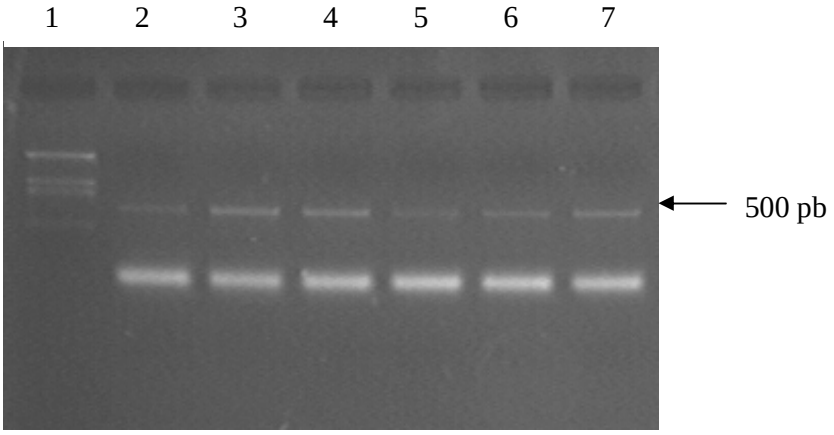

Figure 6. Separation of DNA fragments (C677T) in 1% agarose gel

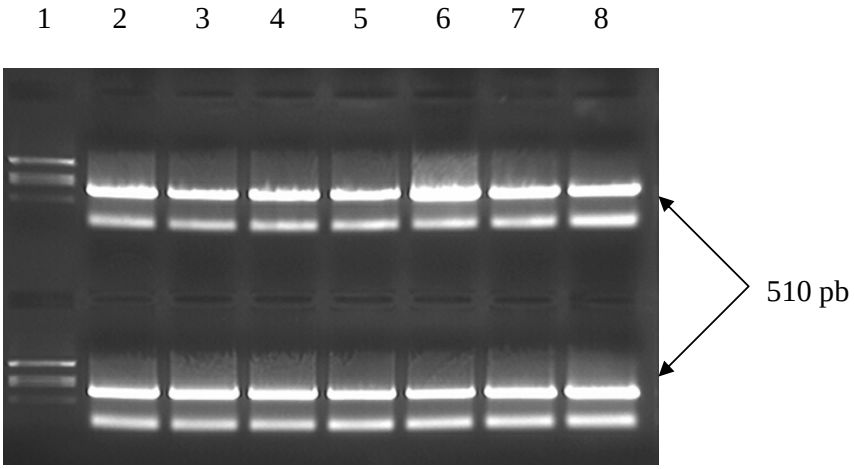

Figure 7. Separation of DNA fragments (A1298C) in 1% agarose gel

We used Wizard ® SV Gel and PCR Clean-Up System (Promega) for amplicon purification.

1. MTHFR gene polymorphism

We analyzed the C677T polymorphism of *MTHFR* gene in 46 samples collected from a group of patients. We found that 13 of them have the wild allele CC, 27 individuals are heterozygous CT, and six individuals are homozygous, having the mutant allele TT. In the analyzed control group were from 35 samples 23 individuals have the wild allele CC, 7 are heterozygous CT, and five individuals are homozygous, with the mutant allele TT (Figure 8).

