Folate cycle (FC) is one of the main metabolic cycles in the human organism, folic acid derivatives are involved in its enzyme systems. Moreover, the biosynthesis of methionine, purine and pyrimidine nucleotides, and DNA methylation takes place [1]. Homocysteine (Hcy) is a sulphur-containing amino acid which is a marker for the efficiency of FC functioning.

Blood Hcy levels have exceeded physiological limits in more than 75.0% of the adolescents living in Ivankivskyi and Poliskyi districts, Kyiv region, in 30 years after the accident at the Chornobyl nuclear power plant (ChNPP) [2]. Disorders in the production of thyroid hormones have also been registered [3].

Taking into account a high prevalence of thyroid cancer among the inhabitants of the Ukrainian-Belarusian Polissia after accident at the Chornobyl nuclear power plant [4], a determination of the FC state and its link with the thyroid system in children, constantly living under radiation risk due to the ChNPP accident, is of high priority.

Forest fires at the territory contaminated with radionuclides may be one of the causes of radiation exposure. In total, 4.0 millions hectares of the forest lands were contaminated with radioactive elements in Ukraine. Zhytomyr region (974.3 thousands hectares), Kyiv region (416.4 thousands hectares), Chernihiv region (725.5 thousands hectares), and Rivne region (728.8 thousands hectares) are the most suffered regions [5]. The greatest amount of radioactive fall-out, in particular 137Cs – 4.4 PBq, 90Sr – 4.0 PBq, 239/240Pu – 32 TBq, fell within 30-km Chornobyl exclusion zone [6].

Thus, the soils of suffering areas, the forest trees, shrubs, and grasses, growing in these areas, currently contain a huge amount of radioactive elements. Forest fires, often occurring in the Chornobyl exclusion zone, are one of the most powerful sources of secondary air pollution.
tion with different radionuclides which have a negative impact on people’s health [7].

Radioactive products of combustion of the forest plants move into the atmosphere with wind currents for long distances. The duration of the existence of radiation smoke aerosols and clouds is less than a week in the lower troposphere (the height up to 1.5 km), about a month in the upper troposphere, and 1-3 years in the stratosphere. At the same time, radioactive products of combustion are deposited on the radiation-free territories. Basically, radioactive aerosols contain $^{137}\text{Cs}$ [7].

The aim of this study was to determine a state of FC and its link with a thyroid system in the children of the Ukrainian Polissia after forest fires in the Chornobyl exclusion zone.

Material and methods. The study was conducted within the implementation of the projects of the European Commission in Ukraine “Health and Ecological Programmes around the Chornobyl Exclusion Zone: Development, Training, and Coordination of Health-Related Projects” and the Rhone-Alpes Regional Council (France).

178 children from Ivankivskyi district and 158 children from Poliskiy district of Kyiv region, underwent laboratory and instrumental examination. According to the findings of dosimetry certification of the settlements, the territory of the regions has remained contaminated with radioactive substances after the Chornobyl accident until the present day (the $^{137}\text{Cs}$ soil pollution density varies 0.17-1.9 Cu/km² [8]).

The average age of the children in the group from Ivankivskyi district was (13.6 ± 0.1) years old (95% CI 13.4-13.8 years old), and (14.8 ± 0.0) years old (95% CI 14.7-15.0 years old) in the group from Poliskiy district.

In the morning, all the children who attended school were taken the blood sampling in an empty stomach from the ulnar vein. Blood sampling among children from Poliskiy district was carried out on 02.04.2015 and in the children from Ivankivskyi district on 18.12.2015.

The investigations of blood samples were agreed with the parents, they were analysed at the laboratory, certified by quality standards. A content of pituitary thyroid-stimulating hormone (TSH), free triiodothyronine ($T_3$), free thyroxine ($T_4$), $H_c$, and the state of the FM genetic system were assessed.

TSH, $T_3$ and $T_4$ concentrations were determined with the help of electrochemiluminescent immunoassay method (ECLIA). Analyser and test kit: Cobas 6000; Roche Diagnostics (Switzerland).

Plasma homocysteine concentrations were measured with the chemiluminescent immunoassay method (CLIA). Analyser and test kit: Architect 1000 (ABBOT Diagnostics (USA)).

