WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 3, Mar., 2022

REMARKABLE ASSOCIATION OF THE HIGHLY FREQUENT rs1801133 SNP OF MTHFR GENE WITH GROWTH HORMONE DEFICIENCY IN CHILDREN

Haider Turky Mousa Al-Mousawi
Al-Qasim Green University/ College of Biotechnology/ Babylon Province.
Corresponding author: dr.haider.almusawi@biotech.uoqasim.edu.iq
*ORCID ID: https://orcid.org/0000-0001-9292-4611

Abstract

This study was conducted to investigate the possible association of the rs1801133 SNP located in the methylenetetrahydrofolate reductase (MTHFR) gene through for the development of growth hormone deficiency in Iraqi children. In this study, one genetic locus of 232 bp was designed for amplification. In this locus, a high-frequency single nucleotide polymorphism (SNP) rs1801133 was targeted. Then, a direct sequencing stratagem was accomplished for designed the PCR amplicons to evaluate the pattern of these SNPs variation among the investigated population. Results identified three forms of the highly polymorphic rs1801133 SNP, namely the normal homozygous (G/G), the mutant heterozygous (G/C), and the mutant homozygous (C/C). Both mutant forms were identified in the majority samples of patients, while the normal G/G form was observed in the majority of the control samples.

In conclusion, rs7007634 SNP is a possible causative SNP for our development of the growth hormone deficiency in the children, which revealed a remarkable association with the progression of this disease.

Keywords: Growth hormone, Growth hormone deficiency, MTHFR gene Polymorphism, PCR, SNP, genetic mutations

1. Introduction

Growth hormone (GH) is a 191-amino-acid polypeptide protein hormone that is synthesis and secreted by somatotroph cells in the anterior lobe of the pituitary gland and is the main regulator of stature during childhood. It promotes the growth of virtually all body tissues, including bone. It is the major contestant in control of various complicated physiological practices that including growth and metabolism [1]. Growth hormone deficiency (GHD), described as insufficient GH synthesis, is a unique but significant cause of short height in very young children, with an occurrence of one in every 4000 during childhood. GHD affects children growth by decreasing the synthesis and secretion of GH dependent growth factors similar to insulin like

growth factor I (IGF-I, IGF-II, and their binding proteins IGFBPs) [2,3]. GHD can be developing as a result of birth defects or intracranial neoplasms (i.e. cranio pharyngioma), infiltrative complaints i.e. trauma, Langerhans cell histio cytosis, cranial or Total Body Irradiation TBI, infection i.e. tuberculosis, HIV, and chemotherapy [4]. In children's, a proper diagnosis of GHD appears to require a multistep process that includes a clinical history and investigation with detailed auxology, biochemical testing, and THE magnetic resonance imaging (MRI) of the hypothalamic pituitary regions, with genetics endeavoring to play an increasing importance in patients with congenital GHD [3]. An autoimmune etiology may be expected based on the progress of circulating ant pituitary antibodies directed against of the GH secreting cells in some cases of GHD [5]. Moreover, early beginnings of growth disappointment, a genetic factors, a stature or less 3 SD below the norm, a very low GH response to aggravation tests, and the exact low IGF-I and IGFBP-3 levels are also indicators of inherited GHD [1,6].

Many genetic mutations have been reported in a number of individuals with GHD, and with the progress of new genetic knowledges, genetic screening may well play a greater influence in diagnostic algorithms in the future years. GH deficiency can be associated with mutations in transcription factors genes involved in pituitary gland development, which are typically linked to multiple pituitary hormone deficiencies; or through autosomal recessive, autosomal dominant, and X-linked recessive forms of GHD (Types I, II, III respectively), and multiple altered mutations in genes encoding growth hormone-1 (GH1) and GH releasing hormone receptor genes (GHRHR) [7]. Methylene tetra hydro folate reductase (MTHFR) is an important enzyme in one-carbon metabolism, which includes the metabolisms of folate and homocysteine. MTHFR catalyzes the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, along with folate and homocysteine (Hcy) conversion, which is linked to methylation of genomic DNA [8]. The MTHFR gene, that is present on chromosome 1 at 1p36.22, is involved in the biosynthesis of amino acids and purines. The complementary DNA sequence has quite a total length of 2.2 kb and has 12 exons of 2.2 kb, encoding a 697-amino-acid protein product [8,9].