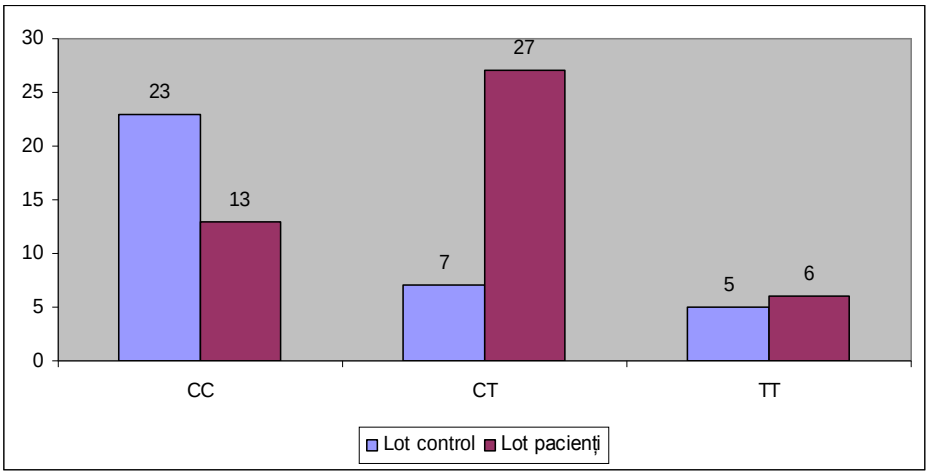

Figura 8. C677T polymorphism of the MTHFR gene

Homozygous mutants TT appear in similar percentages in the two analyzed groups what invalidates the hypothesis that homozygous mutant genotype could be involved in the installation of schizophrenia. There is a reversal proportion of CC and CT genotypes between experimental and control groups (Table 3).

	CONTROLS			**PACIENTS**		
GENOTIPE	**Normal**	**Mutant**		**Normal**	**Mutant**	
	CC	**CT**	**TT**	**CC**	**CT**	**TT**
Number	**23**	**7**	**5**	**13**	**27**	**6**
%	**65,7**	**20,0**	**14,3**	**28,3**	**58,7**	**13,0**
%	**65,7**	**34,3**		**28,3**	**71,7**	

Table 3. C677T polymorphism of MTHFR gene

The A1298C polymorphism of MTHFR gene was analyzed on 42 samples collected from patients. 24 individuals presentes wild alleles AA, 20 are heterozygous AC, and five individuals are homozygous, with mutant alleles CC. The analyzed control group (40 samples) contains 17 persons with the wild alleles AA, 20 individuals are heterozygous AC, and three individuals are homozygous, having mutant alleles CC (Figure 9).

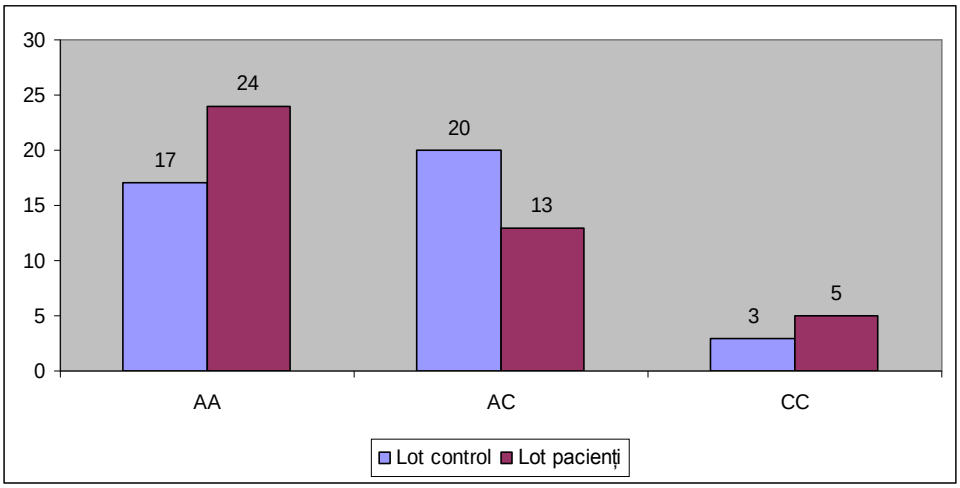

Figure 9. A1298C polymorphism of the MTHFR gene

The small number of homozygous individuals, carriers of the defective allele TT (C677T) / CC (A1298C) may be explained as the result of selection. In these cases results a lower termolabile enzyme activity and negative consequences on the body. Polymorphism of the positions maintained in the population studied in heterozygous form over the generations, but not in homozygous form (Table 4)

	CONTROLS			**PACIENTS**		
GENOTIPE	**Normal**	**Mutant**		**Normal**	**Mutant**	
	AA	**AC**	**CC**	**AA**	**AC**	**CC**
Number	**17**	**20**	**3**	**24**	**13**	**5**
%	**42,5**	**50,0**	**7,5**	**57,1**	**31,0**	**11,9**
%	**42,5**	**57,5**		**57,1**	**42,9**	

Table 4. A1298C polymorphism of MTHFR gene

A similar study on a group of 68 patients, was observed the presence of wild allele in position 677 CC in 32 persons, while 25 patients were heterozygous CT and 11 individuals present the mutant allele in homozygous form TT. Comparing with our results, this group of patients presented the wild alleles CC in 47% of cases, while in our group the CC genotype appears in 28% of cases. CT heterozygotes are present in a proportion of 58.7% in our group while in the otherit is in a 36.8% percentage (Figure 10).

