

CHAPTER 1

INTRODUCTION

The synthesis of trimethylamine, a volatile aliphatic tertiary amine with fish-like odour, was first reported in the literature in 1851 by Hoffmann (quoted by Al-Waiz *et al.*, 1987a) and was obtained in its pure form in 1855. Only in 1951 was it shown to exist in much greater quantities in fish in the form of a nitrogenous oxide by Shewan (Al-Waiz *et al.*, 1987a).

Dessaignes (quoted by Al-Waiz *et al.*, 1987a) reported the presence of trimethylamine in human urine for the first time in 1856. Metabolic studies using trimethylamine, its oxide and choline in animals have shown as early as 1909 that the extra load of these substrates is rapidly excreted in urine, mainly as the nitrogenous oxide (Al-Waiz *et al.*, 1987a).

Trimethylaminuria, colloquially known as the 'fish odour syndrome', is a glycine metabolic disorder that was known for centuries. It is known to be a result of ineffective enzymatic conversion of trimethylamine. Individuals suffering from this disorder often produce a foul smell reminiscent of rotting fish. This is a condition that is thought to be medically insignificant but can cause serious social harm to the affected individual. The enzyme implicated in this disorder is a flavin-containing monooxygenase, which oxidises the nitrogen of the trimethylamine to a less odorous compound, trimethylamine oxide (Christensen, 1999).

There are various methods through which this disorder can be diagnosed. The most effective methods, apart from the sense of smell, require quantitative measurements of trimethylamine ratio to its N-oxide. Several mutations in the FMO3 (flavin-containing monooxygenase 3) gene that are known to cause or assist in projecting symptoms of trimethylaminuria have already been characterised.

Trimethylaminuria is an autosomal recessive disorder (Ziegler, 1993; Cashman, 1995; Mayatepek and Kohlmüller, 1998). It is thus likely that certain mutations will occur more frequently in some populations compared to others, with the available genetic pool being the limiting factor.

In this study, the hepatic FMO (flavin-containing monooxygenase) enzyme was tested for percent functionality using a 600 milligrams load of trimethylamine as a substrate. This was followed by the isolation and amplification of the FMO3 gene, whose protein product malfunction is the primary cause of trimethylaminuria. The amplified exons of the FMO3 gene were then screened for possible mutations. The FMO3 gene's molecular structure from different selected subjects blood samples was compared using the PCR-SSCP/HA (Polymerase chain reaction-single stranded conformation polymorphism/heteroduplex analysis) techniques and to a lesser extent PCR-DGGE (polymerase chain reaction-denaturing gradient gel electrophoresis) method to assess the availability of mutations as well as polymorphisms.

The main aim of this study was to standardise in this laboratory, a reproducible, efficient and clinically acceptable method for detection and identification of mutations and polymorphisms resident in the FMO3 gene. The present study reports on the progress of the methods used so far towards achieving this objective. The methods used do not necessarily give conclusive evidence on the types of mutations found in the nucleotide fragments concerned but show whether certain methods are applicable for detecting mutations in the FMO3 gene. Since this study is continuous, confirmation of mutations would be conducted in a follow-up study wherein the families of the suspected trimethylaminuric heterozygotes would be included.