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Msc Final Paper

Perform a meta-analysis for the variant MTHFR rs1801131 in chronic allograft nephropathy

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Preface

Before I begin, I would like to thank all those people who, in their own way, helped me in order to bring this paper to its completion.

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1. Introduction

Over the years, kidney transplants have faced problems of failure, months or years after having been carried out. Chronic allograft nephropathy, abbreviated CAN is the major cause of this failure. It can happen months to years after the transplant. This problem is characterized by slow decline in the working of the kidney and many a times go together with high blood pressure. CAN is diagnosed by examination of tissue (kidney biopsy). The histopathology is characterized by interstitial fibrosis, tubular atrophy, fibrotic intimal thickening of arteries and glomerulosclerosis. To test for the presence of the chronic allograft nephropathy, examination of tissues such as those from the kidney is done (Cacabelos *et al,* 2013, P.241).

Cardiovascular morbidity and mortality are the most important reasons affecting patient survival after kidney transplantation (Kasiske BL., 1988). Because CAN pathogenesis shares some similarities with the atherogenesis (Ross R. 1999), it has been hypothesized that some gene variants that may play a role in the pathogenesis of atherosclerosis, may also affect kidney graft survival (Suthanthiran M., 2000). Hyperhomocysteinemia is an established, independent risk factor for vascular disease morbidity and mortality. Identification of risk factors, such as hyperhomocysteinemia, is crucial for a better understanding of the events that lead to degenerative processes in the vascular system and for a correct understanding of the potential role of methylene-tetrahydrofolate reductase enzymes (MTHFR) to help in the treatment of vascular disease observed in chronic allograft nephropathy CAN). Total blood levels of homocysteine (tHcy) have been shown to depend on both environmental and genetic factors (Cortese C, Motti C., 2001). Elevated tHcy levels are frequently observed in patients with renal failure and after kidney transplantation. The methylenetetrahydrofolate gene, MTHFR, is a form of a gene that is used as a guide in making the methylenetetrahydrofolate reductase which plays a role in processing amino acids which are the building blocks of proteins, through chemical reactions, to change the amino acids into more useful compounds. Increased levels of homocysteine and the danger of cardiovascular diseases, mostly in people with kidney problems, is a problem associated to mutation in the human methylenetetrahydrofolate gene.

In this paper, the effect and the influence of Methylemetetrahydrofolate is being reviewed, in that it causes gradual kidney dysfunction. Methylemetetrahydrofolate reductase is regarded

as a primary and key enzyme for intracellular folate homeostasis and metabolism which causes an irreversible reaction by catalyzing the conversion of the molecule 5, 10-methylenetetrahydrofolate to molecule 5-metyltetrahydrofolate which is a co-substrate for homocysteine remethylatiom to methionine. Increased levels of homocysteine are said to be linked to mutation in the Methylenetetrahydrofolate gene that is found in the human chromosome 1p36.3. There are two non-synonymous and single nucleotides in the methylenetetrafolate gene that in terms of functioning alter the protein product C677 found in Exon 4 into a reduced thermo labile compound or variant which is decreased in stability and its specificity in the way it reacts and is related to reduce methylenetetrahydrofolate reductase. There is another compound, A1298 that is normally found in Exon 7 and that helps in reducing methylenetetrahydrofolate reductase activity. The polymorphisms highlighted above have recently been associated with a number of diseases such as defects in neural tubes, malignancies, vascular diseases, and cardiovascular diseases.

