MOLECULAR CHARACTERISATION OF SUSPECTED HETEROZYGOTES OF TRIMETHYLAMINURIA

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Sometimes it is better to be kind than to be right

Gandhiji

To my grandmother

Mothele gugu-Mothele

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ABBREVIATIONS

α	Alpha
A	Adenine, a purine nucleotide,
A	Alanine, an amino acid
ADP	Adenosine diphosphate
Asn	Aspartic acid
ATP	Adenosine triphosphate
β	Beta
bp	Base pairs
C	Cytosine, a pyrimidine nucloetide
C	Cysteine, an amino acid; one letter notation
C-terminus	Carbon terminus of a protein
cDNA	complementary deoxyribonucleic acid
°C	degree Celsius
COOH	Carboxylic functional group
CSGE	Conformation sensitive gel electrophoresis
CpG	Cytosine pyrophosphate guanosine
cyt P450	Cytochrome P450
D	Aspartic acid, an amino acid; one letter notation
Da	Dalton
DGGE	Denaturing gradient gel electrophoresis
dH_2O	Sterilised distilled water
dNTP	2'-deoxynucleotide-5'-triphosphate
DNA	Deoxyribonucleic acid
E	Glutamic acid, an amino acid; one letter notation
EDTA	Ethylenediaminetetraacetic acid: $C_4H_{16}N_2O_8$

F	Phenylalanine, an amino acid; one letter notation
FAD	Flavin adenine dinucleotide (oxidised form)
$FADH_2$	Flavin adenine dinucleotide (reduced form)
FAD-OH	Flavin adenine dinucleotide (oxidised state 1)
FAD-OOH	Flavin adenine dinucleotide (oxidation state 1)
FMN	Flavin mononucleotide
FMO	Flavin-containing monooxygenase
FMO1	Flavin-containing monooxygenase 1
FMO3	Flavin-containing monooxygenase 3
FMO5	Flavin-containing monooxygenase 5
G	Guanine, a purine nucleotide
G	Glycine, an amino acid
H^+	Hydrogen ion
H_2O	Water
HA	Hetroduplex analysis
HCC	Heteroatom-containing compound
HOT	Hydroxylamine and osmium tetroxide
I	Isoleucine, an amino acid; one letter notation
K	Lysine, an amino acid; one letter notation
K_m	Michaelis constant
L	leucine, an amino acid; one letter notation
LC-MS	Liquid chromatograph-mass spectrometer
M	Molar
M	Methionine, an amino acid; one letter notation
mAmps	Milliamperes

mg	Milligram
$MgCl_2$	Magnesium chloride
min.	Minutes
mM	Millimolar (1×10^{-3} molar)
mmol/L	Millimole per litre
mL	Millilitre
mRNA	Messenger ribonucleic acid
μ/μL	Units per microlitre
μg/μmol	Microgram per micromole
μg/μL	Microgram per microlitre
μM	Micromole
N	Nitrogen
N-terminus	Nitrogen terminus of a protein
N/A	Not applicable
NaCl	Sodium chloride
NAD^+	Nicotinamide adenine dinucleotide (oxidised)
NADH	Nicotinamide adenine dinucleotide (reduced)
$NADP^+$	Nicotinamide adenine dinucleotide phosphate (oxidised)
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced)
ng	Nanogram
O_2	Oxygen
P	Proline, an amino acid; one letter notation
pI	Isoelectric point
PAGE	Polyacrylamide gel electrophoresis
PCR	Polymerase chain reaction
pH	Percentage hydrogen: numerically equal to the negative logarithm of H^+ concentration expressed in molarity
pmol	Pico mole