The following allelic variants were identified in genetic analysis of FM: $C677T$ and $A1298C$ of the $MTHFR$ gene (synthesis of the methylenetetrahydrofolate reductase enzyme), $A2756G$ of the MTR gene (synthesis of the $B_{12}$-dependent methionine synthase enzyme) and $A66G$ of the MTRR gene (synthesis of the methylone synthase reductase enzyme). PCR method in a real-time regime was used. Analyser and test kit: DT-96 detecting thermocycler, DNA-Technology (Russia).

In order to carry out the correlation studies identifying a link between blood $H_c$ levels and the frequency of genetic changes in the studied cases of each genetic polymorphism, we assessed genetic variants in points (0-3): “0” – homozygous carriership of a neutral allele; “1” – heterozygous carriership of a risk allele; “2” – homozygous carriership of a risk allele.

The statistical processing of the obtained results was performed with help of IBM SPSS Statistics 22 software (USA). The arithmetic mean (M), ± standard error of mean (m), confidence interval for the mean value (95% CI), median (Me), interquartile range (IR), minimum and maximum parameter values and percentiles were calculated for the analysed variables. The distribution hypothesis was tested (Kolmogorov-Smirnov criterion).

All the parameters under study did not conform to the normal distribution law, thus, a non-parametric Mann-Whitney U-test was used to compare values. The statistical significance of variables was assessed by the determination of $p$ significance level with the help of the statistical software programme.

The Student $t$-test was used to compare relative values. The critical level of significance for the null hypothesis ($p$) was taken as 0.05. An link between $H_c$, TSH, $T_3$ and $T_4$ levels in blood and variants of the carrierhip of risk alleles of folate metabolism genetic polymorphisms were identified with the help of the Spearman rank correlation coefficient ($r_{xy}$). The strength of a link was assessed according to a typical scale: weak – from 0 to 0.299; moderate – from 0.3 to 0.699; strong – from 0.7 to 1.0.

Results and discussion. The investigations showed that blood $H_c$ level was significantly higher in the subgroup of the children-carriers of the T risk allele of the $MTHFR$: $C677T$ genetic polymorphism-$MTHFR$: $C/T + MTHFR$: $C/T$ genotypes, living in Ivankivskyi and Poliskiy districts of Kyiv region, than in the subgroup of the children without this allele – $MTHFR$: $C/C$ genotype (tables 1-3).

$H_c$ level was statistically significantly higher in the subgroup with the $MTHFR$: $C/T + MTHFR$: $T/T$ genotypes in comparison with the subgroup that included the MTR:$2756 A/G + MTR:2756 G/G$ genotypes in
THE STATE OF FOLATE METABOLISM AND ITS LINK WITH THE THYROID SYSTEM IN CHILDREN AFTER FOREST FIRES IN THE CHORNOBYL EXCLUSION ZONE

Ivankivsky, Ukraine

Objective. We determined the state of folate cycle and its link with thyroid system in the children of the Ukrainian Polissia after forest fires in the Chornobyl exclusion zone.

Methods: Immunochemical, mathematical and statistical ones.

Results. Blood homocysteine levels were statistically significantly higher in the subgroup of the children who were the carriers of the T risk allele of the MTHFR:677Т genetic polymorphism living in Ivankovskiy and Poliskyi districts than in the subgroup of the children without this allele. The examination of the children from the regions adjacent to the Chernobyl nuclear power plant allowed to identify in them the metabolic processes' changes in the form of increased production of Hc due to the forest fires in the exclusion zone. Blood Hc levels were higher in the children from Ivankivskyi district examined after fires in the Chornobyl exclusion zone than in those from Poliskyi district examined before fires, including the majority of genetic subgroups, with the exception of the genetic subgroup which included cases of homozygous carriership of the T allele.

Conclusions. It can be reasonably assumed that fires in the Chornobyl exclusion zone have a negative effect on the functioning of folate metabolism in a child's organism.

Keywords: folate metabolism, homocysteine, hormones of hypophysis and thyroid gland, forest fires, Chornobyl exclusion zone.