There are various effective and common the single nucleotide polymorphisms (SNPs) have been predictable in the rs1801133 or (C677T), MTHFR gene, a missense mutations by transforms the nitrogen base cytosine (C) to thymine (T) at nucleotide position 677 in exon-4 of MTHFR gene and then involves the replacement of amino acid alanine to valine in the catalytic domain on codon 222 (Ala 222 Val) in the N-terminal catalytic dominion, diminishes its catalytic activity are predominantly give an account to be related with reduces enzyme activity by 35%, subsequent in a relative

deficiency in the remethylation process and intrusive with the metabolism pathway. It have been extensively established that rs1801133 increases blood total homocysteine (tHcy; free and protein bound homocysteine) [10].

There is an approximation that further than 60% of the general populations transmitted one of the two polymorphic alleles, as well as at least 10% of them transmits both the alleles being homozygotes (677TT) and / or multiple heterozygotes infrequently in Cis (CT) more frequently in trans (CT) in consideration of the strong linkage disproportion [11].

2.Methods

2.1 Extraction of Genetic material DNA

Genomic of the DNA from all isolated samples, was extracted using Genaid Equipment, a commercial purification system (Geneaid Biotech, Taiwan). The extraction was done following the manufacture's protocols recommended, Quantification of genomic DNA concentration and purity was determined, using a spectrophotometrically (Nano-drop) instrument to measurment the optical density, (O.D) (BioDrop LITE, BioDrop co., UK), and DNA integrity was checkered using astandard 0.8 percent (w/v) of the agarose gel electrophoresis prestained with higher concentration of the ethidium of bromide (0.7 g/ml) in TAE (40 mM of Tris-acetate; 2 mM of EDTA and pH 8.3) buffer and a 1 kb ladder as a PCR template was made from the isolated DNA.The DNA Extraction has been used to create a PCR template [12].

2.2 Primer design for the (PCR)

The GenBank accession number used in the design was NC 000001.11, as according NCBI Primer-BLAST software [13]. The forward primers seemed to have the sequence 5'-GGACGATGGGGCAAGTGATG-3', while the reverse primer already had sequence 5'-ATCCCTCGCCTTGAACAGGT-3'. The PCR fragment to use in this study was designed to cover 232bp of MTHFR DNA sequences. A specified highly polymorphic SNP identified rs1801133 was positioned in the 96th position of the specified segment.

2.3 PCR Polymerase chain reaction Cycling Profiles

Bioneer was indeed the manufacturer for the lyophilized primers (Bioneer, Daejeon, South Korea). Accu Power PCR premix (Cat # K-2012, Bioneer, Daejeon, South Korea) is used in the PCR reaction. Each 20 µl reaction volume tubes of PCR premix relates directly U of Top DNA polymerase, 250 M of dNTPs, 10 mM Tris-HCl (pH 9.0), 30 mM KCl, and 1.5 mM MgCl2. The illumination was prepared, by the mixing 10 pmol of each primer and was added 50 ng from the genomic DNA. The following, program

design is utilized in the PCR thermocycler (My Genie TM 96/384 Thermal Block, Bioneer , and Daejeon, South Korea.

Conditions of PCR amplification were performed with a thermal cycler specific to each single primer set depending on their reference procedure as following: the initial state is denaturation at 94°C for 5-minute, then the annealing at 61°C, and the elongation at 72°C, and a final extension at 72°C for 10 minutes finished with a 30-cycle. Amplification was resolute by using, a 100 bp ladder (Cat D-1010, Bioneer , Daejeon, South Korea) as the molecular weight markers on an ethidium of bromide (0.5 mg/ml) prestained 1.5 % (w/v) gel of agarose in 1X TBE buffer, (2 mM EDTA, 90 mM Tris Borate with pH at 8.3). To confirm that all PCR resolved bands were distinct and consisted of only one crisp and clear bands [14,15].

2.4 Sequencing of DNA from PCR amplicons

The determined of PCR amplicons was sequenced commercially following the protocols of the laboratories for sequencing (Geumchen, Seoul, Macrogen Inc. South of Korea). The simply pure chromatographs from the ABI sequence files were inspected promote, representative that the comment and variations were not induced by the PCR or the sequencing mistakes. By comparing the observed the sequences DNA of limited the samples with the obtained the sequences DNA of MTHFR gene sequences, the fundamental location and other information of the repossessed PCR fragments existed recognized [15,16].