Pro	Proline; three letter notation
R	Arginine, an amino acid; one letter notation
RFLP	Restriction fragment length polymorphism
RH	Alkane
RNA	Ribonucleic acid
mRNA	Messenger ribonucleic acid
ROH	Alcohol
S	Sulphur
S	Serine, an amino acid; one letter notation
SDS	Sodium dodecyl sulphate: $C_{12}H_{25}NaSO_4$
SDS-PAGE	Sodium dodecyl sulphate-polyacrylamide gel electrophoresis
Ser	Serine, an amino acid; three letter notation
SO	Sulphoxide
SSCP	Single strand conformation polymorphism
T	Thymine, a pyrimidine nucleotide
T	Threonine, an amino acid; one letter notation
Taq polymerase	DNA deoxynucleotidetransferase from *Thermus aquaticus*: *EC 2.7.7.7*
TBE	Tris borate buffer
TEA	Triethylamine
Thr	Threonine, an amino acid; three letter notation
TM	Trademark
T_m	Melting temperature, the temperature at which a double stranded DNA denatures
TMA	Trimethylamine
TMAO	Trimethylamine-N-oxide
TMAuria	Trimethylaminuria

Tris	Tris(hydroxymethyl)aminomethyl: 2-Amino-2-(hydroxymethyl)-1,3-propanediol: $C_4H_{11}NO_3$
Tris-HCl	2-amino-2-(hydroxymethyl)-1,3-propanediol hydrochloride: $C_4H_{11}NO_3.H_2O$
Triton-X100	Triton-X100®: octylphenolpoly(ethylene-glycoether)$_n$: $C_{34}H_{62}O_{11}$, for n = 10.
TSGE	Temporal sensitive gelelectrophoresis
TTGE	Temporal temperature gradient gel electrophoresis
U	Units
UV	Ultraviolet
V	Volt
V	Valine, an amino acid; one letter notation
W	Tryptophan, an amino acid; one letter notation
X	No amino acid, product of a stop codon.
Xaa	Any amino acid
Y	Tyrosine, an amino acid; one letter notation

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ABSTRACT

Trimethylaminuria (McKusick 602079) or Fish odour syndrome is inherited recessively as a defect in hepatic nitrogen-oxidation of dietary derived trimethylamine (TMA), which causes excess excretion of trimethylamine such that affected individuals have a body odour reminiscent of rotten fish (Zhang *et al.*, 1995). Trimethylaminuria is a result of either partial or total incapacity to oxygenate trimethylaminuria to its oxide, trimethylamine oxide (TMAO), by an enzyme known as the flavin-containing monooxygenase 3 (FMO3). Mutations in the gene of the major human liver enzyme isoform, FMO3, are responsible for causing trimethylaminuria (Akerman *et al.*, 1999a and Dolphin *et al.*, 1997b). Clinical symptoms of this disorder of metabolism include fish-like to garbage-like odour of urine (trimethylaminuria), sweat (ishthyhidrosis) and breath (halitosis) as well as psycho-clinical symptoms such as depression (Akerman *et al.*, 1999a).

To establish the percentage of homo- and heterozygous trimethylaminuric individuals, a screening programme was introduced for the Potchefstroom first year students. Evaluation of the screening results through the liquid chromatograph-mass spectrometer, which is based on the accurate determination of the TMA:TMAO ratio, showed a 1.46% of mild trimethylaminuria individuals.

In this study, clinical symptoms induced by the loading test prior to urine evaluation of the TMA:TMAO ratio is described. This was followed by isolation of the FMO3 gene from the blood of suspected individuals and subsequent amplification using the PCR. Amplification was succeeded by restriction fragment length polymorphism analysis for the determination of known common mutations throughout the different exons of the FMO3 gene. Single stranded conformation polymorphism and heteroduplex analysis were performed to validate their applicability towards screening the FMO3 gene. Preliminary work was also done towards establishing the usage of the denaturing gel gradient gel electrophoresis to screen the FMO3 gene for aberrant sequences.

Results obtained through restriction fragment length polymorphism indicated the possible presence of the A52T mutation in exon 3 of both patients that showed symptoms of mild trimethylaminuria. The A52T mutation may be the most prevalent in the South African population although more research still have to be done to investigate this possibility. The main objective of this study was to establish the suitability of different methods towards mutation screening of the FMO3 gene. The methods attempted so far include polymerase chain reaction, restriction fragment length polymorphism, single stranded conformation polymorphism, heteroduplex analysis as well as denaturing gradient gel electrophoresis. All methods were applicable, although to different extents and with different limitations and resolutions. This study was a preliminary evaluation for a bigger study, which will include family members of the suspected heterozygotes. In the subsequent study, all nucleotide sequence fragments suspected of having mutations will be sequenced to confirm the presence and the type of the mutation present.

