

## The Fluctuations in Homocysteine Level Caused by Various Combinations of Folic Acid Cycle Genes Snp Alleles as a Factor in the Course of Pregnancy Violation

Komlichenko EV<sup>1</sup>, Fedotov YN<sup>2</sup>, Uvarova MA<sup>3</sup> and Ivanov AV<sup>2,3\*</sup>

<sup>1</sup>Clinic of Obstetrics and Gynecology, Saint-Petersburg State Medical University named after Academician I.P. Pavlov, Saint-Petersburg, Russia

<sup>2</sup>Clinic of Advanced Medical Technologies, Saint-Petersburg State University named after N.I. Pirogov, Saint-Petersburg, Russia

<sup>3</sup>North-West Centre for Evidence-Based Medicine, Saint-Petersburg, Russia

\*Corresponding author: Andrei Vladimirovich Ivanov, Head of Department, University Hospital of Saint-Petersburg State University, Human Genetics 154, Fontanka embankment, Saint-Petersburg, Russian Federation, Tel: +79313605852; E-mail: [anivanov@omrb.pnpi.spb.ru](mailto:anivanov@omrb.pnpi.spb.ru)

Received date: January 24, 2018; Accepted date: February 15, 2018; Published date: February 22, 2018

Copyright: © 2018 Komlichenko EV, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

### Abstract

The presence of pathological alleles of single nucleotide polymorphisms (SNP) of the folic acid cycle genes is one of the female reproductive system violation factors including habitual miscarriage and pre-eclampsia. The realizing mechanism for this genetic predisposition is hyperhomocysteinemia-homocysteine level increasing in the blood. This study presenting an attempt to find the relationship between the genotype for the four SNPs of the three folate cycle genes - C677T and A1298C of the MTHFR gene, A2756G of the MTR gene and the A66G of the MTRR gene and the homocysteine level in the blood of women with impaired pregnancy.

As a result no direct correlation was found but it was found a statistically significant interrelation between the presence of pathological alleles of the studied SNP and the mean square deviation ( $\sigma$ ) of the homocysteine level fluctuations over time. For the polymorphism C677T of the MTHFR gene  $\sigma$  of the homocysteine blood level fluctuation is increased up to four times in women with a homozygous pathological state TT compared with the normal homozygotes CC.

The clinical importance of monitoring the homocysteine blood level has been shown especially for women with folate cycle genes pathological alleles' presence.

**Keywords:** Homocysteine; Single nucleotide polymorphisms (SNP); Folic acid cycle; Habitual miscarriage of pregnancy; Pre-eclampsia

### Introduction

Female reproductive system disorders are the classic example of the multifactorial diseases. Violations of pregnancy leading to miscarriages and pre-eclampsia, the usual failures of *in vitro* fertilization and other types of assisted reproduction techniques is usually leaded by a host of unfavorable factors. One of these factors is the presence of folic acid cycle genes single nucleotide polymorphisms (SNP) pathological alleles. Previously it was demonstrated that in order to assess the role of polymorphisms in assessing the risk of gynecological disease development an integrated assessment of a number of indicators is desirable [1]. Wherein, the presence of SNP pathological alleles in the clotting factors genes, the renin-angiotensin system genes, the detoxification system genes and the folic acid cycle genes is quite significant. For a fairly narrow group of women with a habitual failure of IVF the polymorphisms C677T of the MTHFR gene and A66G of the MTRR gene exert the greatest influence as well as SNP A2756G of the MTR gene in combination with pathological alleles in the MTHFR and MTRR genes even as heterozygote [2].

Since the presence of SNP pathological alleles in the genome cannot itself lead to the development of adverse physiological consequences it is logical to assume that it is the homocysteine blood level affects the

female reproductive system disorders. This increase in the free homocysteine level is called hyperhomocysteinemia and it can be caused both by hereditary factors (folic acid cycle enzymes disorders caused by mutations in the corresponding genes) and by environmental factors: folate, cobalamine and pyridoxine deficiency, which are necessary co-substrates or cofactors for homocysteine metabolism, use of several drugs for example as methotrexate, as well as physiological factors such chronic renal failure. It is hyperhomocysteinemia is a factor instigating the development of various multifactorial diseases such as cardiovascular disorders [3], metabolic syndrome in all manifestations [4], carcinogenesis [5], neurodegenerative diseases [6], schizophrenia [7].