2.5 Data analysis

The sequence outcomes of various PCR products existed edited aligned and evaluated by Bio Edit Sequence alignment editor software Version7.1, on condition that they matched the required sequencing in the reference databases (Danstar, Madison, WI of USA). The identified variants in every the sample sequencing were numbered in the PCR amplicons and their corresponding locations within the reference genome. The reference genome, location of the target SNP was indicated. As a result, looking at the relevant db SNP position revealed the presence of the targeted SNP. The confirmed SNP's location in db SNPs was then registered.

3. Results and Discussion of Sequencing Report

75 samples have been included in the locus, with 323bp amplicons of the MTHFR locus being designed. These amplicons are located on Chromosome1 and code for a crucial enzyme involved in regulating of the metabolism of folate, which playing a role in carcinogenesis via methylation of the DNA [17]. All amplified MTHFR amplicons

were evaluated for crisp, specific, and clean bands before being sent to sequencing assays. The sequencing reactions revealed the inveterate identity of the amplified products, through using NCBI blasts to determine their identity [18,19].

The sequences of similarity between the sequenced samples as well as this target were extremely high, as per NCBI BLASTn engine. According to the NCBI BLASTn engine, there was 99.9% similarity with the predicted target, which included a section of exon 2 of the MTHFR gene. The particular positions, and the other features of obtained the fragment for the PCR were recognized by associating the observed the sequencing DNA of these local the samples with the recovered the sequences of DNA (Gen Bank acc. NC 000001.11) (Supplementary Fig. 1). In comparison to the referencing MTHFR genetic sequences, the alignment resulting of the 232bp samples exposed the occurrence of the only one genetic variation that was inconsistently distributing in about of the investigated Samples (Fig. 1). Only the targeted rs1801133 SNP was found to have identified variants.

Figure (1) shows the DNA sequence alignment of the studied 232 bp amplicons with the MTHFR DNA sequences' corresponding reference sequencing, the symbol (ref.) was refers to the NCBI reference sequencing, the samples were numbered consecutively, and the target SNP was rs1801133.

The identified substitution variant's sequencing chromatograms were recorded, along with extensive annotations. The Chromatogram parts of the experimental variant were demonstrated conferring to their positions in the PCR amplicons, in which three polymorphic patterns, the wild type homozygous pattern (G/G), the mutant

heterozygous pattern (C/G), and the mutant heterozygous pattern (C/C), were identified in the targeted rs1801133 SNP (Fig. 2).

Only one variant of this polymorphic locus, homozygous G/G, was observed in all of the control samples (table 1). In the meantime, the G/G homozygous pattern is observed in alone twenty of the fifty patient samples analyzed. Only fifteen out of seventy five samples seemed to have the heterozygous pattern of G/C, and all of these samples belonged to patient. The same thing was observed in the C/C form, which was confirmed in only patients with a high rate of occurrence (fifteen out of fifty) (table 2).

Table (1): Distribution of genotype and allele frequency of the rs1801133 SNP in control group

rs1801133 SNP /Healthy	Observed	Expected	P.Value
GG	25	25	1.00
GC	0	0	
CC	0	0	
Allele frequency	p(1.00)	q(0.00)	

Table (2): Dispersal of Genotype and Allele frequency of the rs1801133 SNP in the patients group.

rs1801133 SNP /patients	Observed	Expected	P-Value
GG	20	15.15	0.14
GC	15	24.75	
CC	15	10.15	
Allele frequency	p(0.55)	q(0.45)	
Chi square of allele frequency		3.999	

Thus, the result of this study demonstrated that patient samples showed two variants of this locus, heterozygous G/C and homozygous C/C, whereas the G/G form was only present in control samples. This crucial information referred to the higher probability of this nucleic acid substitution in the individuals currently being studied. The matching position of the MTHFR gene was repossessed from the db SNP website (https: // www. ncbi. nlm. nih. Gov / projects / SNP) to elucidate the positions of the targeted rs1801133 SNP in relation to its put down database of the sequenced 232bp fragments. The graphical represent of the MTHFR gene db SNP database within

WEB OF SCIENTIST: INTERNATIONAL SCIENTIFIC RESEARCH JOURNAL
ISSN: 2776-0979, Volume 3, Issue 3, Mar., 2022

Chromosome1 (Gen Bank consent no. NC 000001.11) was used to investigated the nature of this SNP (Supplementary Figure 2).

