CHAPTER ONE

Introduction

Malignant hyperthermia (MH) is a pharmacogenetic disorder that is related to abnormal skeletal muscle regulation. Denborough and Lovell (1960) first described the disorder in a 21-year-old Australian male with a fractured tibia, who experienced complications following exposure to the then newly introduced anaesthetic, halothane. During surgery the patient became cyanosed and experienced a decrease in blood pressure, an increase in pulse rate and an elevated body temperature. Further investigation revealed that ten of his close relatives had died during or following exposure to anaesthesia. The condition was transmitted through three generations and was the first indication that MH susceptibility (MHS) was heritable. Michael Denborough identified MH as a distinct entity and observed that susceptibility to this disorder was inherited in an autosomal dominant manner. His observations led to a worldwide awareness of this potentially fatal disorder.

In susceptible individuals, commonly used inhalational anaesthetics (e.g. halothane) and depolarising muscle relaxants (e.g. succinylcholine) can trigger an MH episode (Denborough *et al.*, 1962). Although timely recognition and appropriate treatment have reduced the mortality rate, the disorder remains the main cause of anaesthetically induced morbidity and mortality (Robinson *et al.*, 1998) in otherwise healthy patients. The clinical presentation of MH is highly variable and may include one or more of the following symptoms: muscle rigidity, increased body temperature, metabolic acidosis, hypoxia and masseter muscle rigidity (MMR) or generalised muscle contracture (Nelson and Flewellen, 1983). Currently, the MHS phenotype is determined via the *in vitro* contracture test (IVCT), which is performed on a fresh muscle sample obtained by biopsy under regional anaesthetics. A standardised protocol for the IVCT was established by the European Malignant Hyperthermia Group (EMHG) and a similar protocol was implemented in North America (Larach, 1989), using different diagnostic criteria than the EMHG protocol (European Malignant Hyperpyrexia Group, 1984).

The animal model of MH is the pig, as porcine stress syndrome (PSS) mimics most of the characteristics of the MH disorder in man (Hall *et al.*, 1966). Biochemical studies of porcine and human MH have identified that an alteration of calcium (Ca^{2+}) homeostasis in skeletal

muscle plays an important role in these syndromes (MacLennan and Phillips, 1992). Molecular genetic studies in the pig linked halothane sensitivity to the glucose phosphate isomerase (GPI) locus on porcine chromosome 6 (Davies *et al.*, 1988). Human MHS has been mapped to chromosome 19q12-q13.2 in a region syntenic with the location of the locus responsible for porcine MH on chromosome 6. The gene for the skeletal muscle calcium release channel of the sarcoplasmic reticulum (SR), known as the ryanodine receptor (RYR1), is located at this locus, and was designated as the first locus for MHS, hence it is known as MHS-1. However, subsequent studies have demonstrated genetic heterogeneity in MHS, as the MHS-1 locus has been excluded in a number of pedigrees (Levitt *et al.*, 1991), providing strong evidence that other genes beside RYR1 are involved in MHS. It is estimated that approximately 50 percent (%) of cases of MH in European families are not linked to 19q12-q13.2 (Ball and Johnson, 1993). Additional candidate genes suggested to cause MHS have been mapped to chromosomes 17q11.2-q24 (Levitt *et al.*, 1992; Olckers *et al.*, 1992; Vita *et al.*, 1995), 7q21-q22 (Iles *et al.*, 1994), 1q32 (Monnier *et al.*, 1997; Robinson *et al.*, 1997), 5p (Robinson *et al.*, 1997), 2 (Olckers *et al.*, 1999), and 3q13 (Sudbrak *et al.*, 1995).

The broad aim of the MH research programme is to identify all the causative mutations in the RYR1 gene in South African MH patients, to determine if any of the mutations are responsible for the pathogenesis of MH in the South African population. In previous studies, selected individuals from South African MH families have been screened for some mutations in the RYR1 gene (Olckers, 1997; Havenga, 2000; Neumann, 2002; Dalton, 2004). Prior to this study only three reported RYR1 mutations have been detected in the South African MH population. The arginine (Arg)614 cysteine (Cys) alteration was detected in one extended MH family while the valine (Val)2168 methionine (Met) alteration was observed in a single individual. The threonine (Thr)4826 isoleucine (Ile) alteration was also observed in a single MH family. The detection of these mutations will contribute to a description of the aetiology of MH in the South African population. However, the inability to identify common mutations in MHS patients screened so far, suggests that the South African population carries mutations that are different to those documented in other international centres. The study presented in this thesis is the first investigation to screen the entire coding region of the RYR1 in order to identify further possible novel or reported mutations that may be responsible for the MH phenotype in the South African population. In Chapter three, the patient population as well as the methods used for screening all 106 exons of the RYR1 gene are described. These protocols were used to achieve the objectives listed in Chapter two. The results obtained from this study are presented in

Chapter four. The conclusions drawn from these results are described in Chapter five, as well as a model proposing the epigenetic character of MH in South Africa. Appendix A presents a summary of reported functional characterisation studies for the MH phenotype, while single nucleotide polymorphisms (SNPs) observed in the coding and the intron sequence of the RYR1 gene in the South African probands are presented in Appendices B and C, respectively.