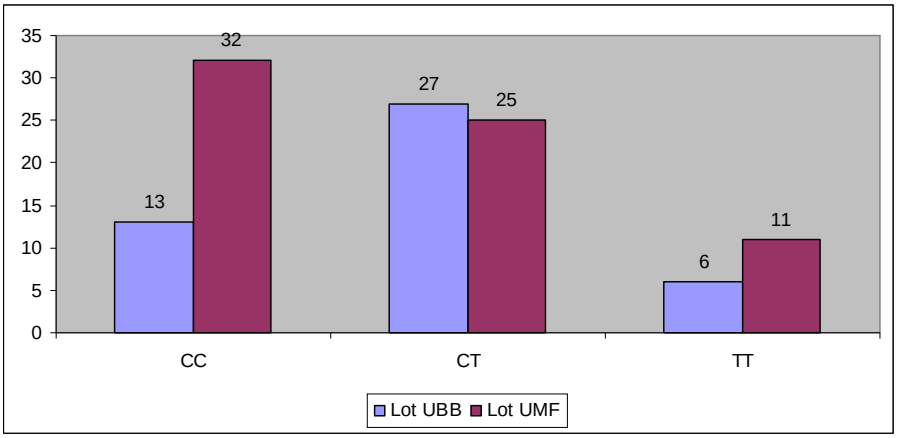

Figure 10. MTHFR gene polymorphism at position 677 in the study group of patients studied at the "Babeş-Bolyai" University and the group studied at "Iuliu Haţieganu" University

In the case of A1298C polymorphism, there are no significant differences between the two groups. The of homozygous wild alleles AA are present in a proportion of 57.1% in our group and 58.2% in the other group. We can noticed that 31% of our patients are heterozygous AC, while in the similar group 41.2% shows both alleles. 11.8% of the patients studied by us are homozygous for the mutant allele CC, but no patients in the other group has this genotype (Figure 11).

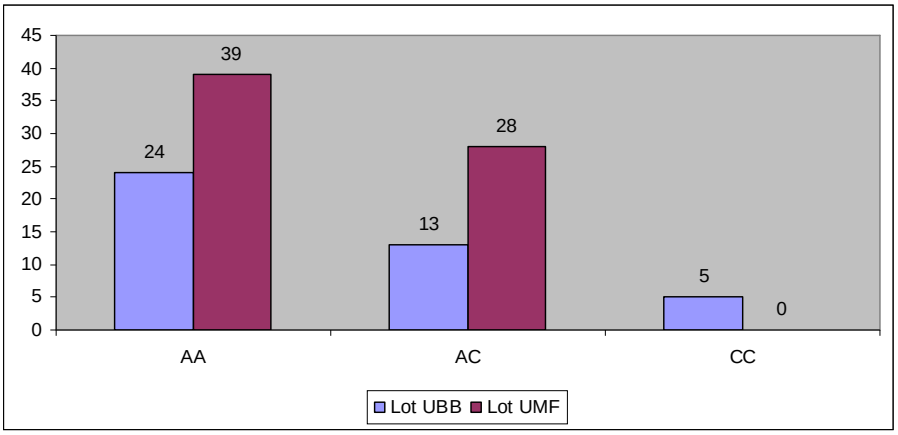

Figure 11. MTHFR gene polymorphism at position 1298 in patients in the study group of patients studied at the "Babeş-Bolyai" University and the group studied at "Iuliu Haţieganu" University

VII. CONCLUSIONS

The analysis of 15 STR loci in a population from north-western Romania (Satu Mare county) and comparing the results with similar data obtained for populations from other regions of Romania, Russia, Montenegro, Tanzania and Venezuela revealed:

- the most significant difference between the studied population and other Romanian population is observed for the general population of Oltenia and Muntenia.
- worldwide, the studied population is closer in the order of the populations of Russia, Venezuela, Montenegro and, finally, Tanzania. The population from Satu Mare presents greater similarity between the population from Venezuela than to the Montenegrin, despite the large geographical distance between the first two. This may be due to the fact that the population of Montenegro is isolated, living in a mountainous area, difficult to access, and inbreed rate is high.

