Molecular Aspects of the *FAH* Mutations Involved in HT1 Disease

3

Geneviève Morrow, Francesca Angileri, and Robert M. Tanguay

Abstract

Hereditary tyrosinemia type 1 (HT1) is caused by the lack of fumarylacetoacetate hydrolase (FAH), the last enzyme of the tyrosine catabolic pathway. Up to now, around 100 mutations in the *FAH* gene have been associated with HT1, and despite many efforts, no clear correlation between genotype and clinical phenotype has been reported. At first, it seems that any mutation in the gene results in HT1. However, placing these mutations in their molecular context allows a better understanding of their possible effects. This chapter presents a closer look at the *FAH* gene and its corresponding protein in addition to provide a complete record of all the reported mutations causing HT1.

Keywords

Hereditary tyrosinemia type 1 (HT1) • Fumarylacetoacetate hydrolase (FAH) • Mutations

3.1 Introduction

Amino acid catabolism provides nitrogen for the synthesis of biologically important compounds like hormones and neurotransmitters as well as energy for the cell. This process occurs mainly in the liver and kidneys and the enzymatic pathway involved depends on the nature of the amino acid.

Phenylalanine and tyrosine are important for protein biosynthesis and intermediates in the biosynthesis of catecholamines. They are both catabolized through the tyrosine degradation pathway, which converts tyrosine into fumarate and acetoacetate,

G. Morrow • F. Angileri • R.M. Tanguay (✉)
Laboratoire de génétique cellulaire et développementale, Département de biologie moléculaire, biochimie médicale et pathologie, Faculté de médecine, Institut de biologie intégrative et des systèmes (IBIS) and PROTEO, Université Laval, 1030 avenue de la médecine, Québec, QC G1V 0A6, Canada
e-mail: genevieve.morrow@fmed.ulaval.ca; francesca.angileri.1@ulaval.ca; robert.tanguay@fmed.ulaval.ca

R.M. Tanguay (ed.), *Hereditary Tyrosinemia*, Advances in Experimental Medicine and Biology 959,
DOI 10.1007/978-3-319-55780-9_3

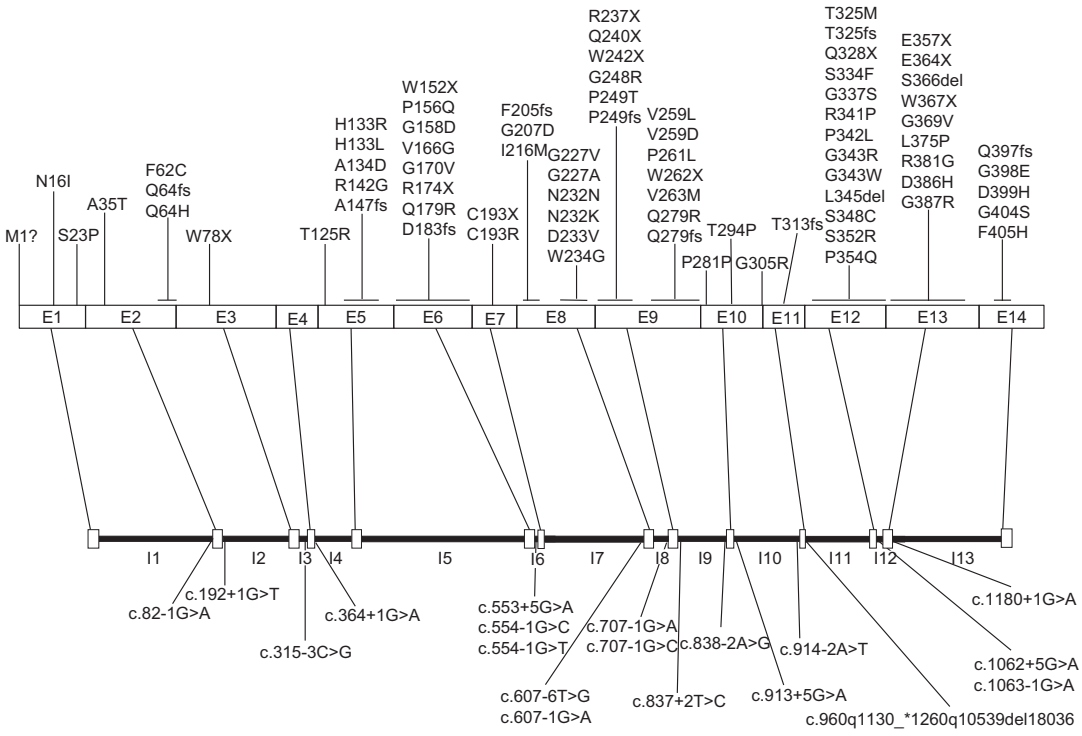

Fig. 3.1 Schematic representation of the *FAH* gene region and the corresponding protein. The location of the 98 mutations identified on the *FAH* gene is presented. *E* exon, *I* intron

