

Methyl Tetra Hydro Folate Reductase Enzyme Polymorphism in the Mothers with Previous Autosomal Aneuploidy Birth in the Indigenous African Population of Northern Region of South Africa

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Abstract

Background and purpose of the study: Aneuploidy births are highly prevalent in our population and some families, recurrent. The study is to investigate the association of MTHFR enzyme polymorphism and levels of vitamin B12, folate and homocysteine in the affected mothers.

Method: A cohort of 30 affected mothers at the (DGMHA) hospital was recruited. Single nucleotide polymorphisms were studied using a High Resolution Melting Curve analysis on the Real Time PCR system, serum vitamin B12 and plasma Homocysteine were analysed on the Abbott Architect i2000 and serum folate levels on the Beckman DXi automated analyser.

Results and discussion: The CC genotype which represent normal enzyme activity of MTHFR was found in 53.3% and the CT genotype with reduced enzyme activity (60% of normal), in 46.6% of mothers were found. There is positive correlation between CT genotype and increased plasma Homocysteine. Although within reference range, the serum B12 and folate levels showed a negative correlation between serum B12 and Homocysteine, folate and Homocysteine.

Conclusion: The data highlights the association of MTHFR abnormal CT genotype and increased plasma Homocysteine in the aneuploidy affected mothers, supporting the recommendation of early referral for pre-natal screening and high dose B12 and folate supplementation in their future pregnancies.

Keywords: Chromosomal aneuploidy; DNA methylation status; B12/homocysteine/folate pathway; MTHFR genotypes

Introduction

Aneuploidy is a genetic disorder where the total number of chromosomes is either less or more than the normal diploid number of 46, XX or 46, XY [1]. The most common aneuploidies in South Africa caused by autosomal chromosomal abnormalities include Down syndrome (Trisomy 21), Edward syndrome (Trisomy 18) and Patau syndrome (Trisomy 13). Globally the incidence for Down syndrome is about 1 in 650 to 1000 live births [2], Edward syndrome is 1 in 6000 live birth [3], and Patau is between 1 in 5000 to 29000 live birth [4]. Currently the prevalence rate is 1 out of 500 live birth for Down syndrome in South Africa with 1886 new cases diagnosed every year [5] and for Edward syndrome the prevalence is 1 in 4000 live birth and Patau is 1 in 8889 live birth [6,7]. The aetiology of these autosomal aneuploidies are believed to be due to non-disjunction of chromosome in maternal ovum during meiosis which is thought to be a sporadic, incidental event usually associated with older age group mothers. Notably, there is an increased incidence of aneuploidy babies has been observed in younger age group mothers with less than 35 years of age in South African black population [8].

There were studies done in the Middle East families with recurrence aneuploidy births to determine if there is a genetic link. Krishna Murthy and Farag reported two unrelated Kuwaiti families each having 3 siblings with trisomy 21 as a result the researchers suggested that some genetic predisposition and gonadal mosaicism could be the underlying cause of recurrent aneuploidy in the younger mothers, thus increasing the risk of recurrence of such pregnancies [9]. There were studies that found that a particular polymorphism of one of these genes Methylene tetrahydrofolate reductase (MTHFR) might be a maternal risk factor for having child with Aneuploidy or Down syndrome and

that a significant increase in plasma homocysteine levels exists in mothers of the children with Aneuploidy or Down syndrome [10,11].

Increased homocysteine may result from dietary deficiency of folate and or vitamin B12 or from genetic polymorphism of Methylene tetrahydrofolate reductase (MTHFR). These genes encode the enzymes involved in the transfer of methyl group from sulphur containing amino acids (homocysteine and cysteine). Decreased transfer results in decrease methylation of DNA around the centromere region of chromosome 21, 18 and 13 proposed to be leading to aneuploidy. This hypothesis has led many researchers to further explore the evidence between DNA methylation of chromosome 21, 18, 13 in the unfertilized ovum but due to invasive nature of method involved in acquiring the pre-implementation studies, it has not been proven [10,12-16].