In addition to the homocysteine level another active agent that causes the adverse effect of the folate cycle genes pathological alleles on the female reproductive system is the epigenetic change in the pattern of a number of genes expressions due to the regulatory regions methylation deficiency [8].

In the present study an attempt has been undertaken to compare the homocysteine level in the blood of women with reproductive disorders with the genotype for the most significant SNP of the folic acid cycle genes network. Previously in this field the advantage of simultaneous analysis of several polymorphisms of the folic acid cycle genes was demonstrated [1,2]. As a result of the analysis of the available data, four polymorphisms were selected for further analysis: 667 C>T (rs1801133) and 1298 A>C (rs1801131) of the MTHFR gene, 2756

A>G of the MTR gene (rs1805087) and 66 A>G of the MTRR gene (rs1801394).

## Materials and Methods

### Study participants

Participants of this study were women of the European population, reproductive age, residents of the North-West region of Russia. Representatives of the problem sample (further – patients) applied to the University Hospital of Saint-Petersburg State University for the violations of pregnancy treatment from January 2016 to March 2017. These women were not pregnant during the study participation and biomaterials collection. In total there were 131 patients. The criteria for inclusion in this sample were: the age of 22-45 years, the absence of pernicious habits (smoking, alcoholism, and drug addiction), the absence of chronic (HIV, HCV) and oncological diseases, the presence of spontaneous interruptions of pregnancy in the anamnesis. The criterion for exclusion was the absence of the most significant for the pregnancy course mutations in the thrombophilia factors genes FII 20210 G>A and FV R506Q G>A (Leiden mutation).

Representatives of the control sample were residents of the North-West region of Russia who had at least one child and did not presenting complaints about the pregnancy course. In this sample there were 46 persons. All study participants have filled informed consent to the data processing; the study was approved by the University Hospital of Saint-Petersburg State University Ethics Committee.

### Genotyping

The determination of the SNP alleles of the folic acid cycle genes was carried out by Real-Time PCR. For this purpose DNA samples were isolated from venous blood samples of patients by absorption on magnetic silica using reagents "AmpliSens" produced by Federal Budget State Organization "Central Research Institute of Epidemiology", Moscow, Russia and the automated installation XIRIL, XIRIL AG, Switzerland. The amount of DNA obtained was monitored with a spectrophotometer and was 50-80 ng. Real-Time PCR was performed using a reagent kit and a thermocycler "DNA-Technology Prime" production of LLC "DNA-Technology", Moscow, Russia in strict accordance with the manufacturer's instructions. The SNP genotype was determined by melting curves analyzing using software LLC "DNA-Technology", Moscow, Russia.

### Homocysteine concentration

Determination of blood homocysteine concentration was carried out by the method of chemiluminescent immunoassay on paramagnetic particles. Blood was taken on an empty stomach by venous puncture with the use of vacuum systems Lind-Vac (Corway, Estonia). Immunoassay was carried out using an automated station and software *ARCHITECT i2000* Analyzer (Abbott, USA) in strict accordance with the manufacturer's instructions.

### Statistical analysis

For statistical processing of the results of genotyping and evaluation of the correlation of the genotype with the blood homocysteine level, and evaluation of the disease developing risk depending on the genotype the software of the Scientific Center of Russian Federation

Research Institute for Genetics and Selection of Industrial Microorganisms (Moscow, Russia) was used.

To assess the differences significance in the parameters of different samples by the genotype, the Pearson  $\chi^2$  criterion was applied. In determining the significance of differences in genotypes the general model of inheritance was used and the multiplicative model of inheritance for allele frequencies was used. With a value of  $p < 0.05$  the results were considered to be statistically significant.

To describe the relative risk of disease developing the odds ratio (OR) was calculated. Wherein OR values close to 1 were considered as a lack of correlation,  $OR > 1$  were considered as a positive association (increased risk of disease developing),  $OR < 1$  were considered as a negative association of a rare allele of polymorphism with a risk of pathological condition developing.

To estimate the individual spread of homocysteine levels in the blood of one patient the mean square deviation  $\sigma$  were calculated. The standard Microsoft EXCEL XP statistical package was used.