two substrates of the mitochondrial tricarboxilic cycle (TCA cycle) (see Chap. 2).

Fumarylacetoacetate hydrolase (FAH, E.C. 3.7.1.2) is the last enzyme of the tyrosine degradation pathway containing 420 amino acids in a homodimeric form (Mahuran et al. 1977; Phaneuf et al. 1991). Up to now, close to 100 mutations in the *FAH* gene have been associated with hereditary tyrosinemia type 1 (HT1; HGMD® Professional 2016.1, accessed on April 2016) (Fig. 3.1) (Angileri et al. 2015). Despite multiple efforts, no clear link between mutation (genotype) and HT1 phenotype has been found. This chapter will focus on molecular aspects of the *FAH* gene and its corresponding protein in addition to give a complete listing of all the mutations identified to date.

3.2 Architecture of the fah Gene

The *FAH* gene is localized on chromosome 15 (15q23–25) and consists of 14 exons spanning over 35 kb of DNA (Awata et al. 1994; Labelle et al. 1993; Phaneuf et al. 1991) (Fig. 3.1). All exon-intron junctions possess the 5′ splice donor (gt) and 3′ splice acceptor (ag) consensus sequence and the major splicing product of the *FAH* gene results in an mRNA of 1260 coding nucleotides (Labelle et al. 1993). Table 3.1 summarizes the length of exons and introns and the number of HT1 mutations found in each of these elements while Fig. 3.1 is a schematic representation of the *FAH* gene region and protein. Exons 9 and 12 have the largest clusters of HT1 disease-causing *FAH* mutations. Interestingly both of these exons contain metal and substrate binding sites.

Table 3.1 Characteristics of *FAH* gene and its corresponding protein

Region	Number of nucleotides	Number of amino acids	Protein features[a]	Number of mutations
Exon 1	81	27	N-terminal domain (PF09298)	3
			N-acetylserine in position 2	
Intron 1	4924	–	–	1
Exon 2	111	37	N-terminal domain (PF09298)	4
Intron 2	1585	–	–	1
Exon 3	122	41	N-terminal domain (PF09298)	2
			Phosphoserine in position 92	
Intron 3	532	–	–	1
Exon 4	50	16	N-terminal domain (PF09298)	0
Intron 4	1786	–	–	1
Exon 5	91	31	FAH C-terminal domain (PF01557)	7
			Active site, Hxx… region	
			Substrate binding	
			Dimerization	
Intron 5	5715	–	–	0
Exon 6	98	32	FAH C-terminal domain (PF01557)	8
			Dimerization	
Intron 6	114	–	–	3
Exon 7	53	18	FAH C-terminal domain (PF01557)	2
			Metal binding	
			Dimerization	
Intron 7	3832	–	–	2
Exon 8	100	33	FAH C-terminal domain (PF01557)	9
			Metal binding	
Intron 8	765	–	–	2
Exon 9	131	44	FAH C-terminal domain (PF01557)	13
			Metal binding	
			Substrate binding	
Intron 9	1871	–	–	2
Exon 10	76	25	FAH C-terminal domain (PF01557)	3
Intron 10	2445	–	–	2
Exon 11	47	16	FAH C-terminal domain (PF01557)	1
			Phosphoserine in position 309	
Intron 11	2540	–	–	1
Exon 12	102	34	FAH C-terminal domain (PF01557)	13
			Substrate binding	
Intron 12	816	–	–	2

(continued)

Table 3.1 (continued)

Region	Number of nucleotides	Number of amino acids	Protein features[a]	Number of mutations
Exon 13	118	39	FAH C-terminal domain (PF01557)	9
			Active site, …xxE region	
Intron 13	4970	–	–	1
Exon 14	213	26	FAH C-terminal domain (PF01557)	5
			Phosphotyrosine in position 395	

[a]Based on Uniprot, PFAM and (Ran et al. 2013; Timm et al. 1999; Bateman et al. 2001, 2007)

3.2.1 Fah Alternative Transcripts

Two minor alternative splicing products of the *FAH* gene have also been found in normal fibroblasts, namely *del100* and *del231* (Dreumont et al. 2005).