The small number of homozygous individuals carriers of the mutant alleles TT for C677T locus and CC for A1298C locus is due to negative selection acting on these genotypes.

In the experimental group, for the C677T locus dominates the heterozygous CT genotype with percentage of 58.7% compared with 20% in the control group. For A1298C locus, dominant in the experimental group with a share of 57.1% is the homozygous normal phenotype while in the control group the percentage is 42.5%. It shows a direct and significant correlation between mutant genotypes and schizoid manifestations.

For neurological diseases because of their complex etiology, it is necessary to study the different genes such as *MTFHR, PON1, COMT*, etc.

Allele	D8S1179	D21S11	D7S820	CSF1PO	D3S1358	TH01	D13S317	D16S539	D2S1338	D19S433	vWA	TPOX	D18S51	D5S818	FGA
4	-	-	-	-	-	0.002	-	-	-	-	-	-	-	-	-
6	-	-	-	-	-	0.224	-	-	-	-	-	-	-	-	-
7	-	-	0.016	-	-	0.159	-	-	-	-	-	0.002	-	0.005	-
8	0.021	-	0.091	0.005	-	0.107	0.131	0.009	-	-	-	0.348	-	-	-
9	0.007	-	0.138	0.019	-	0.243	0.072	0.065	-	0.002	-	0.264	-	0.035	-
9.3	-	-	-	-	-	0.252	-	-	-	-	-	-	-	-	-
10	0.068	-	0.213	0.262	-	0.012	0.051	0.082	-	0.002	-	0.084	0.009	0.082	-
11	0.049	-	0.355	0.341	-	-	0.224	0.250	-	0.002	0.002	0.129	0.012	0.304	-
12	0.124	-	0.138	0.306	-	-	0.266	0.334	-	0.103	-	0.164	0.126	0.369	-
13	0.325	-	0.049	0.051	0.005	-	0.189	0.224	-	0.255	0.002	0.009	0.100	0.192	-
13.2	-	-	-	-	-	-	-	-	-	0.090	-	-	0.019	-	-
14	0.266	-	-	0.016	0.136	-	0.051	0.035	0.004	0.322	0.110	-	0.168	0.014	-
14.2	-	-	-	-	-	-	-	-	-	0.019	-	-	0.014	-	-
15	0.126	-	-	-	0.273	-	0.014	-	-	0.161	0.091	-	0.152	-	-
15.2	-	-	-	-	-	-	-	-	-	0.040	-	-	-	-	-
16	0.014	-	-	-	0.231	-	-	-	0.072	0.040	0.206	-	0.138	-	-