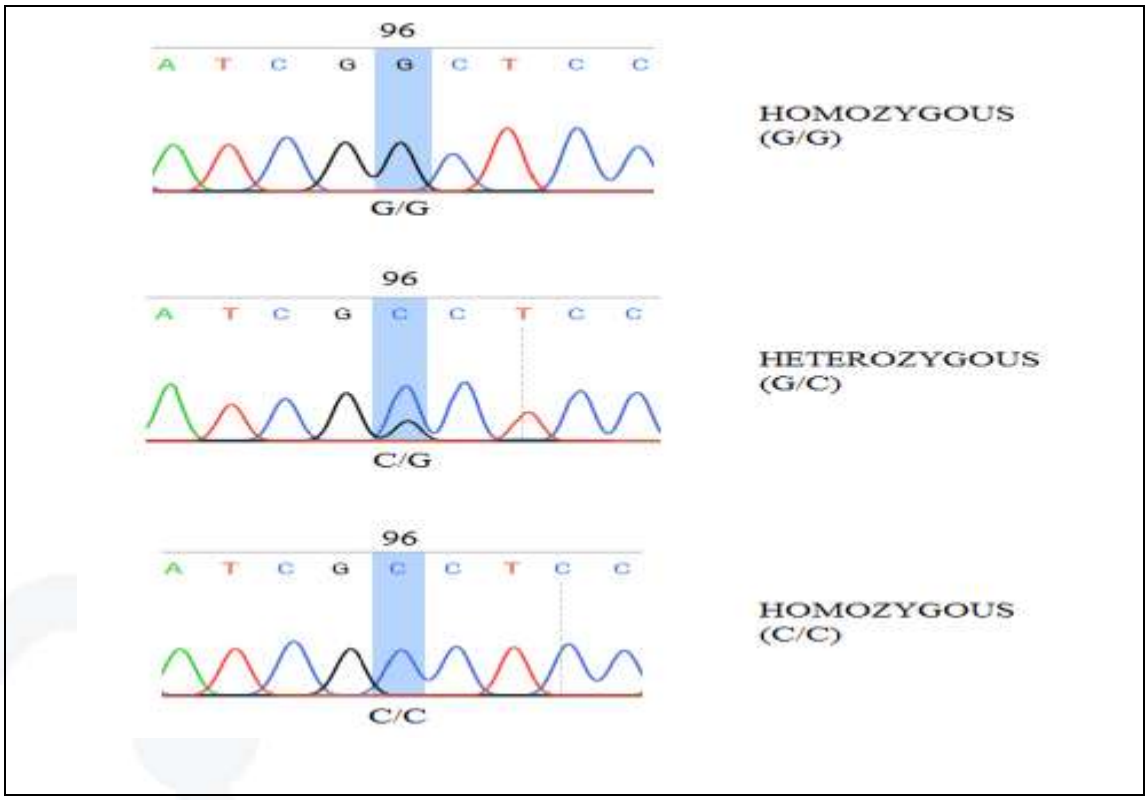

Figure (2) shows the DNA chromatogram patterns of the detected substitution mutations in the 232 bp amplicons inside the MTHFR genomic the sequences of DNA. According to the position in the PCR results, the detected substitution mutation in the targeted rs1801133 SNP was underlined.

Growth hormone GH is the primary regulator of postpartum body growth . Patients with growth hormone deficient GHD commonly have dwarfism as a result of their disorder. GHD is an autosomal dominant characterized the heterozygozygozity by splicing abnormalities that result in the deletion of GH1 exon 3. In humans, GHD severity ensures existed linked to mutant-to-normal transcript ratios, with higher quantities of mutant proteins causing significant short stature [20]. The GHR gene for human Growth Hormone Receptor includes ten exons. Exon2 codes for a signal peptide, while Exons 3–7 code for the extracellular domain, which is 246 amino acids long. Exon8 codes for the transmembrane region, which contains a 24 amino acid peptide, while Exons 9 and 10 code for the cytoplasmic domain, which contains 350 amino acids [21].

As previously stated, the present T allele in the form the CT genotype and the TT genotype of the MTHFR gene Exon4 (677) the rs1801133 SNP is a risk factor for GHD.

At hand the Cytosine (C) to Thymine (T) conversion at the nucleotide position of the (677) in Exon4 of the MTHFR gene and, more importantly, the change of the amino acid Alanine to Valine at a.a 222 of this protein in carriers of the MTHFR (677) C>T polymorphism (the terms C 677 T are recommended; rs1801131 conferring to the database SNP) (p.Ala222Val). The MTHFR gene activiting is the reduced by 40% in the heterozygous form (MTHFR c , CT variations) and 70% in the homozygous form (MTHFR c , TT variants) when this polymorphism is existent [22,23].