The *del100* transcript lacks exon 8 and, as a consequence, the reading frame is shifted and a premature termination codon (PTC) appears in 3′ end of exon 10. While this transcript is subjected to nonsense-mediated mRNA decay (NMD), a small part of it is transcribed into a protein that shares the first 202 amino acids with FAH and as a stretch of 67 amino acids completely different in the C-terminal. The pattern of DEL100 expression differs from the one of FAH and its function remains unknown (Dreumont et al. 2005).

The *del231* transcript is less abundant than *del100* and lacks exons 8 and 9. The corresponding protein should be similar to FAH except for the 77 amino acids encoded by both exons. While this transcript is not subjected to NMD, the corresponding protein has never been observed (Dreumont et al. 2005). The identification of this transcript has led to the hypothesis that intron 8 would be removed before introns 7 and 9 during normal *FAH* splicing (Dreumont et al. 2005).

The biological relevance of these two minor transcripts has not been demonstrated further. Interestingly, the abundance of *del100* and *del231* transcripts changes in presence of mutations affecting splicing donors/acceptors sites (Q279R, c.707-1G>A, c.707-1G>C) or enhancer elements (N232N, V259L) and in presence of the nonsense mutation W262X (Table 3.2) (Dreumont et al. 2001, 2004; Perez-Carro et al. 2014; Morrow et al. submitted).

3.2.2 Splicing Mutations

Up to now, 25 *FAH* mutations associated with HT1 phenotype have been reported to affect splicing (Table 3.2). Among these, four are located at the exon side of the exon/intron junction (p.Q64H, p.Q279R. p.P281P and p.G305R) and three others are located within exons 8, 9 and 12 (p.N232N, p.V259L and p.G337S). While these later mutations do not alter core sequence elements of splicing, they are probably modifying exonic splicing enhancers (ESE) or silencers (ESS) sites. The importance of these sites in splicing efficiency is gaining increasing support (reviewed in Ward and Cooper 2010) and it was recently shown that 20–45% of pathogenic single nucleotide polymorphisms (SNPs) affect splicing (Wu and Hurst 2016). It is therefore likely that other HT1 causing mutations may affect splicing.

3.3 FAH Protein

FAH forms a homodimer that catalyzes the hydrolytic cleavage of a carbon-carbon bound in fumarylacetoacetate (FAA) to yield fumarate and acetoacetate. It is the first member of an expanding family of metalloenzymes characterized by a unique α/β fold and involving a Glu-His-water catalytic triad (Timm et al. 1999). Orthologs of