16.2	-	-	-	-	-	-	-	-	-	0.028	-	-	-	-	-
17	-	-	-	-	0.196	-	-	-	0.189	0.009	0.273	-	0.114	-	-
17.2	-	-	-	-	-	-	-	-	-	0.007	-	-	-	-	-
18	-	-	-	-	0.147	-	-	-	0.098	-	0.196	-	0.068	-	0.009
19	-	-	-	-	0.012	-	-	-	0.117	-	0.091	-	0.047	-	0.072
20	-	-	-	-	-	-	-	-	0.147	-	0.028	-	0.019	-	0.119
21	-	-	-	-	-	-	-	-	0.035	-	-	-	0.012	-	0.152
22	-	-	-	-	-	-	-	-	0.026	-	-	-	0.002	-	0.164
23	-	-	-	-	-	-	-	-	0.091	-	-	-	-	-	0.159
24	-	-	-	-	-	-	-	-	0.112	-	-	-	-	-	0.136
25	-	0.002	-	-	-	-	-	-	0.091	-	-	-	-	-	0.112
26	-	0.002	-	-	-	-	-	-	0.016	-	-	-	-	-	0.061
27	-	0.012	-	-	-	-	-	-	-	-	-	-	-	-	0.014
28	-	0.180	-	-	-	-	-	-	-	-	-	-	-	-	-
29	-	0.245	-	-	-	-	-	-	-	-	-	-	-	-	0.002
29.2	-	0.002	-	-	-	-	-	-	-	-	-	-	-	-	-
30	-	0.194	-	-	-	-	-	-	-	-	-	-	-	-	-
30.2	-	0.044	-	-	-	-	-	-	-	-	-	-	-	-	-
31	-	0.037	-	-	-	-	-	-	-	-	-	-	-	-	-
31.2	-	0.082	-	-	-	-	-	-	-	-	-	-	-	-	-
32	-	0.026	-	-	-	-	-	-	-	-	-	-	-	-	-
32.2	-	0.079	-	-	-	-	-	-	-	-	-	-	-	-	-
33	-	0.042	-	-	-	-	-	-	-	-	-	-	-	-	-
33.2	-	0.035	-	-	-	-	-	-	-	-	-	-	-	-	-
34	-	0.012	-	-	-	-	-	-	-	-	-	-	-	-	-
34.2	-	0.005	-	-	-	-	-	-	-	-	-	-	-	-	-
H	0.827	0.836	0.762	0.696	0.785	0.715	0.738	0.738	0.846	0.785	0.794	0.626	0.832	0.696	0.771
MP	0.078	0.046	0.081	0.130	0.076	0.077	0.058	0.090	0.030	0.075	0.061	0.105	0.029	0.124	0.033
PD	0.922	0.954	0.919	0.870	0.924	0.923	0.942	0.910	0.970	0.925	0.939	0.895	0.971	0.876	0.967
PIC	0.750	0.830	0.750	0.670	0.760	0.760	0.790	0.730	0.870	0.760	0.790	0.720	0.870	0.680	0.860
PE	0.650	0.668	0.530	0.423	0.572	0.452	0.490	0.490	0.687	0.572	0.589	0.323	0.659	0.423	0.546
TPI	2.89	3.06	2.10	1.65	2.33	1.75	1.91	1.91	3.24	2.33	2.43	1.34	2.97	1.65	2.18
P	0.619	0.179	0.321	0.659	0.918	**0.038**	0.433	0.946	**0.015**	0. 458	0.579	**0.000**	**0.034**	0.627	**0.002**