### Table 1

<table>
<thead>
<tr>
<th>Genetic subgroups</th>
<th>Polskyi district</th>
<th>Ivankivskyi district</th>
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<tr>
<td></td>
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<td>IR</td>
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### Table 2

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<tr>
<th>Comparison groups</th>
<th>Comparison group size</th>
<th>Average rank</th>
<th>Mann-Whitney U test value, significance level, p</th>
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<tbody>
<tr>
<td>MTHFR:677 C/T + MTHFR:677 T/T</td>
<td>79</td>
<td>90.16</td>
<td>U = 2278.5; p = 0.003</td>
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<tr>
<td>MTHFR:677 C/C</td>
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<td>68.84</td>
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<tr>
<td>MTHFR:677 C/T + MTHFR:677 T/T</td>
<td>79</td>
<td>73.48</td>
<td>U = 1621.0; p = 0.019</td>
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<tr>
<td>MTR:2756 A/G + MTR:2756 G/G</td>
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<td>57.52</td>
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### Table 3

<table>
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<tr>
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<th>Average rank</th>
<th>Mann-Whitney U test value, significance level, p</th>
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</thead>
<tbody>
<tr>
<td>MTHFR:677 C/T + MTHFR:677 T/T</td>
<td>98</td>
<td>96.67</td>
<td>U = 3217.0; p = 0.04</td>
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<tr>
<td>MTHFR:677 C/C</td>
<td>80</td>
<td>80.71</td>
<td></td>
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</tbody>
</table>

No statistical differences were found in the proportions of risk alleles and neutral alleles of folate metabolism genetic polymorphisms when comparing the groups of children from Poliskyi and Ivankivskiy districts (tables 5, 6).

A weak direct link was found in the manifestation of the impact of the risk allele of the MTHFR:677T and MTRR:A66G polymorphisms in the groups of children from Poliskyi and Ivankivskiy districts (tables 8, 9).

There were no statistical differences in the frequency of cases of carriership of the T allele of the MTHFR:677T polymorphism in the similar genetic subgroups of the children from Ivankivskiyi and Poliskyi districts (table 7).

No link was observed between Hc values and a degree of the...
manifestation of carriership of the risk allele of the MTR:A2756G and MTHFR:A1298C polymorphisms.

Direct links were detected between blood Hc and TSH levels, and Hc and T3 levels in the group of children from Ivankivskyi district in contrast to the group of the children from Poliskyi district.

A direct link was observed between TSH and T3, and an inverse link was found between TSH and T4 in both groups of children, which indicated a TSH participation in the formation of T3 (tables 10, 11).

The obtained findings show that the presence of the T allele of the MTHFR:C677T genetic polymorphism contributes to the increased formation of Hc in the organism of the children living in the areas suffered from the accident at the Chernobyl NPP. Maximum Hc levels were reported in the group of the children who were the carriers of a homozygous variant of T allele.

Statistically significant differences between Hc values were found in both groups of the children under study between a subgroup with the 100 percent carriership of T allele and a subgroup without cases of carriership of T allele. However, blood Hc level was higher in the children from Ivankivskyi district than in those from Poliskyi district both in a total group and in the majority of genetic subgroups with the exception of a genetic subgroup which included cases of homozygous carriership of T allele.

It should be noted that 84 children from the examined group of Poliskyi district were examined again on 18.12.2015 simultaneously with the children from Ivankivskyi district. In this regard, an increase of Hc level in the blood was found in 78.6% of cases in comparison with the state on 02.04.2015 [9].

There were no statistical differences between blood Hc levels in the children with the MTHFR:677 T/T genotype at the marked differences in the children with the MTHFR:677 C/C genotype in this study as well (table 12) [9].

An increase of Hc level was registered in 78.6% of cases among the children of Poliskyi district at the examinations on 02.04.2015 and 18.12.2015.

Thus, it should be stated that a worse course of Hc metabolism is observed in the children from Ivankivskyi district examined on 18.12.2015 in comparison with those from Poliskyi district examined on 02.04.2015.

In addition, the direct links were reported between the Hc and TSH, Hc and T3 levels in the group of children from Ivankivskyi district. Thus, Hc can cause an increase in the production of TSH, and thereby affect the thyroid gland contributing to proliferative processes in its tissues. The correlative links between TSH and T3, T4 registered in both groups of children indicate that the pituitary hormone is involved in the metabolism of thyroid hormones.

