A Pilot Study for the Prevalence of Methylenetetrahydrofolate Reductase Gene Mutation in Egyptian Children with Homocystinuria

Thesis

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ABSTRACT

Objective: To determine the prevalence of C677T MTHFR gene mutation among children suffering from homocystinuria and the severity of clinical manifestations.

Methods: 7 families (7 cases, 9 siblings and 12 parents) & 20 healthy age and sex matched subjects were tested for C677T MTHFR gene mutation using Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP).

Results: The results of this study revealed that the TT genotype frequency was high (42.9%) among homocystinuric cases while 57.1% of cases had the wild type (CC).

Conclusion: This study revealed that the TT genotype frequency was high among homocystinuric cases, indicating a high prevalence of C677T MTHFR gene mutation among Egyptian homocystinuric cases. However, the presence of C677T mutation alone does not necessarily imply poor prognosis as other homocystinuric patients without this mutation had higher plasma homocysteine and worse clinical presentation.

Key words: C677T MTHFR, PCR-RFLP, homocystinuria.

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LIST OF ABBREVIATIONS

A	Adenine
ADMA	Asymmetric dimethyl arginine
AIS	Arterial ischemic stroke
ALP	Alkaline phosphatase
AMP	Adenosine monophosphate
ATP	Adenosine triphosphate
Au	Gold
BIA	Bacterial inhibition assay
BMD	Bone mineral density
Bp	Base pair
C	Cytosine
CBC	Complete blood picture
Cb	Cobalamin
CE	Capillary electrophoresis
CE–EC detection	Capillary electrophoresis with electrochemical detection
CK	Creatine kinase
CMPI	Chloro-1-methylpyridinium iodide
CNS	Central nervous system
CT	Computerized tomography
Cu	Cupper
CUCH	Cairo University Children Hospital
CVCs	Chorionic villus cells
CβS	Cystathionine β synthase
dATP	Deoxyadenosine Triphosphate
DBS	Dried blood spots
dCTP	Deoxycytidine Triphosphate
dGTP	Deoxyguanosine Triphosphate
DHF	Dihydrofolate
DIMP-T&R-MIMS	Direct insertion membrane probe using trap and release membrane introduction mass spectrometry
DNA	Deoxyribonucleic acid
DNTPs	Deoxynucleotides Triphosphate

DTT	Dithiothreitol
dTTP	Deoxythymidine Triphosphate
EC	Electrochemical
EDTA	Ethylenediamine tetra-acetic acid
EEG	Electroencephalogram
EIA	Enzyme-linked immunoassay
EMG	Electromyography
ESI	Electrospray ionization
F	Forward
FAD	Flavin adenine dinucleotide
Fe	Iron
FPIA	Fluorescence polarization immunoassay
FRET	Fluorescence resonance energy transfer
G	Guanine
GC	Gas chromatography
GC–MS	Gas chromatography -mass spectrometry
GNMT	Glycine N-methyltransferase
Hb	Haemoglobin
Hcy	Homocysteine
HDG	Heteroduplex generator
HDL cholesterol	High density lipoprotein cholesterol
Hg	Mercury
HPLC	High pressure liquid chromatography
HPLC–EC	High pressure liquid chromatography with electrochemical detection
HPLC- UV	HPLC with ultraviolet detection
ICL	Chemiluminescence immunoassay
ID	Isotopic dilution
Kcl	Potassium chloride
KD	Kilodalton
LC	liquid chromatography
LC–MS/MS	liquid chromatography-mass spectroscopy
LDL cholesterol	Low density lipoprotein cholesterol
LIF	Laser Induced Fluorescence

Lt	Left
M.W.	Molecular Weight
MAT	Methionine adenosyltransferase
Max	Maximum
MBrB	Monobromobimane
MeOH	Methanol
$MgCl_2$	Magnesium Chloride
$MgCl_2.6H_2O$	Magnesium Chloride Hexahydrate
Min	Minimum
ml	Milliliter
MLT	Methionine Loading Test
MRI	Magnetic resonance imaging
mRNA	messenger RNA
MS	Methionine synthase
MTHFR	Methylenetetrahydrofolate reductase
MTR	5-methyltetrahydrofolate-homocysteine S-methyltransferase
n	Number
NaCl	Sodium Chloride
NADP	Nicotinamide-adenine dinucleotide phosphate
NaOH	Sodium hydroxide
OF	Outer forward
o-PA	O-phthaldialdehyde
OR	Odds ratio
P	Petit (short arm of chromosome)
P Value	Probability Value
PCR	Polymerase chain reaction
PGD	Preimplantation genetic diagnosis
Pte	Pteroic acid
PteGlu	Pteroylglutamic acid
R	Reverse
RBCs	Red Blood Cells
RFLP	Restriction fragment length polymorphism
rHcyase	Homocysteine α,γ-lyase

RNA	Ribonucleic acid
Rt	Right
SAH	S-adenosyl- -homocysteine
SAHase	S-adenosyl- -homocysteine hydrolase
SAM	S-adenosyl-methionine
SD	Standard deviation
SDS	Sodium Dodecyl Sulphate
SNP	Single nucleotide polymorphism
SSCP	Single strand conformation polymorphism
T	Thymine
TBE	Tris-borate EDTA
TCEP	Tris(2carboxyethyl) phosphine.
TE	Tris EDTA
tHcy	Total homocysteine
THF	Tetrahydrofolate
Tris-HCl	Tris – Hydrochloric Acid
UV	Ultraviolet
Y	Years
µg	Microgram
µmole	Micromole
14C	14 radioactive carbon
5-methyl-THF	5-methyltetrahydrofolate

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The enzyme MTHFR plays a critical role in homocysteine metabolism by catalyzing the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate, the methyl-group donor in the B_{12}-dependent remethylation of homocysteine to methionine ***(Frosst et al., 1995).***

Methylenetetrahydrofolate reductase (MTHFR) deficiency is an autosomal recessive inborn error of metabolism characterized by varying degrees of developmental delay, seizures, motor and gait abnormalities, and thrombosis. The presentation of affected individuals is variable, ranging from severely affected neonates to mildly affected adults. The majority of patients present within the first few years of life. The degree of severity correlates with residual enzyme activity, with severely affected patients having little or no residual MTHFR specific activity in cultured fibroblasts. Biochemical features include homocystinuria, hyperhomocysteinemia, low or normal plasma methionine levels and decreased levels of S-adenosylmethionine ***(Morel et al., 2005).***

The C to T change at nucleotide position 677, whose functional consequences are dependent on folate status, has been extensively studied for its clinical consequences. Neural tube defects and pregnancy complications appear to be linked to impaired MTHFR function ***(Schwahn and Rozen, 2001)***