## Results

### Demographic characterization of the participants

The average age of the patients was  $33.36 \pm 6.6$  years. The average age of the control group participants was  $35.44 \pm 6.9$  years.

Genotyping results for the four SNP in genes whose corresponding proteins are actively involved in homocysteine metabolism are presented in Tables 1 and 2.

Gene, SNP	Patients, % (n)	Control, % (n)	p	χ <sup>2</sup>	OR (95% CI)
MTHFR C677T					
C/C	53.44 (70)	67.4 (31)	0.08	5.08	0.56 (0.27-1.12)
C/T	29.77 (39)	28.3 (13)			1.08 (0.51-2.26)
T/T	16.79 (22)	4.3 (2)			4.44 (1-19.69)
MTHFR A1298C					
A/A	45.04 (59)	65.2 (30)	0.06	5.59	0.44 (0.22-0.88)
A/C	42.75 (56)	26.1 (12)			2.12 (1.01-4.45)
C/C	12.21 (16)	8.7 (4)			1.46 (0.46-4.62)
MTR A2756G					
A/A	64.89 (85)	47.8 (22)	0.06	5.76	2.02 (1.02-3.98)
A/G	30.53 (40)	50 (23)			0.44 (0.22-0.87)
G/G	4.58 (6)	2.2 (1)			2.16 (0.25-18.44)
MTRR A66G					
A/A	18.32 (24)	39.1 (18)	0.02	8.26	0.35 (0.17-0.73)
A/G	52.67 (69)	37 (17)			1.9 (0.95-3.78)
G/G	29.01 (38)	23.9 (11)			1.3 (0.60-2.82)

**Table 1:** Identified genotypes of four SNPs in three folic acid cycle genes in patients and control group representatives.

Allele frequencies	Patients, % (n)	Control, % (n)	p	$\chi^2$	OR (95% CI)
<b>MTHFR C677T</b>					
C	68.32	81.5	0.02	5.85	0.49 (0.27-0.88)
T	31.68	18.5			2.05 (1.14-3.68)
<b>MTHFR A1298C</b>					
A	66.41	78.3	0.03	4.51	0.55 (0.31-0.96)
C	33.59	21.7			1.82 (1.04-3.18)
<b>MTR A2756G</b>					
A	80.15	72.8	0.14	2.15	1.51 (0.87-2.61)
G	19.85	27.2			0.66 (0.38-1.15)
<b>MTRR A66G</b>					
A	44.66	57.6	4.58	0.03	0.59 (0.37-0.96)
G	55.34	42.4			1.68 (1.04-2.72)

**Table 2:** Identified allele frequencies of four SNPs in three folic acid cycle genes in patients and control group representatives.

In general the detected allele frequencies coincide with database of allele frequency data U.S. National Science Foundation, placed <http://alfred.med.yale.edu/alfred/index.asp>. The data obtained correspond to the Hardy-Weinberg principle. From the results of genotyping it follows that the presence of pathological alleles of SNP in the genes of the folic acid cycle is significantly higher in patients with pathological pregnancy than in the control group. This observation corresponds to the literature data.

Search for the association of the blood homocysteine level and various variants of the genotype according to the SNP of the folic acid cycle genes.

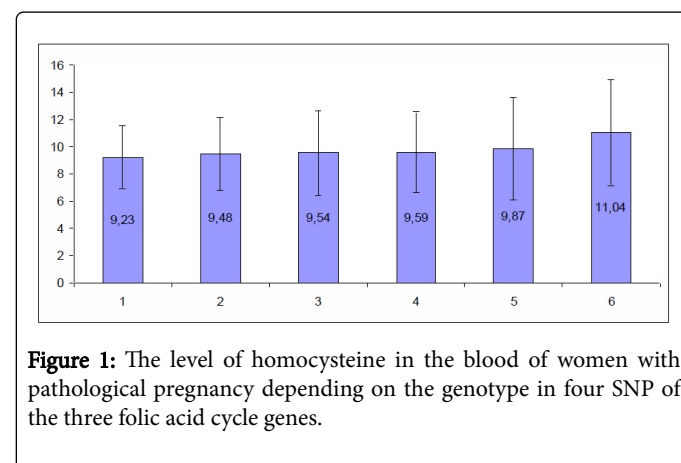
In all patients' blood sampling to measure homocysteine level was performed at the same time as blood sampling for genotyping. In 88 patients this was determined once, in 43 - several times within several months. For these patients the mean value was calculated. As a result the average homocysteine level for all participants in the problem sample was  $9.57 \pm 3.08 \mu\text{mol/l}$ .