Indirect evidence of younger mothers having high level of plasma homocysteine with or without genetic polymorphisms would be the closest link to identifying the risk of recurrence aneuploidy in the

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next pregnancy for these mothers. In one the study that was done by James et al. [10] it was stated that a polymorphism on one of these genes; methyl tetrahydrofolate reductase (MTHFR) is a maternal risk factor for having a child with Down syndrome. This polymorphism CT (C677T where cytosine is replaced by Thymine at the codon 677) is known to reduce the function (60% of normal) of MTHFR enzyme [17]. Researchers assume that another gene encoding the enzyme; cystathionine beta synthase (CBS) located on the chromosome 21 and its polymorphisms may be involved in the pathogenesis of Trisomy 21 birth especially when it is aggravated by deficiency of folate [17]. Despite the high prevalence of aneuploidy and reports from other parts of the world suggesting the links between DNA methylation and these disorders, there has been no study regarding the polymorphisms of MTHFR genes in the Aneuploidy birth affected mothers in our Black indigenous population.

Materials and Methods

A sample of the cohort of 30 mothers was recruited for this project. The inclusion criteria were mother's <42 years old at the time of conceiving the aneuploidy babies (Trisomy 13, 18 and 21). About 5 ml of blood was drawn from each participant using vacutainer sets into 3 samples tubes (2x EDTA tubes; 1 for Red blood folate and 1 for homocysteine and 1 serum separator tube for vitamin B₁₂). Determination vitamin B₁₂ and serum folate test was done immediately post sample preparation while homocysteine was batched and stored at -20°C fridge until 10 patients samples are obtained due to cost implications of the test kit. Plasma homocysteine is stable at -20°C for 3 months according to the test kit manufacturer. The analysis of B₁₂, homocysteine (post complete thawing) was done using an automated immunochemistry analyser called Architect i8200 from Abbott and for serum folate test was analysed with automated immunochemistry analyser from Beckman DXi 800.

Cells that were left over from the EDTA tube after centrifugation of plasma was used to extract DNA using a High Pure PCR Template preparation kit from Roche company by following their manufacturer's protocol [18]. In this product the probe that matched the sequence of the wild type genotype and the presence of a mutation variant it reduced Tm as result. The reading of the genotype result was based on the melting temperatures compared to the supplied standards [19].

Results

The statistical analyses were done using Statal Package for Social Sciences (SPSS) version 23 software. The chi-square test with Pearson statistics was used in order to determine statistical differences for this study. In the present study a total of 30 participants, including mothers of 27 children with Down syndrome, 2 with Edward syndrome and 1 with Patau syndrome. Of the total sample the frequency for CC genotype which represents normal enzyme activity of MTHFR was found in mothers of 50% with Down syndrome children and 3.3% with Patau children, meanwhile the frequency for CT genotype which represents 60% of normal enzyme activity was found in mothers of 40% with Down syndrome children, 3.3% with Edward syndrome children and 3.3% with Patau children. The present study revealed that none of the mothers who were participating in the study had TT genotype (Table 1).

MTHFR Genotype	Down syndrome	Patau syndrome	Edward syndrome
Homozygous (CC- Normal genotype) - normal enzyme activity	50% (N=15/30)	0%	3.3% (N=1/30)
Heterozygous (CT- Abnormal genotype) - 60% of normal enzyme activity	40% (N=12/30)	3.3% (N=1/30)	3.3% (N=1/30)
Homozygous TT (mutant) - 30% of normal enzyme activity	0%	0%	0%

Table 1: MTHFR genotype for the participating mothers.

