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Molecular Analysis of MTHFR (C677t) Polymorphism in Patients Facing Infertility Issues

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Abstract: Methylenetetrahydrofolatereductase (MTHFR) is a regulatory enzyme which has ability to break down the amino acid homocysteine. MTHFR is coded by MTHFR gene. Variations in MTHFR gene can interfere with the enzyme's ability to function normally or it can completely inactivate it. C677T and A1298C are the two most common types of MTHFR mutations. There are several factors that lead infertility like advanced maternal age of the patient, abnormal hormonal production in males and females, environmental factors and also their genetic changes. Most of the couples prefer IVF.

One of the patients showed TT polymorphism for C677T.Two pathogenic variants where the male patient is carrying 4 pathogenic variants. The couple are planning for IVF. The couple is advised to go for targeted testing by amniocentesis after the successful IVF procedure. Most of the patients with C677T are facing infertility issues. It is Advisable to check C677T Polymorphism in individuals planning for IVF.

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Index Terms*: *MTHFR, C677T, A1298C, IVF, Mutations, amniocentesis.

I. INTRODUCTION

Methylenetetrahydrofolatereductase which in short MTHFR is an enzyme which breaks down the amino acid homocysteine. Methylenetetrahydrofolatereductase is coded by MTHFR gene which has the ability to mutate. MTHFR gene can either hinder with the enzyme's ability to function normally or completely inactivate it.

Generally, every human has two of these MTHFR genes, inheriting one from father and the other from mother, and these genes can be affected by mutations. If one gene is affected then it is heterozygous and if both the genes are affected then it is homozygous. Mainly there are two most common or the general types of this MTHFR mutation, which are **C677T** and **A1298C.**

If you think you got MTHFR variant don't take the cure yourself consult a doctor who can determine whether a person has an MTHFR variant by examining their medical history, considering their current symptoms and performing a physical examination. A doctor may recommend running a blood test to check the levels of homocysteine of that person if it is necessary.

Symptoms of MTHFR mutation

Symptoms changes from variant to variant and from person to person and it can directly result in many conditions or health issues.

The research related to MTHFR and its effects is still emerging. Proofs linking most of the health conditions to the MTHFR variants are currently lacking or has been disproven.

You will never ever going to know your MTHFR mutation status until you found the symptoms or you have gone for medical testing.

Conditions that have been advised to be correlated with MTHFR include:

- Recurrent miscarriages in women of child-bearing age
- Acute leukemia
- Migraines
- Bipolar disorder
- Schizophrenia
- Anxiety
- Cardiovascular and thromboembolic diseases (includes blood clots, stroke, embolism, and heart attacks)

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• Colon cancer
• Nerve pain
• Depression
• Chronic pain and fatigue
• Pregnancies with neural tube defects, like spina bifida and anencephaly

The risk is possibly increased if a person has two gene variants or is homozygous for the MTHFR mutation.

Women who test positive for an MTHFR mutation are most likely to be infected or exposed to preeclampsia, blood clots, recurrent miscarriages, or giving birth to a baby with congenital disabilities. Many studies have been done to find the relationship between the mutation and pregnancy complications, but the data is not sufficient in their conclusions.

ENVIRONMENTAL AND BIOCHEMICAL REASONS FOR INFERTILITY

The research conducted by many scientists states that life style of partners can also affect the fertility and that life style includes the place they live, the food they take, the sleep hours and the way they behave. They also may lead to other diseases apart from infertility.

To be more specific lifestyle factors like no proper nutrition, weight (overweight, skinny), stress that include both physical and mental, exposure to bad environments, usage of drugs (like marijuana) and medications has a tremendous effect on infertility.[6]

The results of research are as follows:

Main reason for infertility in men is obesity which reduce the sperm count and its quality.

For better likeliness of ovulation in obese women with polycystic ovary syndrome, reducing their weight by 5% is recommended and it also increases the chances of getting pregnancy.

For women being skinny can lead to ovarian disfunction which results in infertility.

Physical stress and undergoing too many medications at a time can leads to decrease the sperm count in males.[7]

In case of women, extreme exercise can affect the fertility by affecting ovulation.

Results of this research recommend that using drugs that can enhance your body weight or androgens have great effect on sperm count and its formation.

Consuming too much tobacco products includes cigarette and usage of other products like marijuana, consuming too much alcohol and using banned drugs like heroin cocaine can increase the chances of infertility in both men and women.