This SNP was located in the exonic sections of MTHFR gene sequences, and it was exerted the transition of cytosine to guanine, according to the obtained dbSNP-based database. In the examined the methylenetetrahydrofolate reductase Isoform 1 protein (NC 000001.10:g.11856378G>A), this SNP had a missense impact of Ala to Val at the 263 amino acid position. This targeted SNP has been reported to be localized with high Occurance in several researched populations due to its high frequency, which accounts for 0.31 to 0.33 in the Genome Aggregation Database Exomes (Gnom AD Exome) and ALFA Project, respectively. According to the ClinVar database, the highly common exonic rs1801133 SNP is significantly linked to human health. It has also been extensively characterized in the literature, having a definite function in a wide range of symptoms in a wide range of people. Furthermore, an increasing number of papers point to this SNP as a likely causal factor in a variety of fetal development, folliculogenesis, choline intake, polycystic ovary syndrome, and breast cancer dysfunctions [24,25,26,27]. However, due to the high discovered frequency in the currently researched group, the clear genetic polymorphism of the rs1801133 SNP in this study could not be disregarded.

In conclusion, the targeted rs1801133 SNP is a possible causative SNP for our investigated samples, with a remarkable association with the development of growth hormone deficiency in the children of Iraq.

References

1. Varuna Vyas ., Anil Kumar., Vandana Jain. "Growth Hormone Deficiency in Children: From Suspecting to Diagnosing. Indian Pediatr". Nov 15;54(11):955-960, 2017. doi: 10.1007/s13312-017-1190-3.
2. Reiter, E.O. and Rosenfeld, R.G. "Williams Textbook of Endocrinology (11th Edition) ". Philadelphia, USA: Saunders Elsevier; p. 849–968, 2008.

3. Mauro Bozzola and Cristina Meazza. "Growth Hormone Deficiency: Diagnosis and Therapy in Children. Restricted Growth - Clinical, Genetic and Molecular Aspects". http://dx.doi.org/10.5772/64803. 2016.
4. Argente J. "Challenges in the management of short stature". Horm Res Paediatr.85:2–10. DOI: 10.1159/000442350. 2016.
5. De Bellis A., Colao A., Tirelli G Ruocco G., Di Somma C., Battaglia M, Bizzarro A and Bellastella A. "Autoimmunity as a possible cause of growth hormone deficiencyž J Endocrinol Invest".1:1132–1134. 2008.
6. Floris M, Sanna D, Castiglia P, et al. "MTHFR, XRCC1 and OGG1 genetic polymorphisms in breast cancer .a casecontrol study in a population from North Sardinia". BMC Cancer, 20, 1-15. 2020.
7. Wit J.M, Kiess W, Mullis P. "Genetic evaluation of short stature". Best Pract Res Clin Endocrinol Metab ;25(1):1–17. 2011.
8. Lorenzo D. Botto and Quanhe Yang. "5,10-Methylenetetrahydrofolate Reductase Gene Variants and Congenital Anomalies: A HuGE Review". American Journal of Epidemiology. Vol. 151, No. 9. 2021.
9. Yang B., Fan S., Zhi X., Wang D., Li Y., Wang Y. et al. "Associations of MTHFR C677T and MTRR A66G gene polymorphisms with metabolic syndrome: a case-control study in Northern China". Int. J. Mol. Sci. 15, 21687–21702 10.3390/ijms151221687. 2014.
10. Yafeng Wang , Gongfeng Zheng , Mingqiang Kang , Weifeng Tang3, Yanan Wang , Wei Cai5, Ziyang Huang. "Methylenetetrahydrofolate reductase rs1801133 C>T polymorphism is association with nonsyndromic cleft lip with or without cleft palate susceptibility: a meta-analysis". Int J Clin Exp;10(2):1734-1749 www.ijcem.com /ISSN:1940-5901/IJCEM0043554. 2017.
11. Shan Liu , Mengwei Liu , Qian Li, Xiuping Liu, Yue Wang, Michael Mambiya, Kaili Zhang, Luping Yang, and Wanyang Liu. "Association of single nucleotide polymorphisms of MTHFR, TCN2, RNF213 with susceptibility to hypertension and blood pressure". Portland Press, https://doi.org/10.1042/BSR20191454. 2019.
12. AL-Hajjar, Q. N., and Al-Mousawi, H. T. M. (2021). Immunological and Molecular Diagnosis of Cytomegalovirus Infection between Aborted & Pregnant Women in Babylon City. Baghdad Science Journal, 18(2 ملحق).
13. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden T. "Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction.BMC Bioinformatics". 13:134. 2012.