Discussion

In this pilot study, mothers with Down syndrome birth constitutes a majority at 90% and Edward syndrome at 6.7% and Patau syndrome as the smallest group at 3.3%. The mothers in the study were all black African although there was a significant difference in ethnicity amongst them. 53.3% Tswana, 16.6% Sotho, 6.7% Zulu, 3.3% Ndebele and 20% Pedi. Most of them come from the nearest township to the hospital, Soshanguve (30%). This is a reflection of population residing around the hospital and may not infer the association of the disorder and the ethnic group or geographical situation.

Approximately 80% of the mothers are unemployed and admitted to consuming alcohol and smoking snuff at the time of conception and throughout early pregnancy. This may relate to more poor socio-economic status as well as psychological stress of unemployment. Drinking during pregnancy is known to cause birth defect [20]. Despite unemployment, all the mothers reported having normal diet and consumed dairy products although the adequacy per daily requirement cannot be confirmed. This was included in the study questionnaire as B12 deficiency is common in vegetarian. Most Black people in the townships nearby our hospital consume locally grown green vegetables (such as spinach) thus folate deficiency is rare in this population. Moreover, due to the recent evidence that the red blood cell folate levels do not offer better discrimination in folate deficiency than the serum folate does [21]. This qualitative data suggests that the participants in this group are not likely to have B12 and folate deficient from their dietary history. This was in agreement with the measured levels of serum B12 and folate which are in the normal reference ranges although the levels negatively correlated with those of plasma homocysteine in the mothers who were positive for MTHFR mutations. This draws a conclusion that the increased homocysteine levels were rather related to the mutation instead of dietary deficiencies of B12 and folate.

In family history of the study group, it reveals 26.7% of the mothers have a family history of aneuploidy birth (one family with Down syndrome and the other family with Patau syndrome). According to the literature, this is one of the factors that increases the chance of having baby with aneuploidy due to fact that the maternal one of the parents might be a carrier for translocation chromosome [22,23].

On the medical profile, the data reports 40.3% of the participating mothers were infected with HIV and are on ARV treatment and 6.7% suffered from Hypertension. Interestingly, 16.6% of the mothers with aneuploidy children took traditional medicine and 20% ate soil early in their pregnancy. There have been studies that associate taking traditional medicine with foetal abnormalities, uterine rupture, low birth weight [24,25]. HIV and its association of aneuploidy birth have not been reported.

In other studies, B12 deficiency with increased homocysteine levels has been reported to associate with aneuploidy birth [26-28]. One study showed that vitamin B12 and folate deficiency contributed to high levels of homocysteine in those mothers who gave birth to children with aneuploidy and it also implicated as risk factor for aneuploidy births [29,30]. In my study, all mothers showed serum B12 and folate levels within normal reference ranges inferring to the dietary adequacy

(Table 2). Therefore the conclusion can be drawn that the increased plasma homocysteine levels in 46.6% of the mothers (Figure 1) were due to the presence of CT polymorphism of MTHFR where only 60% of enzyme activity is expected. There is positive correlation between positive CT polymorphism and the level of plasma Homocysteine, with the significance of ($p < 0.05$) in our study group and a few studies done in other parts of the world seem to concur with our results [31,32]. Whilst 3 mothers with CT genotype in our group presented with recurrent of aneuploidy in the family, studies done in different population groups in the world also found the same correlation between the CT genotype and the recurrence of aneuploidy birth suggesting the possible mechanism of inheritance of the disorder [10,33,34]. This study shows a positive correlation ($p < 0.05$) between eventful birth history and MTHFR polymorphism. They include 13.3% (3/30) miscarriages, 3.3% (1/30) of the previous birth with Down syndrome. The fact that we found a slightly higher number of mothers with a CC genotype (53%) with a normal enzyme activity in our cohort, indicate that other factors (over and above MTHFR polymorphism) such as maternal age may play a role in development of foetal aneuploidy in this population (Table 3).