Blood pressure can also affect the fertility, many researches showed that high blood pressure can impact the sperm shape, count and which can ultimately leads to infertility.

Research also concludes that the type of underwear a man chooses can affect his fertility is just a myth and it's not true. Men's infertility does not depend on the type of underwear he wears.[8]

Cancer curing medications including radiation therapy and chemotherapy have a huge impact on infertility. It is recommended that who is taking these medications are suggested to go for fertility preservation.

Research conducted by NICHD states that long disclosure to organic pollutants and certain chemicals like endocrine disrupting chemicals can increase the chances of infertility in both men and women.

Among all the other chemicals only persistent organic pollutants are considered to affect the fertility in men and women when they are exposed to those chemicals because they stay much longer in environment than the other chemicals which are released during industrial processes. Studies related to causes of long time exposure to those POP's can lead to infertility. Fecundity is ability to reproduce which is ability of couples to become pregnant and give birth to a baby. Longitudinal Investigation of Fertility and the Environment is a study conducted by NICHD which fecundity. This study focuses on

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how exposer to chemicals can affect the infertility in both the partners who are trying to get pregnancy for one year.

This study concludes that exposure to pesticides which are made by using chlorine such as organochlorine and poly chlorinated biphenyls can decrease the fecundity there by increasing the time to become pregnant. It also concludes that chemicals that can affect the time for pregnancy are found in male where as in female there are chemicals that can affect the fecundity. Some related studies concluded that exposure to TCCD dioxin and some diethers and some perfluoro chemicals can reduce the fecundity.[8]

The Longitudinal Investigation of Fertility and the Environment study conducted by NICHD found that a chemical called EDC methyl paraben change how the hormonal system works there by decrease the chances of fertility in women. Where as in men fertility is affected by chemicals such as UV filter benzophenone-2 and phthalates. [8][9][10]

II. RELATED WORK

Infertility is condition where a couple is not able to deliver a baby after sexual interaction. This infertility might cause due to weak reproductive system of men or women which finally results in failed pregnancies in women more than once. After many researches and studies suggest that 15% of couples are not able to get their pregnancies after 1 year of having sex without any protection, and 10% of couples are not able to get pregnancy after unprotected sex [11]. 20% to 37% of healthy couples younger than age 30 are able to get pregnancy in their first 3 months of having sex. According to a study conducted by scientists over one third of infertility cases recorded are due to male reproductive issues and another one third of those cases are due to female reproductive problems and the other one third of cases are due to both male and female systems or by unknown factors. And the final conclusions of those studies says that infertility can occur as a result of single cause or by many causes or in strange cases there is no identity of that cause.[12]

The MTHFR gene mutation hinders the way our body deals with folic acid and other important B vitamins. Altering the supplementation of this nutrient is a probable and main focus in countering its effects.Folic acid is indeed a man-made version of folate, which is a naturally existing nutrient generally found in foods. Consuming the readily available and bioavailable form of folate methylated folate might help your body to absorb it easily.

Most people are recommended to take or to have a multivitamin that contains at least 0.4 milligrams of folic acid daily.Pregnant women are not recommended to swap prenatal vitamins and care based on their MTHFR status by themselves .which means consuming the usual dose of 0.6 milligrams of folic acid daily.Women having history of neural tube defects should consult their doctor in order to get specific recommendations.

Male infertility is connected with polymorphisms in the genes encoding enzymes in the folate metabolism pathway which can also cause chromosome abnormalities. The intent of current study is to evaluate and understand how the distribution of the methylenetetrahydrofolatereductase (MTHFR), methionine synthase (MTR), and methionine synthase reductase (MTRR) polymorphisms among themselves both in fertile men and infertile men with non-obstructive azoospermia (NOA). Scientists had personally conducted a study among 85 infertile men with NOA and 246 fertile men to understand this by using the polymerase chain reaction restriction fragment length polymorphism technique they found the following polymorphisms MTRR c.66A > G (rs1801394), MTHFR c.677C > T (rs1801133), MTHFR c.1298A > C (rs1801131), and MTR c.2756A > G (rs1805087). After employing Bonferroni correction, they observed some important differences in AC + CC