Table 3.2 Splicing mutations involved in HT1 disease

	cDNA/protein location	Information	HT1 alleles	References
1	c.82-1G>A/ – I1/E2 junction	mRNA absent, probably degraded by NMD	1	Perez-Carro et al. (2014)
2	c.192G>T/p.Q64H E2/I2 junction	Decreased level of mRNA, retention of 94 pb from intron 2 and PTC after 9 missense amino acids, absence of protein	41	Rootwelt et al. (1994a, 1996), Angileri et al. (2015), and Ijaz et al. (2016)
3	c.192 + 1G>T (IVS2 + 1G>T)/ – E2/I2 junction	No experimental data on mRNA or protein	1	Bergman et al. (1998)
4	c.315-3C>G (IVS3-3C>G)/ – I3/E4 junction	No experimental data on mRNA or protein	8	Dursun et al. (2011)
5	c.364 + 1G>A[a] (IVS4 + 1G>A)/ – E4/I4 junction	No experimental data on mRNA or protein	8	Imtiaz et al. (2011)
		Predicted to completely abolish splicing donor site		
6	c.553 + 5G>A (IVS6 + 5G>A)/ – E6/I6 junction	Abnormal mRNA, absence of protein	1	Timmers and Grompe (1996)
7	c.554-1G>C (IVS6-1G>C)/ – I6/E7 junction	No experimental data on mRNA	1	Bergman et al. (1998)
		Protein absent		
8	c.554-1G>T (IVS6-1G>T) I6/E7 junction	3 distinct mRNA all lacking 5 nt of exon 7; additionally the first 13 nt are lost in one transcript and all exon 8 in the other transcript	155	Angileri et al. (2015), Arranz et al. (2002), Bergman et al. (1998), Couce et al. (2011), Dursun et al. (2011), Kim et al. (2000), la Marca et al. (2011), Laszlo et al. (2013), Ploos van Amstel et al. (1996), Poudrier et al. (1999), Rootwelt et al. (1996), Timmers and Grompe (1996), and Vondrackova et al. (2010)
9	c.607-1G>A (IVS7-1G>A)/ – I7/E8 junction	mRNA bearing a deletion of a single G at the beginning of exon 8 due to a shift of the acceptor splice site	6	Ploos van Amstel et al. (1996), GQET
10	c.607-6T>G (IVS7-6T>G)/– I7/E8 junction	No experimental data on mRNA or protein	?	Sniderman King et al. (2006)
11	c.696C>T/p.N232N 11 bp before E8/I8	mRNA lacking exon 8 (del100 transcript)	1	Ploos van Amstel et al. (1996)
		Creation and alteration of ESE predicted[b]		
12	c.707-1G>A (IVS8-1G>A)/ – I8/E9 junction	mRNA lacking exons 8 and 9 (del231 transcript)	13	Arranz et al. (2002), Couce et al. (2011), and Imtiaz et al. (2011)
		Alteration of the splice acceptor site of intron 8		
13	c.707-1G>C (IVS8-1G>C)/ – I8/E9 junction	mRNA lacking exons 8 and 9 (del231 transcript)	16	Bergman et al. (1998) and Elpeleg et al. (2002)
		Alteration of the splice acceptor site of intron 8		
14	c.775G>C/p.V259L 63 bp before E9/I9	Slightly affect splicing of exons 8 and 9 (del231 transcript)	1	Angileri et al. (2015) and Morrow et al. (submitted)
		Recombinant protein: same solubility and activity as wt, decreases FAH activity when combined with G398E		

(continued)

Table 3.2 (continued)

	cDNA/protein location	Information	HT1 alleles	References
15	c.836A>G/p.Q279R E9/I9 junction	mRNA lacking exons 8 and 9 (del231 transcript)	2	Dreumont et al. (2001), Kim et al. (2000), and Perez-Carro et al. (2014)
		Recombinant protein: same solubility and activity as wt		
16	c.837 + 2T>C (IVS9 + 2T>C)/ – E9/I9 junction	No experimental data on mRNA or protein	4	Dursun et al. (2011)
17	c.838-2A>G (IVS9 -2A>G) I9/E10 junction	No experimental data on mRNA or protein	2	Angileri et al. (2015) and Heath et al. (2002)
18	c.843 C>A/p.P281P[c] I9/E10 junction	No experimental data on mRNA or protein	2	Imtiaz et al. (2011)
		Creation and alteration of ESE predicted[b]		
19	c.913G>C/p.G305R E10/I10 junction	mRNA lacking exon 10	1	Perez-Carro et al. (2014)
20	c.913 + 5G>A/ – E10/I10 junction	No experimental data on mRNA or protein	1	Choi et al. (2014)
21	c.914-2A>T (IVS10-2A>T)/ – I10/E11 junction	No experimental data on mRNA or protein	1	Arranz et al. (2002)
		Possible alteration of exon 11 splicing		
22	c.1009G>A/p.G337S Middle of E12	3 distinct mRNA	30	Bergman et al. (1998), Bliksrud et al. (2005, 2012), Haghighi-Kakhki et al. (2014), Prieto-Alamo and Laval (1998), Rootwelt et al. (1994b, 1996), and St-Louis et al. (1995)
		One lacking first 50 nucleotides of exon 12		
		One having all exon 12 plus 105 nucleotides from intron 12		
		One normal transcript		
		No protein detected in patients		
23	c.1062 + 5G>A (IVS12 + 5G>A)/ – E12/I12 junction	3 distinct mRNA	305	Angileri et al. (2015), Arranz et al. (2002), Bergman et al. (1998), Couce et al. (2011), Dursun et al. (2011), Grompe and al-Dhalimy (1993, 1994), Hahn et al. (1995), Heath et al. (2002), Ijaz et al. (2016), Imtiaz et al. (2011), Perez-Carro et al. (2014), Ploos van Amstel et al. (1996), Poudrier et al. (1996), Rootwelt et al. (1994d, 1996), Timmers and Grompe (1996), and Vondrackova et al. (2010), GQET
		One lacking exon 12		
		One lacking exons 12 and 13		
		One with a 105 base pair retention of intron 12		
		No protein detected in patients		
24	c.1063-1G>A (IVS12-1G>A)/ – I12/E13 junction	No experimental data on mRNA or protein	2	Mak et al. (2013)
		Suggested to abolish the acceptor site by the authors following in silico analysis		
25	c.1180 + 1G>A/ – E13/I13 junction	No experimental data on mRNA or protein	2	GQET

