

A missense mutation (Q279R) in the Fumarylacetoacetate Hydrolase gene, responsible for hereditary tyrosinemia, acts as a splicing mutation

## ABSTRACT

These data suggest that the putative missense mutation Q279R in the FAH gene acts as a splicing mutation in vivo. Moreover FAH expression can be partially restored in certain liver cells as a result of a reversion of the Q279R mutation and expansion of the corrected cells.

## INTRODUCTION

Background Tyrosinemia type I (HTI, McKusick 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. In the absence of FAH, metabolites such as maleylacetoacetate (MAA), fumarylacetoacetate (FAA), and succinylacetone (SAC) accumulate during tyrosine degradation. FAA has been shown to have a mutagenic activity in a mammalian cell assay, to induce cell cycle arrest at G2/M, and apoptosis. Tyrosinemia is characterized by hepatic failure, cirrhosis, renal dysfunction, hepatocarcinoma, and neurologic crisis. Liver is the most severely affected organ. Two distinct forms of the disease have been described according to the symptoms and age of onset. The acute form of tyrosinemia is diagnosed in the first months of life and results in rapid deterioration of hepatic and renal functions leading to early death due to hepatic failure. In the chronic form, symptoms appear later in childhood and often culminate with development of hepatocarcinomas. Molecular studies on some patients presenting either the acute or the chronic form revealed identical genotypes suggesting that genotypic variability does not account alone for the different clinical forms observed in tyrosinemia. The gene encoding the FAH protein has been cloned and mapped to chromosome 15, region q23-q25. It contains 14 exons, spanning over 35 kb of DNA. At this time, 34 different mutations of the FAH gene have been identified in HTI patients: 18 missense mutations, 5 nonsense mutations, and 10 splicing mutations. One mutation (R341W) causes a pseudodeficient phenotype with a reduced amount of FAH immunoreactive material. FAH is a cytoplasmic homodimeric enzyme. Its crystal structure shows that each subunit of 46 kDa consists of a 120 residue N-terminal domain and a 300 residue C-terminal domain. These subunits interact through their C-terminal domains and the active site is located in close proximity to the dimer interface. It has been shown that the FAH active site, which is highly conserved from fungi to human, comprises two metal ions that participate in substrate binding and catalysis. Recently, Kim et al. described the case of a HTI patient who showed few of the symptoms associated with HTI until the age of 37 when hepatocellular carcinoma was diagnosed. This patient is one of the few reported cases of HTI who lived over 30 years and has been genotyped as a compound heterozygote for a frequent splice mutation, IVS6-1g->t, and a new mutation, Q279R (836A->G). Since this patient showed an almost normal phenotype during the first 36 years of her life, a molecular analysis of both mutations was carried out in vivo and in vitro to determine if this particular phenotype was caused by a neutral missense mutation (Q279R) like in the pseudodeficiency phenotype or by other mechanisms such as mutation reversion, as described in a number of HTI patients. It was shown that FAH was expressed in a mosaic pattern in the patient's liver, with non-tumoral regions expressing FAH. Here we report that the Q279R mutation acts as a splicing mutation in vivo and that correction of this mutation in some cells leads to restored FAH function and partial liver repopulation by corrected

cells.

## CONCLUSION

This clinical case of HTI is particularly interesting since the patient ceased dietary restriction at age 14 and showed no evidence of sickness until 37 of age. One explanation for this particularly mild phenotype was that the missense Q279R allele was enzymatically functional like in the case of the pseudodeficient allele (R341W), which produces low levels of catalytically active FAH. Our data rather suggest that the Q279R mutation disturbs mRNA splicing in vivo with the result that very little if any functional mRNA is made. Finally, analysis of transcripts in a FAH expressing region suggests that mutation reversion in the Q279R allele leads to the production of functional FAH and clonal expansion of the corrected cells and that this restoration may account for the mild phenotype observed.