H-heterozigoția, MP-probabilitate de potrivire, PD-putere de discriminare, PIC-informativitatea polimorfismului, PE-puterea de excluziune, TPI- indicele de paternitate, p- testul exact pentru echilibrul Hardy Weinberg

Table 1. Allele frequencies for 15 STR loci in the North-Western population of Romania for 214 individuals

Marker	Test	Population data	CJ-Hu	CV-Sze	HR-Sze	HR-Csn	B-Ro	Trsi	West	Moldova	Dobrogea	Oltenia and Muntenia
			n=146	n=278	n=257	n=220	n=243	n=1977	n=219	n=1321	n=569	n=1910
D8S1179	Fst	SM-Ro vs	0.58398+-0.0143	0.49707+-0.0152	0.31543+-0.0206	0.70215+-0.0138	0.25977+-0.0140	0.16016+-0.0108	0.44238+-0.0158	0.36621+-0.0159	0.19727+-0.0123	0.27246+-0.0165
	Exact test	SM-Ro vs	0.90666+-0.0051	0.80224+-0.0131	0.39188+-0.0197	0.60221+-0.0113	0.41800+-0.0165	0.23989+-0.0114	0.78764+-0.0111	0.65771+-0.0168	0.30948+-0.0200	0.36312+-0.0190
D21S11	Fst	SM-Ro vs	0.14453+-0.0115	0.31641+-0.0155	0.41895+-0.0141	0.10352+-0.0113	**0.03809+-0.0053**	**0.04297+-0.0066**	0.08496+-0.0092	0.11328+-0.0111	**0.04492+-0.0063**	**0.01562+-0.0042**
	Exact test	SM-Ro vs	**0.03935+-0.0065**	**0.01303+-0.0042**	0.10699+-0.0080	**0.02369+-0.0049**	**0.00041+-0.0005**	**0.00000+-0.0000**	**0.01099+-0.0021**	**0.00000+-0.0000**	**0.00002+-0.0000**	**0.00000+-0.0000**
D7S820	Fst	SM-Ro vs	**0.01758+-0.0042**	**0.01465+-0.0037**	**0.00195+-0.0014**	**0.00000+-0.0000**	nd	0.05273+-0.0061	0.08984+-0.0114	**0.03906+-0.0055**	0.06934+-0.0100	0.15723+-0.0111
	Exact test	SM-Ro vs	0.10347+-0.0070	0.07884+-0.0084	**0.01037+-0.0022**	**0.00122+-0.0009**	nd	0.06343+-0.0061	0.08678+-0.0087	0.10949+-0.0122	0.16529+-0.0075	0.26625+-0.0191
CSF1PO	Fst	SM-Ro vs	0.98633+-0.0027	0.24219+-0.0138	0.51172+-0.0146	0.87988+-0.0091	nd	0.90039+-0.0070	0.77246+-0.0105	0.69824+-0.0143	0.94141+-0.0063	0.97559+-0.0048
	Exact test	SM-Ro vs	0.93996+-0.0049	**0.04996+-0.0085**	0.44186+-0.0151	0.36834+-0.0135	nd	0.55183+-0.0210	0.85288+-0.0031	0.47035+-0.0104	0.86094+-0.0105	0.75232+-0.0143
D3S1358	Fst	SM-Ro vs	0.33691+-0.0105	0.20215+-0.0117	0.26465+-0.0120	0.28516+-0.0131	0.85156+-0.0084	0.35742+-0.0122	0.48438+-0.0148	0.48145+-0.0153	0.60645+-0.0120	0.15723+-0.0103
	Exact test	SM-Ro vs	0.51354+-0.0118	0.28015+-0.0163	0.35603+-0.0152	0.15460+-0.0093	0.90081+-0.0072	0.33840+-0.0130	0.59505+-0.0156	0.38096+-0.0100	0.83136+-0.0109	0.24812+-0.0135
TH01	Fst	SM-Ro vs	0.32422+-0.0113	0.18555+-0.0135	0.72852+-0.0153	0.14551+-0.0101	**0.01758+-0.0037**	0.30469+-0.0153	0.05469+-0.0076	0.16895+-0.0133	0.38477+-0.0110	0.12988+-0.0098
	Exact test	SM-Ro vs	0.50545+-0.0171	0.30468+-0.0223	0.76938+-0.0080	0.30201+-0.0142	**0.03951+-0.0168**	0.15612+-0.0211	0.06102+-0.0098	0.16656+-0.0068	0.43041+-0.0314	0.10284+-0.0089
D13S317	Fst	SM-Ro vs	**0.01172+-0.0030**	**0.01660+-0.0046**	**0.02148+-0.0053**	**0.00000+-0.0000**	nd	**0.02637+-0.0050**	**0.00586+-0.0022**	**0.00586+-0.0026**	**0.00684+-0.0023**	**0.00195+-0.0014**
	Exact test	SM-Ro vs	**0.02277+-0.0031**	**0.00682+-0.0013**	**0.01849+-0.0029**	**0.00000+-0.0000**	nd	**0.00101+-0.0010**	**0.00450+-0.0016**	**0.00000+-0.0000**	**0.00012+-0.0001**	**0.00008+-0.0001**