In addition, in the presence of carriership of the G allele of the MTRR:66 A/G polymorphism, Hc can be converted to cysteine through cystathionine β-synthase, and then to selenocysteine, which forms the active center of deiodinase 5-DI, which catalyzes the conversion of T4 to T3. Due to this mechanism, Hc levels are lower in this genetic subgroup than in others. At the same time, increased production of T3 can cause serious cardiac abnormalities.

The absence of statistical differences in the occurrence of FC

Table 4

<table>
<thead>
<tr>
<th>Genetic subgroups</th>
<th>Comparison groups</th>
<th>Comparison group size</th>
<th>Average rank</th>
<th>U test value, significance level, p</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTR:2756 A/A</td>
<td>1</td>
<td>104</td>
<td>94.65</td>
<td>U = 4384.0; p = 0.001</td>
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<tr>
<td></td>
<td>2</td>
<td>106</td>
<td>116.14</td>
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<tr>
<td>MTR:2756 A/G+</td>
<td>1</td>
<td>54</td>
<td>50.25</td>
<td>U = 1228.5; p = 0.0001</td>
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<tr>
<td>MTR:2756 G/G</td>
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<td>72</td>
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</tr>
<tr>
<td>MTHFR:1298 A/A</td>
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<td>82</td>
<td>73.93</td>
<td>U = 2659.5; p = 0.002</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>89</td>
<td>97.12</td>
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</tr>
<tr>
<td>MTHFR:1298 A/C+</td>
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<td>76</td>
<td>72.19</td>
<td>U = 2560.5; p = 0.007</td>
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<tr>
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<td>92.23</td>
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<tr>
<td>MTHFR:677 C/C</td>
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<td>66.18</td>
<td>U = 2068.5; p = 0.0001</td>
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<tr>
<td></td>
<td>2</td>
<td>80</td>
<td>93.64</td>
<td></td>
</tr>
<tr>
<td>MTHFR:677 C/T +</td>
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<td>79</td>
<td>81.53</td>
<td>U = 3281.0; p = 0.082</td>
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<td>80</td>
<td>95.02</td>
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<tr>
<td>MTHFR:677 C/T</td>
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<td>62.45</td>
<td>U = 1917.0; p = 0.019</td>
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<tr>
<td></td>
<td>2</td>
<td>83</td>
<td>78.90</td>
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</tr>
<tr>
<td>MTHFR:677 T/T</td>
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<td>15</td>
<td>17.05</td>
<td>U = 134.0; p = 0.768</td>
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<tr>
<td>MTRR:66 A/A</td>
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<td>32</td>
<td>26.33</td>
<td>U = 314.5; p = 0.074</td>
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<td>MTRR:66 A/G+</td>
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<td>MTRR:66 G/G</td>
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<tr>
<td>Total group</td>
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<td>158</td>
<td>145.64</td>
<td>U = 10450.5; p = 0.0001</td>
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<tr>
<td></td>
<td>2</td>
<td>178</td>
<td>188.79</td>
<td></td>
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Table 5

<table>
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<th>Gene, polymorphism</th>
<th>&quot;Neutral&quot; allele</th>
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<td>Absolute number (n)</td>
<td>Percentage, %</td>
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<td>MTR:A2756G</td>
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<td>65.8</td>
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<td>51.9</td>
</tr>
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<td>MTHFR:C677T</td>
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<td>50.0</td>
</tr>
<tr>
<td>MTRR:A66G</td>
<td>32</td>
<td>20.3</td>
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genetic variants among examined groups allows to suggest that an increase in Hc concentrations in the body of children may be associated with the effect of an environmental factor during the period between the two examinations. This factor is related to forest and peat fires in the Chornobyl exclusion zone in an area of 10.127 ha during the period from 29.04.2015 to 05.07.2015 [11].

In spring and summer of 2015, air currents containing products of combustion of wood, peat, and grass, including radioactive elements spread over long distances from fire sites and affected the population of Ivankivskyi and Poliskyi districts of Kyiv region.