Then all the patients were divided into six groups depending on the genotype. A graphical representation of the homocysteine level in each of these groups is shown in Figure 1.

The first group included patients with at least one pathological homozygous condition in any of the four SNPs tested. Such patients were 67, No. 3 in Figure 1. In contrast, the second group consisted of 64 patients without pathologic homozygous conditions, only normal homozygous and heterozygous (No. 4 in Figure 1). Apart from these two groups were distinguished group No. 3 comprising the carriers of only normal homozygous genotype at polymorphism C677T MTHFR gene without homozygous pathological states of other polymorphisms (value is 38, No. 2 in Figure 1), its opposite group No. 4 comprising only carriers of pathological homozygous genotype at polymorphism C677T MTHFR gene (22 persons, No. 6 in Figure 1), the group No. 5, comprising only carriers of pathological homozygous genotype at

polymorphism A2756G MTR gene (6 persons, No. 1 in Figure 1) and the group 6, comprising only carriers of pathological homozygous genotype at polymorphism A66G MTRR gene (value is 38, No. 5 in Figure 1).

Despite a visible tendency to increase the homocysteine level in carriers of pathological homozygous SNP states, especially in polymorphism of C677T of the MTHFR gene, no statistically significant differences between the groups were detected.



**Figure 1:** The level of homocysteine in the blood of women with pathological pregnancy depending on the genotype in four SNP of the three folic acid cycle genes.

On the abscissa axis: Group No.:

1. Pathologic homozygous in A2756G of MTR gene (6 persons),
2. Normal homozygous in C677T of MTHFR gene (38 persons),
3. Pathologic homozygous in any of SNP tested (67 persons),
4. Normal homozygous and heterozygous in any of SNP tested (64 persons),
5. Pathologic homozygous in A66G of MTRR gene (38 persons),
6. Pathologic homozygous in C677T of MTHFR gene (22 persons).

On the ordinate axis: Average homocysteine level in the blood,  $\mu\text{mol/l}$ . The value is also indicated in the middle of each column.

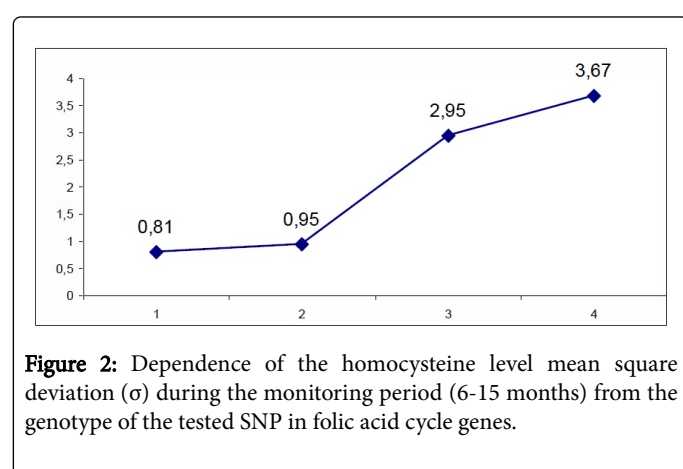
Individual fluctuations of homocysteine levels in patients.

Since there was found no direct correlation between the blood homocysteine level from the genotype by the main SNP in folate cycle genes the question arises about the mechanism for the realization of the destructive effect of these polymorphisms pathological alleles presence on the course of pregnancy. Since for some patients the homocysteine level monitoring for some time (6-15 months) were prescribed it was an opportunity to analyze the data on the individual variations in homocysteine level. As the initially defined homocysteine levels in the overwhelming majority did not exceed the threshold values defined for women of this age group as  $13.56 \mu\text{mol/l}$  (except for 8 cases) no compensatory therapy was prescribed and measured homocysteine levels fluctuations in patients are due only to natural physiological processes.