GQET Groupe québécois d'étude de la tyrosinémie/Quebec HT1 study group
[a]Reported as c.442-1G>A in (Angileri et al. 2015)
[b]Prediction made with Human Splicing Finder (Desmet et al. 2009)
[c]Reported as T281P in (Imtiaz et al. 2011)

FAH are found in different species and share a high degree of homology (Fig. 3.2). The protein can be separated in two distinct domains; the FAH N-terminal domain and the FAH C-terminal domain.

3.3.1 FAH N-Terminal Domain

The N-terminal domain of FAH consists of ~100 residues that are encoded by exons 1–4 (Timm et al. 1999) (Pfam: PF09298, InterPro: IPR015377) (Fig. 3.3a). Little is known about this structural domain except that it forms a structure consisting of an SH3-like barrel. This domain is not involved in dimerization or in active site formation, but it could have a regulatory function given its contacts with the C-terminal domain (Bateman et al. 2001, 2007; Timm et al. 1999).

The FAH N-terminal domain contains two identified post-transcriptionally modified amino acids (N-acetyl-S2 and phospho-S92), but the reasons/effects of these modifications have not been investigated (UniProt: P16930) (Huttlin et al. 2010; Vaca Jacome et al. 2015).

Among the nine exonic *FAH* mutations found in this domain (Fig. 3.1), five can be linked to aberrant mRNA processing and two results in the p.W78X nonsense mutation yielding a protein lacking the entire FAH catalytic C-terminal domain (Figs. 3.1 and 3.3b) (Table 3.3). Among the five mutations that can be linked to aberrant mRNA processing, the c.1A>G (p.M1?/exon 1) mutation results in the start codon loss, while p.Q64H (exon 2) has been shown to affect splicing by promoting the retention of 94 nucleo-