In particular, the 137Cs concentration amounted to 2.5x10^-3 Bq/m³ in an air sample taken directly in the fire area on the outskirts of the Poliskyi settlement, which substantially exceeded the reference level established by the exposure standards "Basic reference, clearance and action levels with regard to the radioactive contamination of the facilities of the zone of exclusion and zone of unconditional (obligatory) resettlement" [11].

It is known that inhalation of combustion products, as well as smoking, induces the formation of Hc in the organism [1, 12].

Thus, a deterioration of Hc metabolic processes in the children from Ivankivskyi district in comparison with those from Poliskyi district is not associated with homozygous carriergership of the T allele of the MTHFR:C677T polymorphism. One can reasonably suggest that there is a connection between fires in the Chornobyl exclusion zone and abnormal functioning of FM in a child’s organism resulting in increased blood levels of Hc.

The impact of the forest fires in the Chornobyl exclusion zone as an environmental factor on the developing organism contributes to hyperhomocysteinemia and identification of direct link between Hc and TSH, H3, and T3.

Conclusions

1. Blood Hc level were statistically significantly higher in the subgroup of the children who were the carriers of T risk allele of the MTHFR:C677T genetic polymorphism, living in Ivankivskyi and Poliskyi districts of Kyiv region, than in the subgroup of the children without this allele.

2. The examination of the children from the regions adjacent to
the Chornobyl nuclear power plant allowed to identify changes in metabolic processes among them as the increased production of H₃ due to the forest fires in the exclusion zone.

3. Blood H₃ levels were higher in the children from Ivankivskyi district examined after the fires in the Chornobyl exclusion zone than in those from Poliskyi district examined before fires, including the majority of genetic subgroups, with the exception of the genetic subgroup which included cases of homozygous carriership of T allele.

4. Direct links were detected between blood H₃ and TSH levels, and H₃ and T₄ levels in the group of children from Ivankivskyi district who had higher blood H₃ concentrations in comparison with the group of the children from Poliskyi district.

5. It can be reasonably assumed that fires in the Chornobyl exclusion zone have a negative effect on the functioning of folate metabolism in a child’s organism.

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Table 10

<table>
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<th>Parameter</th>
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<tr>
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<td>H₃</td>
<td>TSH</td>
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<tr>
<td>H₃</td>
<td>Spearman’s 1.000</td>
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</tr>
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<td>Sign. (2-tailed), p .</td>
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<tr>
<td></td>
<td>N</td>
<td>158</td>
</tr>
<tr>
<td>TSH</td>
<td>Spearman’s -0.002</td>
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<tr>
<td></td>
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<tr>
<td>T₃</td>
<td>Spearman’s 0.130</td>
<td>0.211**</td>
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<td>T₄</td>
<td>Spearman’s 0.0001</td>
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Table 11

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<td>H₃</td>
<td>Spearman’s 1.000</td>
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<td>Sign. (2-tailed), p .</td>
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<tr>
<td>TSH</td>
<td>Spearman’s 0.206**</td>
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<td>Sign. (2-tailed), p 0.006</td>
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<td>T₃</td>
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<td>Sign. (2-tailed), p 0.037</td>
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Table 12

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<tr>
<th>Genotype</th>
<th>Number of Cases</th>
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<th>Blood H₃ levels, µmol/L</th>
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<tr>
<td></td>
<td>H₃</td>
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<td>Me</td>
</tr>
<tr>
<td>C/C MTHFR:677</td>
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<td>20</td>
<td>45.5</td>
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<tr>
<td>T/T MTHFR:677</td>
<td>11</td>
<td>9</td>
<td>81.8</td>
</tr>
</tbody>
</table>

Note: Measurement I - 02.04.2015; Measurement II - 18.12.2015; 1 - statistical differences between Measurements I and II, p<0.05; 2 - statistical differences between Measurements I and II (Wilcoxon T-test standardized score, Z – 4.773, asymptotic significance (two-tailed), p=0.0001); 3 - statistical differences between Measurements I and II (Wilcoxon T-test standardized score, Z – 1.156, asymptotic significance (two-tailed), p=0.248).
and Lifshitz G.I.

tenparticles are associated with
Normative Aging Study.
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еlevated homocysteine. The VA
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