OPSOMMING

Trimetielamienurie (McKusick 602079) of "Fish Odour Sindroom" word resessief oorgeërf as 'n defek in die hepatiese stikstofoksidasie van trimetielamien (TMA) wat deur die dieet ingeneem word. Die oormaat trimetielamien word uitgeskei en veroorsaak dat geaffekteerde individue 'n liggaamsreuk van vrot vis het (Zhang et al., 1995). Trimetielamienurie ontstaan as gevolg van 'n gedeeltelike of 'n algehele onvermoë om trimetielamien na die oksied te oksideer, deur 'n ensiem bekend as die flavien-bevattende mono-oksigenase 3 (FMO3). Mutasies in hierdie geen in die mens se lewer die oorsaak van trimetielamienurie (Akerman et al., 1999a en Dolphin et al., 1997b). Kliniese simptome van hierdie afwyking van die metabolisme sluit in vis- na vullis tipe reuke in die uriene (trimetielamienurie), sweet (igtihidrose) en asem (halitose) in, sowel as sielkundige simptome soos depressie (Akerman et al., 1999a).

Die persentasie homo- en heterosigoot trimetielamienurie individue is bepaal deur 'n siftingsprogram met Potchefstroom eerstejaarstudendte as studiegroep. Die vloeistofchromatograaf-massa spectrometer is gebruik om die sifting te evalueer, omdat dit die TMA:TMAO-verhouding baie akkuraat bepaal. Daar is waargeneem dat 1.46% van getoetsde individue milde trimetielamienurie gevalle is.

In hierdie studie is die kliniese simptome geinduseer deur 'n beladingstoets volgens die vooraf evaluering van die TMA:TMAO-verhouding wat beskryf is.

Die FMO3 geen is uit die bloed van persone geïsoleer wat vermoedelik die defek het, waarna geenamplifisering gebruik is deur middel van PCR-tegniek. Amplifiseering van die relevante geengedeeltes is gevolg deur RFLP-analiese om te toets vir die teenwoordigheid van bekende mutasies in die verskillende aksons van die FMO3-geen. Enkelstring-konformasiepolimorfisme en heterodupleksanalises is gebruik om die toepasbaarheid van hierdie tegnieke te evalueer vir die sifting van die FMO3-geen. Voorlopige werk is gedoen om die gebruik van die denaturerings-gradiëntgelelektroforese te vestig om die FMO3-geen te bestudeer vir ongewone volgordes.

Resultate wat verkry is deur die restriksie fragmentlengte polimofisme dui daarop dat die teenwoordigheid van die A52T mutasie in ekson 3 in beide ouers aanleiding gee tot simptome van milde trimetielamienurie. Die A52T mutasie kan die mees algemene mutasie in die Suid-Afrikaanse populasie wees, alhoewel meer navorsing gedoen moet word voordat hierdie feit bevestig kan word. Die hoofdoelwit van hierdie studie was om die geskiktheid van verskillende metodes te toets tydens die sifting vir mutasies in die FMO3-geen. Die metodes wat gebruik is, is die polimerasekettingreaksie, restriksiefragmentlengtepolimorfisme, enkelstring-konformasiepolimorfisme, heterodupleksanalise en die denatureringsgelgradiëntgelelektroforese. Al hierdie metodes kan gebruik word, maar vir verskillende gebruike want elkeen het sy beperkinge. Hierdie studie is 'n voorlopige evaluering vir 'n groter studie wat onderneem sal word, wat die familielede van die vermoedelike heterosigote insluit. In hierdie studie sal al die nukleotiedfragmente wat vermoedelik mutasies bevat se volgordes bepaal word om die teenwoordigheid en die tipe mutasie wat teenwoordig is te bevestig.