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genotype (OR = 1.9, 95% CI = 1.1–3.2) and C allele frequencies (OR = 1.8, 95% CI = 1.2–2.8) of MTHFR c.1298A > C polymorphism between NOA patients and controls. However, 1298AC genotype, 1298AC + CC genotype, and 1298C allele frequencies were statistically significant in NOA with chromosomal unusualness and a Y chromosome deletion compared to the controls (AC genotype: OR = 3.0; AC + CC genotype: OR = 3.0; C allele: OR = 2.3). In consideration of all the other polymorphisms, there were no differences were observed between cases and controls. Our results recommend that the MTHFR c.1298A > C polymorphism is related or connected with high risk of male infertility. i.e., NOA.(3)

The intent of this study was to observe the distributions of methylenetetrahydrofolatereductases (MTHFR s) C677T and A1298C genotype in couples suffering with unexplained fertility problems (UFP) and healthy controls (healthy couples), and also to evaluate the distribution of the genotype and haplotype in spontaneously aborted embryonic tissues (SAET) by adopting allele specific polymerase chain reaction (PCR) in 200 couples suffering with infertility with UFP, 353 samples of SAET and 222 healthy couple samples. The results of this study reported that 677T allele in male probands from couples with UFP (p=0.036). The mixed genotype distribution for both MTHFR polymorphisms was also altered (χ 2 21.73, p <0.001) though there is no contribution from female pro bands (χ 2 1.33, p = 0.72). The complete representation of the 677T allele was more obvious in SAET (0.5 vs. 0.351 in controls, p <0.001) despite of the karyotype status (aneuploidy vs. normal karyotype). The frequencies of the CA and CC haplotypes were noticeably lower than in the control group (p = 0.021 and p = 0.001, respectively), the frequency of the TC haplotype was comparatively higher than in controls (p <0.0001). The results of this study states that only male probands devote to the union or association of MTHFR mutations with fertility problems in adults and also exhibit a high popularity of mutated MTHFR genotypes in SAET.

Keywords: Methylenetetrahydrofolatereductase (MTHFR), Genotype, Haplotype, Infertility , Miscarriage.(2)

III. MATERIALS AND METHODS ANALYSIS

A. Isolation of DNA by Salting Out Method.

The genomic DNA isolation needs to separate total DNA from RNA, protein, lipid, etc. Initially the cell membranes must be disrupted in order to release the DNA in the extraction buffer. Chloroform is also a protein denaturant, which stabilizes the rather unstable boundary between an aqueous phase and pure phenol layer.

In general, they aim to separate DNA present in the nucleus of the cell from other cellular components. Isolation of DNA is needed for genetic analysis, which is used for scientific, medical, or forensic purposes.

B. Quantification of DNA – QUBIT Fluorometer .

The Qubitfluorometer is a lab instrument in use that, among other applications, is used for the quantification of DNA along with others. The Qubitfluorometer uses fluorescent dyes that emit signals only when bound to the specific target molecules (DNA or RNA) even in the presence of free nucleotides, degraded nucleic acids, or protein contaminants.

C. Polymerase Chain Reaction -PCR

Usually, Polymerase Chain Reaction(PCR) is done to amplify small sections of DNA. To perform PCR entire DNA sequence is not required. Specific targeted region of DNA is amplified.

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Usually, we use vectors for targeting the specific sequence of DNA, by using PCR we can target and amplify the sequence in few hours.

DENATURATION: In this step, DNA is heated to high temperature(92°C) to make double stranded DNA to single stranded DNA

ANNEALING: Here the temperature is lowered to enable the DNA primers to attach at specific location on the template DNA (ss DNA)

EXTENSION: When the temperature (72°C) is raised tag polymerase enzyme helps to synthesize new DNA strand which adds DNA bases.

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D. GEL Electrophoresis

Gel electrophoresis separates DNA fragments by size in a solid support medium (an agarose gel). The rate ofmigration is proportional to size: smaller fragments move more quickly, and wind up at the bottom of the gel. DNA is visualized by including in the gel an intercalating dye, ethidium bromide.

The negatively charged DNA molecules migrate towards the positive charge under the influence of constant current, thus the separation depends on the mass and charge of DNA. The DNA molecules are forced to move through the agarose gel pores.

E. DNA Sequencing

DNA sequencing is a laboratory technique used to determine the exact sequence of bases (A, C, G, and T) in a DNA molecule. We follow four different steps for DNA Sequencing.