In total homocysteine levels were monitored in 43 patients. In 23 of them homocysteine was measured twice, in eight patients it was three times, in another eight it was four times and in four women there were five dimensions during the observation period. For each of these patients the homocysteine level mean square deviation ( $\sigma$ ) was calculated as the function most accurately describing the fluctuations in the homocysteine level. A graphical representation of the dependency  $\sigma$  of homocysteine level versus genotype according to the

SNP folic acid cycle genes is shown in Figure 2. For perception convenience and in connection with a smaller number of patients four groups were identified: Normal homozygous CC in C677T of the MTHFR gene, Normal homozygous and heterozygotes in all polymorphisms studied, pathological homozygotes in any of the polymorphisms studied pathological homozygotes TT in the SNP C677T of the MTHFR gene.

In this case a clear dependence of  $\sigma$  to the presence of pathological alleles in the SNP of the folic acid cycle genes is revealed especially for the studied SNP in the pathological homozygous state. For the polymorphism C677T of the MTHFR gene fluctuations in the homocysteine blood level  $\sigma$  is increased up to four times (3.67 vs. 0.81) in women with a homozygous pathological state TT compared with the homozygotes of the CC.



**Figure 2:** Dependence of the homocysteine level mean square deviation ( $\sigma$ ) during the monitoring period (6-15 months) from the genotype of the tested SNP in folic acid cycle genes.

On the abscissa axis: Group No.:

1. Normal homozygous in C677T of MTHFR gene (24 persons),
2. Normal homozygous and heterozygous in any of SNP tested (18 persons),
3. Pathologic homozygous in any of SNP tested (25 persons),
4. Pathologic homozygous in C677T of MTHFR gene (8 persons).

On the ordinate axis: homocysteine level mean square deviation ( $\sigma$ ).

## Discussion

The increase in the frequency of pathological alleles of the folic acid cycle genes in women with disorders of the pregnancy course, as compared to the control group, revealed in the present study is supported by numerous literature data, for example [9]. However, the lack of direct correlation between the genotype of the four most clinically significant SNPs and the blood homocysteine level detected is in some contradiction with the literature data, for example [10]. This phenomenon can be pretty explained, on the one hand, by the features of the sample being analyzed and on the other hand, by the difference in the mean square deviation of the homocysteine level fluctuation over time in specific patients.

The presence of pathological alleles in the folate cycle genes does not directly increase the level of homocysteine in the blood but causes its instability, considerable fluctuations from physiological values up to levels several times higher than normal. Apparently the amplitude of such oscillations is determined precisely by the SNP genotype of the folate cycle.

The role of genetic variability in the folic acid cycle genes and the homocysteine level in the various pathological processes development.

Features of the synthesis of homocysteine, the multistage nature of its regulation, the multiplicity of its molecular precursors are the cause of the multifaceted action of this component on a variety of biological processes. This phenomenon is most vividly demonstrated in experimental models using laboratory animals but a significant amount of data has been accumulated with respect to humans. This primarily affects cardiovascular disorders from which the role of homocysteine has been described since the 1960s, review [3]. The influence of homocysteine and folate cycle genes on the developmental features of the neural crest and neural tube during embryogenesis is described [11], the toxic effect of increased blood homocysteine concentration on the vascular epithelium, leading to ischemic strokes and neurotoxicity is shown [12]. It is demonstrated that the effect of increased homocysteine doses on the developing cardiac neural crest leads to the congenital heart defects development [13].

Recently the role of homocysteine in the osteoporosis and bone diseases development has been noted [14]. As the proposed molecular mechanism the authors consider the modulation of osteoclastogenesis which exerts its negative impact on the bone by degradation of the extracellular matrix mediated by oxidative stress as well as the degradation of blood vessels and a decrease in blood flow in bone tissue.

Among the multitude of SNP in folic acid cycle genes the genotype of each polymorphism has a particular effect on the homocysteine level and various pathological conditions development. It is noted that the greatest clinical significance have the rare alleles of polymorphisms in the MTHFR gene, especially rs1801133. A statistically significant effect was also noted for polymorphisms in the genes RFC1, TCN2, BHMT, CBS [15], MTR, MTRR [1]. For the polymorphisms in other folate cycle genes such as MTHFD1, SHMT1 and SLC19 no statistically significant effect was found.

