

# Chapter 1: Introduction

---

Inherited metabolic diseases are identified by characteristic metabolic profiles, which have its origin in the block of a metabolic pathway due to a defective enzyme. The deficiency prevents the normal metabolism of a nutrient and can result in the accumulation (to toxic levels) of the substrate, upstream accumulating metabolites, and/or intermediates originating from induced alternative metabolic pathways. The accumulation of these metabolites gives rise to a specific metabolic profile. Each of the inherited metabolic diseases has clinical features by which they can be characterized.

Hereditary tyrosinemia type 1 (HT1; OMIM 276700) is an autosomal recessive disease, caused by a deficiency in fumarylacetoacetate hydrolase (FAH, EC 3.7.1.2), the last enzyme of the catabolic pathway of tyrosine (Holme, 2003:141, Mitchell *et al.*, 2001:1777). In the absence of FAH, metabolites such as maleylacetoacetate (MAA), fumarylacetoacetate (FAA), succinylacetone (SA), and *p*-hydroxyphenylpyruvic acid (pHPPA) accumulate during tyrosine catabolism (Mitchell *et al.*, 2001:1777).

Characteristic of HT1 is a high incidence of hepatocellular carcinoma and a high frequency of mutation reversion (Demers *et al.*, 2003:1313, Hirschhorn, 2003:721, Holme, 2003:141, Mitchell *et al.*, 2001:1777, Sniderman King *et al.*, 2008, Youssoufian and Pyeritz, 2002:748). Molecular studies of HT1 patients revealed that identical genotypes present with different phenotypes, and even family members could present different phenotypes, suggesting that genotypic variability alone does not account for the different clinical forms observed in tyrosinemia (Mitchell *et al.*, 2001:1777, Ploos van Amstel *et al.*, 1996:51, Poudrier *et al.*, 1998:119). A remarkable observation was made by Kvittingen *et al.* (Kvittingen *et al.*, 1993:1816, Kvittingen *et al.*, 1994:1657) when they described liver nodules in HT1 patients in which FAH activity was restored to normal. They proposed that nodule formation was due to a growth advantage of reverted cells. It was later suggested that the presence of varying numbers of reverted cells contributes towards the varying phenotypes observed for the same FAH mutations (Demers *et al.*, 2003:1313). Reversions to wild-type have been reported for only four HT1 mutations: IVS12+5g→a, Q64H, G337S, and Q279R (Demers *et al.*, 2003:1313, Dreumont *et al.*, 2001:9, Kvittingen *et al.*, 1993:1816, Kvittingen *et al.*, 1994:1657, Poudrier *et al.*, 1998:119), but reversion to wild-type of other HT1 mutations cannot be excluded. It is generally accepted that the reversion to wild-type of HT1 mutations are the result of a true back mutation (Blikrud *et al.*, 2005:406, Dreumont *et al.*, 2001:9, Hirschhorn, 2003:721,

Kvittingen *et al.*, 1994:1657, Pasmooij *et al.*, 2005:727). The mechanism underlying the true back mutations, and the ensuing genetic mosaicism, is, however, still unresolved.

The aim of this study was therefore to investigate the molecular basis of the characteristic genetic mosaicism in hereditary tyrosinemia type 1. Specifically, to determine if base- and nucleotide excision repair pathways are affected in HT1, and to what extent, and to determine if microsatellite instability is present in HT1.

To achieve the aims of the study, a two pronged approach was followed. The approaches that were followed in parallel were to: develop a hepatic cell culture model of HT1, through RNAi; and to use HT1 related models and HT1 patient material. The HT1 related models are the *fah*<sup>-/-</sup> mouse model and metabolite exposed hepatic cell cultures.

To understand the mosaicism occurring in HT1, the molecular mechanisms underlying the mutation reversion needed to be investigated. Previous results indicated the accumulating metabolites may affect the ability of cells to repair DNA damage (van Dyk, 2005, van Dyk and Pretorius, 2005:815). To elaborate on these studies and to evaluate the effects on DNA repair capacity and genome stability in the HT1 hepatic cell model, different assays were adapted to local laboratory conditions. These assays include the modified comet assay (to measure to capacity of cells for base- and nucleotide excision repair), relative quantification of gene expression (to determine gene expression of DNA repair proteins), microsatellite analyses (to measure genome stability), and HRM and sequencing (to assess mutation accumulation).

Chapter 2 of the thesis reviews the relevant and current literature available on hereditary tyrosinemia type 1, genetic mosaicism, and DNA repair.

Chapter 3 gives a description of the development of a HT1 hepatic cell model. This chapter encompasses both the methodology used for the development of the HT1 hepatic cell model, and the results obtained during the development of the model. The development of the HT1 hepatic cell model was a collaborative study, with equal conceptual, intellectual and technical input by Mrs. Chrisna Gouws and myself. The development of a HT1 hepatic cell was part of a larger study, with different objectives and outcomes.

In chapter 4, the technical procedure of the methods used to assess DNA repair capacity, and genome stability, is given. In specific, technical procedures for cell culturing, DNA and RNA extraction, the comet assay, RT-PCR and real-time PCR, MSI assaying, and high resolution melting and sequencing, are provided.

The results and discussion of the results, obtained after the assessment of the HT1 models and HT1 patient material using the above mentioned methods, are presented in chapter 5. This

chapter includes results on the recuperation time needed for adherent cells to be usable in the comet assay, and the gene expression levels of the DNA repair proteins, hOGG1 and ERCC1. Also included are the results from analysis of microsatellite DNA, and results of high resolution melting and sequencing of different mouse and human *fah* and *hprt1* gene fragments.

In chapter 6, the paper: “Hereditary tyrosinemia type 1 metabolites impair DNA excision repair pathways.” is presented. The paper was published in *Biochemical and Biophysical Research Communications* in October 2010. The authors all equally contributed to the intellectual and technical work of the paper.

Chapter 7 contains the paper: “Impaired DNA repair and genomic stability in hereditary tyrosinemia type 1”. This manuscript was published in *GENE*.

A summary of and a conclusion to the study are given in chapter 8. In this chapter, relevant literature and results are briefly summarised. Concluding remarks regarding the results are given and the scientific contribution of the results explained.

Chapter 9 contains the paper: “Point mutation instability (PIN) mutator phenotype as model for true back mutations seen in hereditary tyrosinemia type 1 – a hypothesis”. The paper presents a hypothesis that stems from this study, i.e. that the mechanism underlying the true back mutations seen in HT1, is the result of a PIN mutator phenotype that develops in HT1 cells. The paper was published in *Journal of Inherited Metabolic Disease*.

Subsequent sections include a list of references used in the thesis, and various appendices.

Work from this study was presented at the South African Society for Human Genetics (SASHG) Conference in Stellenbosch (2009). The conference contribution was in the form of a poster presentation, under the following title: “Effect of HT1 metabolites on NER and/or BER repair of DNA damage.” The abstract from this contribution can be found in appendix E.

Referencing in this thesis was done with RefWorks web based bibliographic management software (version 2.0) and the reference output style was NWU Harvard 6.