In the study done by Scholtz et al. [35] reported a high prevalence of 1.43 (1/70) family hypercholesterolaemia (FH) in the South Africa Afrikaner population, due to MTHFR polymorphism (677C→T and 1298A→C gene) which was shown to be accountable for the disease in about 90% of affected Afrikaners, while 10-20% of affected coloured and were absent in the black population FH patients. Our finding of nearly half of the mothers in this cohort being positive for the CT genotype

Sub-group of mothers	Tests	Median	Range
CC genotype	Homocysteine (umol/l)	7.65	03-Dec
	Vitamin B12 (pmol/l)	254	47-604
	Serum folate (nmol/l)	24.75	Nov-36
CT genotype	Homocysteine (umol/l)	11.3	Jun-15
	Vitamin B12 (pmol/l)	236	80-451
	Serum folate (nmol/l)	21.6	Sep-38

Table 2: Mean values and ranges of blood variables amongst participants (mothers with aneuploidy births).

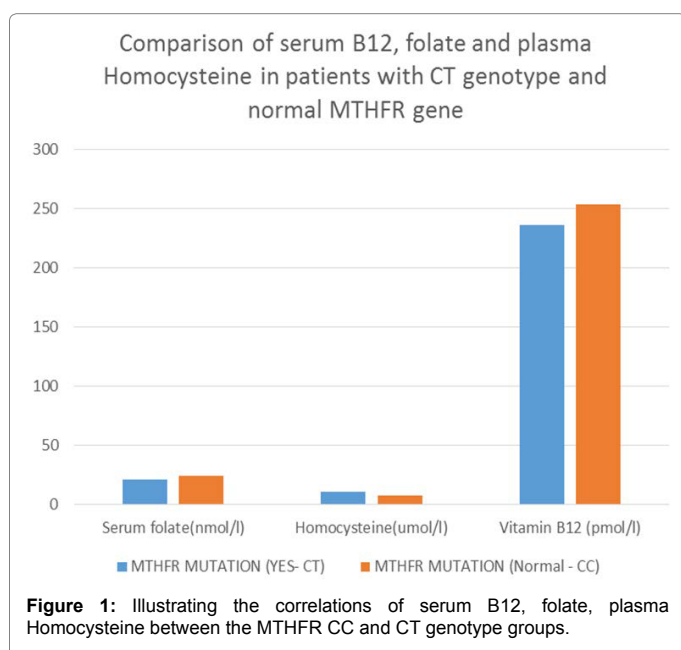


Figure 1: Illustrating the correlations of serum B12, folate, plasma Homocysteine between the MTHFR CC and CT genotype groups.

Variables	Bivariate (MTHFR) polymorphism	p-value
Age (23-41)	0.144	0.446
Homocysteine	0.538**	0.002
Serum folate	0.054	0.821
Vitamin B12	0.253	0.177
Medications (ARV)x 13	0.062	0.743
Past and Present disease (HPT x2, HIV)	0.134	0.481
Eating soil (4 of total)	0.033	0.861
Drinking (alcohol) x2	0.018	0.925
Smoking x2	0.286	0.126
Family history of aneuploidy x2	0.191	0.311
Miscarriage x4 previous aneuploidy birth x1	0.590**	0.001

** Correlation is significant at $p < 0.05$ level

Table 3: Bivariate correlation between MTHFR and different variables.

is significant addition to the knowledge body of the country's MTHFR status and its risks for aneuploidy in the mothers of reproductive age.

A limitation of this study was the small sample size due to limited time for recruitment in submission for the Honours degree.

Conclusion

The data confirms the presence of CT mutation (C677T) in the MTHFR gene which is known to have an estimated 60% reduction of the enzyme activity are found in the nearly 50% of the mothers with aneuploidy birth(s) in this study group. There is also association with increased homocysteine levels in this group. Whether this association infers any risk of recurrence in aneuploidy in their future pregnancies can only be ascertained at their future pregnancies. The authors have requested the mothers to communicate should there be any recurrence in the next pregnancies. The affected mothers with the CT genotype have been advised to see their doctors early in the pregnancy for supplementing with high folate and B12.

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