Hyperhomocysteinemia has been discovered as a problem suffered by patients of kidney disease, more particularly those with end stage renal disease on dialysis, and it is thus said to play a role in their increased cardiovascular disease peril. According to Cacabelos *et al* (2013, p.321) when combined with fibrinogen as risk factors, homocysteine may explain almost up to 40% of the attributable mortality as far as chronic kidney disease is concerned. Methylenetetrahydrofolate dehydrogenase 1 and methylenehydrofolate reductase 5 10 are the two important folate metabolizing enzymes involved in the folate metabolic pathway. The polymorphisms, MTHFR and the MTHFD are suspected to be associated with congenital heart disease susceptibility (Garre & Hernández p 13). A number of mutations in the MTHFR gene are said to have been found in people with homocystinuria which can be described as a disorder that renders the body unable to process certain compound in amino acids properly (Fung et al, 2012 p.241). The effect of most of this mutation is that they change single amino acids in MTHF reductase. These changes in turn impair the functioning of the enzyme and may eventually cause the enzyme to be inactivated. Sometimes, a different form of the mutations may cause production of a too small and non-functioning type of the enzyme. Normally, without the MTHF reductase playing its role, homocysteine cannot be converted to methionine. Due to this reason, the amount of methionine is reduced, as homocysteine accumulates in the blood streams. Excess

homocysteine is excreted through urine (Cacabelos *et al*, 2013 p.343). Research has not yet discovered how changing levels of homocysteine and methionine cause different health problems affecting numerous parts of the body of the people with homocystinuria. This study aimed at performing a meta-analysis for the variant MTHF reductase rs1801131 in chronic allograft nephropathy and to determine its role in the event rate for chronic allograft nephropathy.

2. Literature review

The MTHFR gene mainly provides guidelines for making an enzyme called methylenetetrahydrofolate reductase. This enzyme plays a major function in processing amino acids, which are the building blocks of proteins. MTHFR reductase is essential for chemical reactions involving various forms of the vitamin B9 (Currie *et al*, 2007, p.50). This enzyme converts a molecule called 5, 10-MTHFR to a molecule called 5-MTHFR. The reaction is essential for the multistep process that breaks amino acid homocysteine into another amino acid and methionine. The proteins and other important compounds are made from methionine. About 40 mutations in the MTHFR gene have been discovered in people who have homocystinuria, a disorder that makes the body unable to process certain amino acids properly in the body. These changes occurring in the body impair the function of the enzyme, and some other causes that make the enzyme inactivated in the body. Other mutations occurring lead to the production of an abnormally non-functional version of the enzyme (Cacabelos *et al*, 2013, p.45). Without functional MTHFR reductase, homocysteine is unable to be converted to methionine. Homocysteine builds up in the bloodstream and the amount of methionine is reduced in the body. The excess homocysteine is excreted in urine. Although researchers have conducted some research but they have not yet determined how altered levels of homocysteine and methionine lead to some health problems affecting the bodies of the people.

There are some variations in the MTHFR gene which have been associated with an increased risk of neural tubes defects, a group of defects that occur mainly during the development of the spinal cord and brain. There are most well-studied polymorphisms related to neural tube defects changes a single DNA which is the building block in the MTHFR gene. It replaces the nucleotide cytosine with the nucleotide thymine at position 677. This common variant results in a form of MTHFR reductase that has reduced activity at higher levels of temperatures.

Hyperhomocysteinemia is mainly associated with the increased venous thrombosis and cardiovascular disease (CVD). Mutations which are carried in the human MTHFR gene have been associated with increased homocysteine levels and involved risks of CVD including people who are suffering from kidney disease. Previous studies that have been carried out, have

indicated that this gene is associated with some morbid conditions including cardiovascular and cerebrovascular disease. This is the first study that evaluates two common polymorphisms within the gene and also associates them with those coming from iothalamate. Few studies have evaluated MTHFR polymorphisms which are associated with renal diseases. Additionally, MTHFR A1298C was associated with UPCR as one of the potential confounder to the association, together with GFR decline over time. Also proteinuria is an important risk factor affecting kidney disease progression (Jamison *et al*, 2009, p.738). Therefore, polymorphisms from the associated gene, appear with homocysteine levels and have been associated with cardiovascular risk factors which include hypertension and renal function, which are responsible for causing these conditions to people.