The importance of the homocysteine level monitoring instead of a single measurement becomes particularly important in event of the administration of a number of folic acid antagonist drugs such as methotrexate. Chinese researchers have shown that hyperhomocysteinemia caused by the pathological allele T of the C677T polymorphism in MTHFR gene causes increased toxicity of methotrexate during the rheumatoid arthritis treatment. Wherein there was not marked the dependency of the drug effectiveness against the MTHFR genotype. In total 6436 cases of methotrexate administration were analyzed in this study [16]. The authors also note a significant variability in the severity of methotrexate toxicity among different ethnic groups and draw attention to the independence of this effect from compensatory therapy with folic acid preparations.

## The gene network of the folic acid cycle as a component of the suertal system

Such a multifaceted influence of different allelic variants in several genes in combination with the regulation of their activity mechanisms not yet fully understood allow us to talk about them as a predetermining, suertal genes (from Spanish "Suerte" – in different translation options: fortune, chance, destiny, fate). Significant role in epigenesis, the dependence of expression on a variety of epigenetic and environmental factors, peculiarities of the individual behavior, allow one to draw conclusions about the stochastic multifactor character of the manifestation of inherent genetic information. A suchlike

phenomenon brings folate cycle genes close together the with a number of other hemostatic regulators such as some surface antigens, for example CD44 [17] and CD99 [18], transcription factors MED29 [19] and JAK2 [20,21].

A special role among such a selection of suertal genes is engaged by epigenetic factors: regulators of CpG islets methylation [8], histones acetylation [22] and siRNA [23]. Is a matter of principle it is to this group that genes of the folic acid cycle should be included.

This conclusion is summed up by the above facts about the multifaceted role of the folic acid cycle genes for human physiology. And the blood homocysteine level is not the only indicator controlled by this gene network. The dependence of homocysteine level fluctuations amplitude on the SNP genotype of the folic acid cycle genes revealed in the presented study only emphasizes the need for using data integration methods to search for genotype-phenotype interactions with the use of several OMIK technologies [24].

### Possible clinical application of the study results

The present study shows the role of homocysteine level fluctuations as a negative factor for the course of pregnancy. It was also demonstrated that there was no direct correlation between the blood homocysteine level and the genotype for the most clinically significant polymorphisms of the follic acid cycle genes. This observation points to the undoubted clinical importance of the homocysteine level monitoring not allowing its sharp jumps. It seems rather likely that often a defined once certain homocysteine level can mislead the treating doctor which can lead to mistakes in the treatment tactics choice.

For example, in Russia, genetic testing for pregnancy problems is often prescribed only when observing an elevated homocysteine level. But as already mentioned, homocysteine is a component of a complex multistage system and at certain times its concentration is within the physiological norm even in the presence of an unfavorable SNP genotype in the folic acid cycle genes. The reasons may be the features of the diet, hormonal and neurological status of women. The slightest deviation from a harmonious combination of factors leads to a sharp increase of the homocysteine level often not noticed by the doctor and leads to the pathological physiological states development.

### Conclusion

Monitoring the homocysteine level for a sufficiently long period of time together with the definition of the main clinically significant SNP (not only in folate cycle genes but also in thrombophilic factors, hypertension, detoxification system) is able to correctly display the current situation. This view for the clinical role of homocysteine is consistent with [25].

### Conflict of Interests

The authors declare the conflict of interests absence. The study was partially presented on the 9th World Biomarkers Congress, Madrid, Spain, 06-09 December 2017.

### References

- Ivanov AV, Dedul AG, Fedotov YN, Komlichenko EV, et al. (2016) Toward optimal set of single nucleotide polymorphism investigation before IVF. *Gynecol Endocrinol* 32: 11-18.