D16S539	Fst	SM-Ro vs	0.56152+-0.0138	0.11426+-0.0090	0.70117+-0.0131	0.24707+-0.0136	0.09766+-0.0068	**0.03711+-0.0048**	0.49609+-0.0193	0.23047+-0.0116	0.06250+-0.0087	**0.00488+-0.0020**
	Exact test	SM-Ro vs	0.84081+-0.0072	0.09541+-0.0065	0.68951+-0.0097	0.35600+-0.0227	0.07079+-0.0053	0.08556+-0.0084	0.68294+-0.0107	0.43492+-0.0150	0.14369+-0.0097	**0.01482+-0.0027**
D2S1338	Fst	SM-Ro vs	0.95605+-0.0058	0.90723+-0.0099	0.59766+-0.0178	0.39453+-0.0162	0.57227+-0.0155	0.62402+-0.0150	0.93066+-0.0083	0.62598+-0.0162	0.87305+-0.0109	**0.49707+-0.0176**
	Exact test	SM-Ro vs	0.99409+-0.0008	0.95735+-0.0061	0.59530+-0.0149	0.55020+-0.0162	0.44333+-0.0163	0.06738+-0.0114	0.88256+-0.0085	0.14671+-0.0181	0.66312+-0.0187	0.08443+-0.0129
D19S433	Fst	SM-Ro vs	0.83789+-0.0109	0.90625+-0.0103	0.59473+-0.0162	0.45215+-0.0190	0.38574+-0.0161	0.30664+-0.0141	0.41797+-0.0154	0.94824+-0.0077	0.37988+-0.0194	0.53125+-0.0145
	Exact test	SM-Ro vs	0.36183+-0.0110	0.92205+-0.0066	0.66935+-0.0179	0.42362+-0.0186	0.44635+-0.0211	0.59229+-0.0163	0.38028+-0.0157	0.92115+-0.0105	0.68269+-0.0108	0.60449+-0.0209
vWA	Fst	SM-Ro vs	0.90723+-0.0079	0.73242+-0.0156	0.61914+-0.0144	0.36621+-0.0128	0.91797+-0.0092	0.69043+-0.0123	0.98047+-0.0044	0.85840+-0.0098	0.93359+-0.0061	0.55273+-0.0169
	Exact test	SM-Ro vs	0.94020+-0.0030	0.52834+-0.0105	0.53429+-0.0200	0.15078+-0.0127	0.79558+-0.0155	0.35741+-0.0158	0.65802+-0.0149	0.77206+-0.0149	0.85927+-0.0119	0.17599+-0.0271
TPOX	Fst	SM-Ro vs	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	nd	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**
	Exact test	SM-Ro vs	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	nd	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**	**0.00000+-0.0000**
D18S51	Fst	SM-Ro vs	0.57910+-0.0156	0.62500+-0.0147	0.55957+-0.0140	0.77246+-0.0113	0.24316+-0.0159	0.80762+-0.0118	0.36426+-0.0150	0.93359+-0.0082	0.41406+-0.0128	0.73145+-0.0124
	Exact test	SM-Ro vs	0.61956+-0.0105	0.21570+-0.0169	0.32835+-0.0230	0.63413+-0.0184	0.15329+-0.0100	**0.00118+-0.0009**	0.17251+-0.0104	**0.01545+-0.0030**	0.08280+-0.0113	**0.00171+-0.0007**
D5S818	Fst	SM-Ro vs	0.86523+-0.0095	0.98730+-0.0034	**0.00000+-0.0000**	0.96875+-0.0061	nd	0.56543+-0.0161	0.91406+-0.0095	0.99707+-0.0014	0.65625+-0.0126	0.87598+-0.0090
	Exact test	SM-Ro vs	0.94839+-0.0050	0.97169+-0.0019	**0.00000+-0.0000**	0.96260+-0.0015	nd	0.71274+-0.0209	0.81045+-0.0121	0.89155+-0.0079	0.87704+-0.0059	0.90950+-0.0124
FGA	Fst	SM-Ro vs	0.33496+-0.0179	0.45996+-0.0169	0.54199+-0.0152	**0.04590+-0.0063**	0.13672+-0.0088	0.07715+-0.0073	0.44434+-0.0144	0.11426+-0.0102	0.27832+-0.0148	0.23047+-0.0119
	Exact test	SM-Ro vs	0.28976+-0.0233	0.39253+-0.0283	0.26907+-0.0258	**0.00052+-0.0003**	**0.00638+-0.0020**	**0.00811+-0.0036**	0.47217+-0.0208	**0.01929+-0.0045**	0.09060+-0.0147	**0.03515+-0.0077**

Table 2. Comparative study of the population from Satu Mare county with other populations from Romania

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