Elevation of the homocysteine levels could be brought about by either genetic or dietary factors. In a study that was conducted on an Indian population, the majority of the population were found to have a lower level of vitamin B12. This is mainly due to their dependence on a vegetarian diet which is one of the critical factors that leads to hyperhomocysteinemia. Therefore, polymorphisms in those genes that are found to be responsible for the metabolism of homocysteine, have been considered to have a greater influence when it comes to hyperhomocysteinemia in the Indian population. For such reasons, effects of both methylenetrahydrofolate reductase (MTHFR) polymorphism and diet are significantly associated with high homocysteine levels (ACTON, 2012, p.21).

Many publications have established statistically significant correlation between tacrolimus and Single Nucleotide Polymorphisms (SNP) (HARZALLAH, 2011, p.56). Some working groups, researchers and consortia have even recommended guidelines for initial doze-adjustment with the SNP but are always being followed by TDM.

Over the last decades, there has been unprecedented advancement in assessing the diversity of human diversity across the major human being population in development of the deep sequencing and high throughput genotyping technologies similar to the advancement of the scale maps of the population including international Hap Map project (ACTON, 2012, p. 52). Such tools have been greatly utilized in deeply characterizing the architecture of the genotypes of rare and common diseases and brought about some other conditions such as adverse events and

severe drug effects. However, up to date, the organ transplantation field has not yet benefited in any way from these advancements. Whereas more than a thousand studies on organ rejection have already been published, there tend to be mostly candidate based and they suffer from the many pitfalls of genetic association. These pitfalls include: lack of the required sample size, failure to include covariates like ethnicity, retrospective study designs, improper statistical correlation and replication especially in cases where multiple hypotheses are involved (ACTON, 2012, p. 57).

According to ENVER *et al* (2012, p. 12), the MTHFR C677T polymorphism brings about amino-acid alterations from alanine to valine making the enzymes to become half-deceased and thermo labile. However, the prevalence varies among populations globally. Therapeutic drug monitoring (TDM) acts as an indispensable and essential instrument for the dosing of calcineurin inhibitor and in the reduction of pharmacokinetic variability component via controlling the concentrations of the drug in blood. However, this is possible only after the administration of drugs and patient compliance and achievement of a steady state. This implies that some complementary strategies are required. If the genetic variants are characterized, they can help in establishing effective doses which will minimize adverse reactions (ACTON, 2012, p. 33).

Chronic Allograft Dysfunction is a very common outcome in transplantation of kidneys (LIU, 2001, p.17). However, its pathogenesis has been unclear. Its natural history has been explained to be as a result of an earlier of tubulointerstitial injury initiation that eventually makes interstitial fibrosis and tubular atrophy to become worse. Earlier phases of chronic allograft dysfunction may occur within the three months after the transplant. It is an irreversible damage that may lead to the declining of the kidney function as well as allograft failure. Nevertheless, the rate at which the functioning of the kidney declines as well as the development of the failure of the allograft may vary in different CGD recipients. Therefore, there exists a need for a further characterization of chronic allograft dysfunction phenotype to provide a better understanding of those phenotypes which will cause rapid development of allograft failure. Methylenetetrahydrofolate reductase (MTHFR) remains a very critical enzyme in the folate metabolism. It is required in DNA repair, DNA methylation as well as DNA synthesis. Additionally, it has also remained a potential risk factor for neural tube defects. Association of

C677T polymorphism in MTHFR gene and susceptibility to neural tube defects has already been widely established although the findings are still inconclusive (LIU, 2001, p.19).

Methylenetetrahydrofolate reductase (MTHFR) is a crucial enzyme for metabolism of folate in humans and it is coded by the gene MTHFR. This research has studied the assessment of association between polymorphism of MTHFR C677T and congenital heart defects risks while its findings were not consistent (D'Alessandro & Mital, 2013, p.398). The congenital heart disease (CHD) is the type of birth defect which is common. Polymorphism in metabolism of folate is suspected to be associated the increased risks of congenital heart disease although it is not clear.