- Uvarova MA, Ivanov AV, Dedul AG, Sheveleva TS, Komlichenko EV (2015) The effect of single nucleotide genetic polymorphisms of folic acid cycle on the female reproductive system disorders. *Gynecol Endocrinol* 31: 34-38.
- Durand P, Prost M, Loreau N, Lussier-Cacan S, Blache D (2001) Impaired homocysteine metabolism and atherothrombotic disease. *Lab Invest* 81: 645-672.
- Ntaios G, Savopoulos C, Chatzopoulos S, Mikhailidis D, Hatzitolios A (2011) Iatrogenic hyperhomocysteinemia in patients with metabolic syndrome: A systematic review and meta-analysis. *Atherosclerosis* 214: 11-19.
- Keshteli AH, Baracos VE, Madsen KL (2015) Hyperhomocysteinemia as a potential contributor of colorectal cancer development in inflammatory bowel diseases: A review. *World J Gastroenterol* 21: 1081-1090.
- Sharma M, Tiwari M, Tiwari RK (2015) Hyperhomocysteinemia: Impact on Neurodegenerative Diseases. *Basic Clin Pharmacol Toxicol* 117: 287-296.
- Moustafa AA, Hewedi DH, Eissa AM, Frydecka D, Misiak B (2014) Homocysteine levels in schizophrenia and affective disorders-focus on cognition. *Front Behav Neurosci* 8: 343.
- Esteller M (2002) CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Oncogene* 21: 5427-5440.
- Mtiraoui N, Zammiti W, Ghazouani L, Braham NJ, Saidi S, et al. (2006) Methylenetetrahydrofolate reductase C677T and A1298C polymorphism and changes in homocysteine concentrations in women with idiopathic recurrent pregnancy losses. *Reproduction* 131: 395-401.
- Ni J, Zhang L, Zhou T, Xu WJ, Xue JL, et al. (2017) Association between the MTHFR C677T polymorphism, blood folate and vitamin B12 deficiency and elevated serum total homocysteine in healthy individuals in Yunnan province, China. *J Chin Med Assoc* 80: 147-153.
- Murphy MM, Fernandez-Ballart JD (2011) Homocysteine in pregnancy. *Adv Clin Chem* 53: 105-137.
- Lehotsky J, Tothova B, Kovalska M, Dobrota D, Benova A, et al. (2016) Role of homocysteine in the ischemic stroke and development of ischemic tolerance. *Front Neurosci* 10: 538.
- Rosenquist TH (2013) Folate, homocysteine and the cardiac neural crest. *Dev Dyn* 242: 201-218.
- Behera J, Bala J, Nuru M, Tyagi SC, Tyagi N (2017) Homocysteine as a pathological biomarker for bone disease. *J Cell Physiol* 232: 2704-2709.
- Liang S, Zhou Y, Wang H, Qian Y, Ma D, et al. (2014) The effect of multiple single nucleotide polymorphisms in the folic acid pathway genes on homocysteine metabolism. *Biomed Res Int* 560183.
- Shao W, Yuan Y, Li Y (2017) Association between MTHFR C677T polymorphism and methotrexate treatment outcome in rheumatoid arthritis patients: A systematic review and meta-analysis. *Genet Test Mol Biomarkers*. 21: 275-285.
- Louderbough JM, Schroeder JA (2011) Understanding the dual nature of CD44 in breast cancer progression. *Mol Cancer Res* 9: 1573-1586.
- Scotlandi K, Zuntini M, Manara MC, Sciandra M, Rocchi A, et al. (2007) CD99 isoforms dictate opposite functions in tumour malignancy and metastases by activating or repressing c-Src kinase activity. *Oncogene* 26: 6604-6618.
- Kuuselo R, Savinainen K, Sandström S, Autio R, Kallioniemi A (2011) MED29, a component of the mediator complex, possesses both oncogenic and tumor suppressive characteristics in pancreatic cancer. *Int J Cancer* 129: 2553-2565.
- Leroy E, Constantinescu SN (2017) Rethinking JAK2 inhibition: towards novel strategies of more specific and versatile janus kinase inhibition Leukemia.
- Chen E, Mullally A (2014) How does JAK2 V617F contribute to the pathogenesis of myeloproliferative neoplasms? *Hematol Am Soc Hematol Educ Program* 1: 268-276.
- Sabari BR, Zhang D, Allis CD, Zhao Y (2017) Metabolic regulation of gene expression through histone acylations. *Nat Rev Mol Cell Biol* 18: 90-101.

- 
23. Joh RI, Palmieri CM, Hill IT, Motamedi M (2014) Regulation of histone methylation by noncoding RNAs. *Biochim Biophys Acta* 1839: 1385-1394.
  24. Ritchie MD, Holzinger ER, Li R, Pendergrass SA, Kim D (2015) Methods of integrating data to uncover genotype-phenotype interactions. *Nat Rev Genet* 16: 85-97.
  25. Newton LA, Sandhu K, Livingstone C, Leslie R, Davis J (2010) Clinical diagnostics for homocysteine: A rogue amino acid? *Expert Rev Mol Diagn* 10: 489-500.