

CHAPTER 3

APPROACH TO THE STUDY

3.1. Introduction

This study presents efforts within a model system to determine the feasibility of using different mutation detection methods in a clinical environment for the detection of mutations and/or polymorphisms in the FMO3 gene relevant for the diagnosis of trimethylaminuria. The apparent association of cardiovascular diseases such as hypertension and migraine to the malfunctioning of the liver FMO3 enzyme leading to deficient biogenic amine metabolism warrants further research into this matter (Brunelle *et al.*, 1997; Akerman *et al.*, 1999b). Yet, before the apparent link can be investigated further, cheaper but effective screening techniques need to be put in place for easy screening of mutations in the FMO3 gene. This study seeks to develop a protocol through a combination of the already known methods in such a way that screening for mutations in the exons as well as the flanking intronic sites of the FMO3 gene is relatively simple with regards to diagnosis of trimethylaminuria.

This study aims to establish the following:

- To screen for trimethylaminuria homozygotes as well as simple and compound heterozygotes by directly challenging the hepatic FMO3 enzyme using the loading test coupled with the recently developed LC-MS (liquid chromatography-mass spectrometer) method (Erasmus *et al.*, 2000).
- To develop a new protocol that will be cost effective, relatively simple to perform yet efficient for the specific diagnosis of mild or severe congenital trimethylaminuria caused by mutations or polymorphisms in the FMO3 gene.
- To determine similarities and frequency of polymorphisms in the FMO3 genes of a pre-selected Potchefstroom community.
- To characterise available FMO3 gene sequences and their mutations.

3.2. Approach

In this study, urine samples were collected from volunteers to quantify the TMA and TMAO ratios and thereby reflect on the functionality of the liver FMO3 enzyme using the loading test.

This was followed by blood sample collection from those volunteers that exhibited a low ratio associated with mild trimethylaminuria as well as those with a very low ratio corresponding to severe trimethylaminuria. Although subsequent analysis used the white blood cell genome, the degree of functionality of the liver FMO3 enzyme would have been established by the loading test, hence a mutation or polymorphism in the white blood cell genome would invariably be reminiscent of its hepatic counterpart's molecular structure.

PCR amplification was then standardised for the eight coding exons. With the knowledge of the disease-causing mutations, relevant restriction enzymes were used as diagnostic tools to indicate the presence or absence of a specific mutation. The fragments were then electrophoresed through the SSCP gel. The gels were optimised so as to analyse the fragments with regards to their three dimensional conformation as single stranded or double stranded. This is called single stranded conformation polymorphism (SSCP) and heteroduplex analysis (HA), respectively. As the absence of the distinction between the conformers in SSCP and HA does not necessarily advocate the absence of a mutation, an attempt was made to use a more sensitive mutation detection system called the denaturing gradient gel electrophoresis (DGGE). Although the DGGE method was not fully completed in this study, preliminary results using this technique will certainly help in the future towards mutation detection with regards to trimethylaminuria.