3. The kidney and MTHFR genetic variation

Vascular disease in renal transplant patients has been suggested to have a similar pathophysiology as vascular aging and atherosclerotic processes. MTHFR polymorphism has been evaluated by certain studies as associated with renal disease. In renal failure, association of CVD and MTHFR polymorphisms has shown mixed findings. Two universal genetic mutations were studied, A1298C and C677T, and were both shown to decrease the enzyme activity of MTHFR. Increased levels of plasma homocysteine are associated with the polymorphism of C677T itself and in combination with the mutation of A1298C, as these levels of elevated homocysteine and polymorphism have been associated with morbidity and mortality of CVD. As it was hypothesized, that pathophysiologic mechanism might be mediated via levels of elevated homocysteine (Bosó *et al*, 2013 p.483). Effective lowering of the levels of homocysteine with combination of treatment of vitamin like folate, B6 and B12 have not yet shown to decrease mortality in patients of renal failure who bear the burden of this disease, to be modified successfully. Moreover, it is suggested that homocysteine may be vascular disease maker rather than causative agent (Tayeb *et al*, 2015, p.89). C677T polymorphism has been reported to be 5-MTHF marker, circulating metabolite of folic acid which participates in the metabolism of homocysteine that seems to be the main controller of endothelial nitric oxide coupling synthase and nitric oxide available in the human vessels. This may then motivate oxygen free-radical formation, which contributes to vascular oxidative stress, causing alteration in thrombogenicity and endothelial function and ultimately leading to atherothrombosis (Bosó *et al*, 2013, p.486). Therefore, plasma homocysteine which is associated with CVD and cerebrovascular might be an indirect 5-MTHF marker other than primary endothelial function regulator. Consistent with the hypothesis are the CVD and CKD mortality rates. It is not clear whether the Polymorphism of MTHFR A1298C also increases levels of homocysteine. It is not easy to evaluate levels of plasma homocysteine as they were defined at the AASK start trial and not available in the VAHC.

Hyperhomocysteinemia is highly associated with rise of cardiovascular disease (CVD) and venous thrombosis (D'Alessandro & Mital, 2013, p.403). Mutations in the human MTHFR reductase gene have been associated with rise in the levels of homocysteine and risks of

cardiovascular disease in certain populations with inclusion of those having kidney disease. Therefore, MTHFR-coding polymorphism is associated with renal decline at A1298C in African-Americans having hypertensive nephrosclerosis, it is assisted by cohort having primary hypertension of care diagnosis.

4. Research Questions

- What is MTHFR?
- What is the normal function of MTHFR?
- How are changes in MTHFR related to CAN?

5. Data Collection Methods

5.1. Patients and Study Design

Donors and their respective recipients who underwent surgery were included in study. All recipients had under gone renal transplantation at least 12 months prior the investigations. Patients having biopsy proven subclinical acute rejection were not included in the studies. Plasma homocysteine concentrations were measured by liquid chromatography-tandem mass spectrometry and MTHFR polymorphisms were investigated by the PCR-RFLP technique. After surgery, immunosuppression regiment was based in tacrolimus. After transplantation, patients were followed up for the first two weeks for pharmacokinetic data, then again for 18 months for clinical data. Clinical data, analytical data and length of stay in hospital after transplant were obtained from hospital records. Genetic data and other additional information were obtained from study population with no disturbance to patient management and were handled in line with regulations in data registration, preservation and use of patient privacy.

5.2. Measurement of tacrolimus concentrations and baseline immunosuppression

The immunosuppression regimen was composed of corticosteroids, tacrolimus, mycophenolate and mofetil. Treatment was started orally, 24 hours after surgery if there were no complications. Patients received tacrolimus (orally) as a primary immunosuppression drug at initial doses of 0.1-0.2 mg/kg/day, subdivided to two doses. Tracolimus dose was then individualized by TDM so as to maintain target blood through concentration 10-15 ng/mL. After this, Tacrolimus blood samples were measured in blood samples obtained before tacrolimus dose administration in the morning. Through clinical chemistry, a system of a minimum of four samples per patient was obtained.