1. **Exosap:** Among all other cleanup reagents, ExoSAP-IT single-step PCR is the best reagent which uses two hydrolytic enzymes. And the two hydrolytic enzymes, Shrimp Alkaline Phosphatase which are added together in certain ratio in order to form a buffer solution which is used to eliminate undesired or not required dNTP's and primers from PCR products. Exonuclease I eliminate single-stranded primers and any other unrelated single-stranded DNA developed in the PCR and leaves no residuals. Whereas SAP eliminates the rest of dNTPs in the PCR batter or PCR products.
2. **Cycle sequencing**: It is a technique to improve the sensitivity of DNA sequencing process by allowing to use tiny amounts of DNA starting material and this technique is best known for its robust and easy performance. And the whole process is achieved by applying temperature cycling process which is completely identical to polymerase chain reaction.
3. **Post PCR Cleanup**: Polymerase Chain Reaction is a method commanly used to create millions of copies of a specific DNA sample on a rapid basis allowing scientists to take a very small sample of DNA and amplify it to a large enough amount to study in detail.
4. **Sequencing**: It is used to determine the nucleotide order of a given DNA fragment. Both laboratory process and computational analysis plays major role in DNA sequencing.

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IV. RESULTS AND DISCUSSION

Screening 10 individuals for MTHFR (C677T) variant who are suffering from infertility and are planning for IVF. Isolated the DNA from the whole blood sample and then quantification of DNA is performed followed by PCR with targeted primers for C677T. Out of 10 individuals one patient showed homozygous mutation for C677T.

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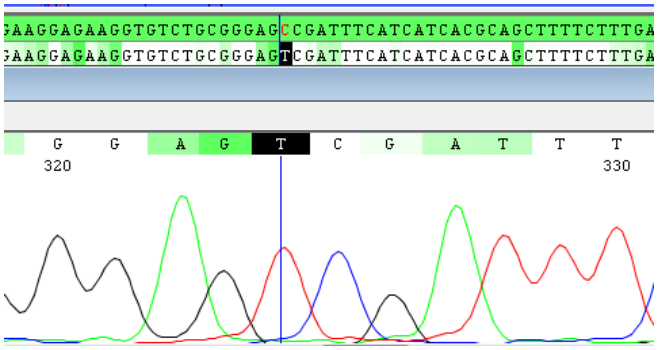

Fig 1: Electropherograms of subject 1

Gene and Transcript	Location	Variant	Zygosity	Classification	Disease	Inheritance
FLNA (NM_001456.3)	Exon5	C.862G>A (P.GLY288Arg)	Heterozygous	Likely pathogenic	Cardiac valvular dysplasia, X-linked	X-Linked Dominant
STRC (NM_153700.2)	Exon24	C.4701+G>A	Heterozygous	Likely pathogenic	Deafness,Autosomal Recessive 16	Autosomal Recessive

Table 1Heterozygous Likely Pathogenic Variants Detected

Theabove individual has shown a CC genotype for C677T polymorphism.Here we report one couple who has planned for IVF and whose MTHFR C677T is homozygous CC, have gone for further whole exome sequencing which revealed that the female is carrying the following.

Gene and Transcript	Location	Variant	Zygosity	Classification	Disease	Inheritance
SDHA (NM_004168.3)	Exon1	c.63+1G>3	Heterozygous	Likely pathogenic	Paraganglioma5	Autosomal dominant
ALDH7A1 (NM_001182.4)	Exon14	c.1279g>c (p.Glu427Gln)	Heterozygous	pathogenic	Epilepsy,pyridoxine_dependent	Autosomal recessive
SERAC1 (NM_032861.3)	EXON 11	c.1112_1113delTG (P.val371Alafs*22)	Heterozygous	Likely pathogenic	3-methylglutaconic aciduria with deafness, encephalopathy,and Leigh-like synydrome	Autosoma; recessive
PIEZO1 (NM_001142864.3)	Exon 9	C.1107+1G>A	Heterozygous	Likely pathogenic	Lymphatic malformation6	Autosomal recessive

Table 2: Heterozygous Pathogenic and Likely Pathogenic variants Detected.

The couple is advised to go for targeted testing by amniocentesis after the successful IVF procedure to study the association of MTHFR C677T variant with infertility in couples planning for IVF.

V. CONCLUSION

It is advisable to check C677T Polymorphism in individuals planning for IVF, So that they can start vitamin B6 B12 medication if they have C677T. Our analysis demonstrated the important role of genetic mutations in folate-related enzyme genes in male infertility. Well established genetic makers surely would contribute to the early screening and prediction of male infertility.

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