14. Abdulhussein, T. M., Abed, A. S., Al-Mousawi, H. M., Abdallah, J. M., & Sattar, R. J. (2020). Molecular genotyping survey for bla TEM Virulence Gene of Acinetobacter baumani isolates, Iraq. Plant Archives, 20(2), 413-415.
15. Al-Azzawie, H. F., Nadhim, M. H., Al-Kharasani, A. M. R., & Albermani, M. S. K. (2018). Genetic study of AHR exonic part and GNAS intronic part mutations in some of iraqi acromegalic patients. Journal of Pharmaceutical Sciences and Research, 10(7), 1663-1667.
16. Nadhim, M. H. (2020). GENETIC POLYMORPHISM IN NOS GENE AND CORRELATED WITH PEROXYNITRITE LEVEL VARIANT IN T2DM OF IRAQI PATIENTS. Plant Archives, 20(2), 1537-1543.
17. Zhang Z, Schwartz S, Wagner L, and Miller W. A greedy algorithm for aligning DNA sequences. J Comput Biol 2000, 7(1-2):203-14
18. Potter. A., Phillips. J.A. and Rimoin. D.L . "Genetic Disorders of the Pituitary Gland. Biomedical Sciences". https://doi.org/10.1016/B978-0-12-801238-3.05555-0. 2014.
19. Al-Mousawi, H. T. M., Mushtaq, A. B. N., & Bohan, A. H. Molecular and immunological activity of Terminalia chebula extracts.2022.
20. Lei, W.; Wei-Cheng, C.; Yuhsi, T..; Yu-Tsun, K.; Yao-Yuan, H.; Cheng-Chun, L.; and Fuu, J. T. "Growth Hormone (GH) Receptor C.1319 G>T Polymorphism, But Not Exon 3 Retention or Deletion Is Associated With Better First-Year Growth Response to GH Therapy in Patients With GH Deficiency". PEDIATRIC RESEARCH. Vol. 62, No. 6, 0031-3998/07/6206-0735. 2007.
21. Anetta Undas, Krzysztof Chojnowski, Anna Klukowska, Magdalena Łętowska, Andrzej Mital, Maria Sąsiadek, and Tomasz Urasiński. "Determination and interpretation of MTHFR gene mutations in gynecology and internal medicine". POLISH ARCHIVES OF INTERNAL MEDICINE .doi:10.20452/pamw. 2019.
22. Cardoso-dos-Santos, A. C., Tagliani-Ribeiro, A., Matte, U., & Schuler-Faccini, L. "Genetic variants linked to folliculogenesis and successful pregnancy are not associated with twin births in a twins' town". The Journal of Maternal-Fetal & Neonatal Medicine, 1-8. 2020.
23. Aly, R. M., Taalab, M. M., & Ghazy, H. F. "MTHFR A1298C and C677T gene polymorphisms and susceptibility to chronic myeloid leukemia in Egypt". International journal of clinical and experimental pathology, 7(5), 2571. 2014.
24. Chmurzynska, A., Seremak-Mrozikiewicz, A., Malinowska, A. M., Różycka, A., Radziejewska, A., KurzawiŃska, G. & Drews, K. "Associations between folate and

choline intake, homocysteine metabolism, and genetic polymorphism of MTHFR, BHMT and PEMT in healthy pregnant Polish women". Nutrition & Dietetics, 77(3), 368-372. 2020.

25. Kos, B. J., Leemaqz, S. Y., McCormack, C. D., Andraweera, P. H., Furness, D. L., Roberts, C. T., & Dekker, G. A. "The association of parental methylenetetrahydrofolate reductase polymorphisms (MTHFR 677C> T and 1298A> C) and fetal loss: a case–control study in South Australia". The Journal of Maternal-Fetal & Neonatal Medicine, 33(5), 752-757. 2020.
26. Mo, W., Ding, Y., Zheng, Y., Zou, D., & Ding, X. "Associations between folate metabolism enzyme polymorphisms and breast cancer: A meta-analysis". The breast journal, 26(3), 484-487. 2020.
27. Nadhim, M. H., Al-Mousawi, H. T., Al-Saad, N. F. N., & QabasNeamalt, A. L. (2019). Vitamin D Receptor Polymorphisms and Correlated with Some Chronic Diseases among Iraqi Women. Indian Journal of Public Heal Iii Research & Developmenl, 10(10).