5.3. Genotyping

From EDTA-anti-coagulated blood of transplanted recipients and donor, genotypic DNA was removed from 200 ul of blood. DNA was extracted using a commercial kit which was based on centrifugation in micro-columns. Quantification was then done using a spectrophotometer.

So as to determine purity and concentration, DNA was stored until use at -20 degrees C. Genetic analysis program was then used to genotype the samples. 34 SNPs in 16 different genes was analyzed.

6. Statistical analysis

Statistical calculations and analyses were performed by use of SPSS software and prism. Categorical variables obtained were then expressed as percentage. Variables which were continuous were expressed as mean and median or interquartile range depending on the result on Kolmogorow-Smirnoff or Shapiro-Wilk normality test, depending on the sample size. Associations between categorical variables or genotypes were assed using a chi-square test. For continuous variables Mann-Whitney U test was performed to compare groups and Kruskal-Wallis test to compare several groups was used.

7. Results

The following articles are used in meta-analysis of the topic of interest. They are coded to provide ease of developing tables.

1. Association of methylenetetrahydrofolatereductase T677 allele with early development of chronic allograft nephropathy (Viklický et al., 2004). Coded study 1.
2. Hyperhomocysteinemia and MTHFR C677T and A1298C polymorphisms are associated with chronic allograft nephropathy in renal transplant recipients (Pavarino-Bertelli et al., 2004). Coded study 2.
3. Polymorphism of the methynetetrahydrofolate reductase C677T gene with allograft nephropathy in renal tranplants recipients. Coded study 3.

From these sources, the tables provided below are developed.

Name of article	Author's name	Publication year	Type of observation study	No. of Cohorts/cases	No. of controls
Study 1	Viklický et al.	2004	Cohort-control	92	365
Study 2	Pavarino-Bertelli et al.	2004	Cross-sectional	53	57
Study 3	Azapira N et al.	Unknown	Unknown	Unknown	Unknown

Table 1 Presentation of articles

Name of article	95% confidence interval (CI)	Odds ratio (OR)	p-value
Study 1	1.11 to 13.8	3.91	0.02
Study 2	1.26 to 6.98	2.97	0.005

Table 2 Analysis of articles

Note: study 3 has not been included in table 2 since the article could not be retrieved (not available online). Therefore, meta-analysis could not be properly carried out due to lack of

sufficient data to analyze. However, even without a proper population of data, as only two articles were available, there is strong evidence that rs1801131 is closely linked with CAN.

Genomic DNA obtained from renal transplant recipients and donors was genotyped. It was then noted that the allele frequencies seen were consisted with those described in the SNP PubMed Database for the Caucasian population. No associations between patients' characteristics and SNPs studied were found.

8. Discussion

Upon reviewing our results, we see that our research indicates that the coding of polymorphism at A1298C for MTHFR is somehow associated with renal decline. In spite of polymorphisms from this gene that are said to appear and be associated or linked with homocysteine levels, and additionally linked with cardiovascular risk factors that include a high tension and also renal function. The factors that contribute to these huge conditions are changes in MTHFR gene and environmental factors.

By examining the articles we analyzed, we conclude that the p-values from the two articles are less than 0.05 and OR are between 2.97 and 3.98. Therefore, the hypothesis is not rejected. So a deduction can be made that variant-MTHFR-rs1801131 is linked to chronic-allograft-nephropathy.

9. Recommendation

More research needs to be done about proteinuria, since it is an important factor of risk for kidney disease progression that may provide a link to the genotype. We recognized the study of polymorphisms in MTHFR gene as potential risk factors for a range of well-known conditions that include; stroke, heart disease, high blood pressure during pregnancy, eye disorder, certain types of cancer, high blood pressure, psychiatric disorders and cleft lip and palate. Thus it remains unclear on what occurs during MTHFR gene play in these disorders, but a large number of environmental and genetic factors, which remain unknown, may likely facilitate the development of risk for most common, complex conditions.

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