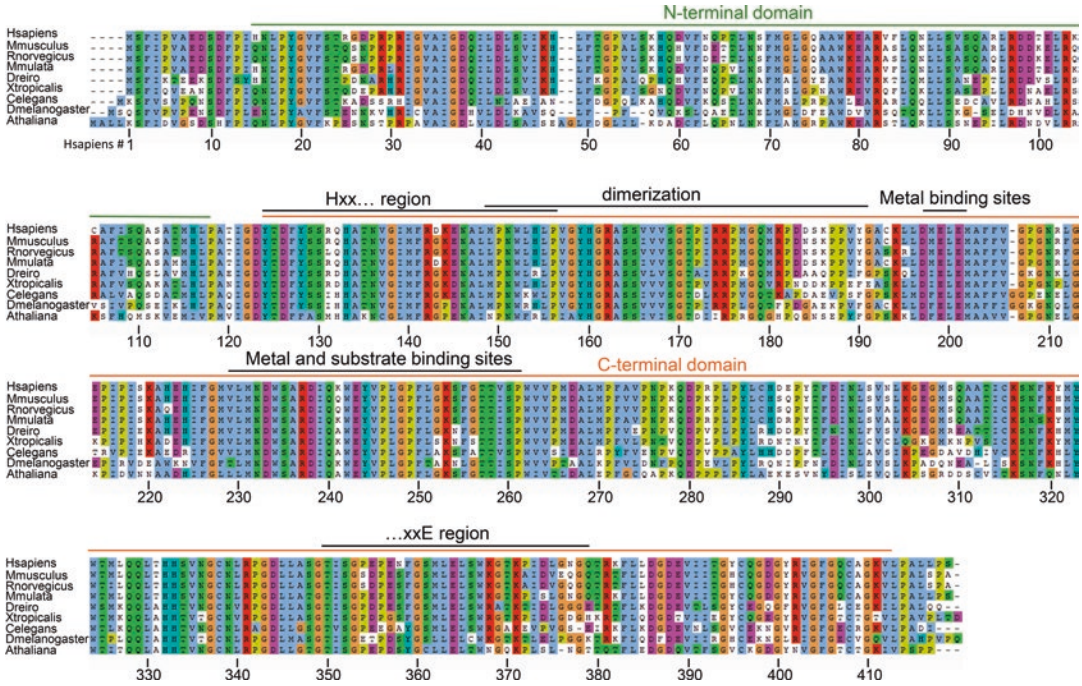

Fig. 3.2 Alignment of FAH orthologs. The protein sequence were retrieved from the NCBI website; *Homo sapiens* (NP_000128.1), *Mus musculus* (NP_034306.2), *Rattus Norvegicus* (NP_058877.1), *Drosophila melanogaster* (NP_524830.2), *Arabidopsis thaliana* (NP_172669.2), *Danio rerio* (NP_955895.1 with N-terminal of Fisher et al. 2008), *Caenorhabditis elegans* (NP_509083.1), *Xenopus tropicalis* (NP_001107523.1), *Macaca mulatta* (NP_001244458.1). The multiple sequence alignment was performed with MUSCLE tool (http://www.ebi.ac.uk/Tools/msa/muscle/) from the EMBL-EBI website. Numbers at the bottom of the figure correspond to *H. sapiens* amino acids sequence. N-terminal and C-terminal domains of FAH are presented, as well as region of interest for FAH activity

tides from intron 2 and resulting in the apparition of a PTC after nine missense amino acids. In addition to these two documented mutations, p.S23P (exon 1), p.F62C (exon 2) and p.Q64fs (exon 2) are likely to affect splicing due to the predicted alteration of an ESE site (S23P), activation of an exonic cryptic donor site (F62C) and direct alteration of the wild-type cryptic donor site (p.Q64fs) (Desmet et al. 2009). However, no experimental data on mRNA and protein are available for the later three mutations preventing a conclusion on their real effect on *FAH* mRNA, protein stability or activity. Of note, S23P was proposed to possibly affect FAH dimerization (Heath et al. 2002) and recombinant F62C was found to be an insoluble/inactive protein (Bergeron et al. 2001). The two remaining disease-causing mutations of the N-terminal domain give rise to normal *FAH* mRNA and are therefore likely to have a structural effect. Indeed, p.N16I (exon 1) produces an inactive and insoluble protein as shown from expression analysis of FAH in patient liver extract and from recombinant expression in cultured cells (Bergeron et al. 2001; Phaneuf et al. 1992) while p.A35T (exon 2) was shown to be expressed at low level and to have a decreased activity both in cultured fibroblasts and liver extracts (Cassiman et al. 2009).

3.3.2 FAH C-Terminal Domain

The FAH C-terminal domain is composed of ~300 residues that are encoded by exons 5–14 (Fig. 3.3a) (Pfam: PF01557, InterPro: IPR011234). It is shared between members of the FAH family of metalloenzymes and characterized by a ß-sandwich fold forming a deep pocket in the catalytic domain and containing a metal ion at its base (Ran et al. 2013; Timm et al. 1999). All enzymes of the FAH family share the ability to cleave C-C bond of their substrate through a Glu-His-water triad involving either a HxxE or Hxx…xxE motif. For extensive alignments between members of the FAH family please refer to (Ran et al. 2013).

The FAH C-terminal domain has functional roles in metal-ion binding, catalysis and dimerization. The dimer formation is needed to form the pocket of the active site, while multiple residues have been shown to be involved in FAH dimerization, the longest stretch of amino acids located at the dimer interface is spanning from the end of exon 5 through exon 6 and the first half of exon 7 (Timm et al. 1999). From the crystal structure of mouse FAH, it was shown that the metal ion is coordinated by residues present in exons 5, 7, 8 and 9 (Bateman et al. 2001, 2007; Timm et al. 1999). Moreover, based on the study of Ran and co-workers, the FAH active site corresponds to the Hxx…xxE motif (Ran et al. 2013) (Figs. 3.2 and 3.3a). The first part of this motif (Hxx…), which would correspond to the lid domain of the active site, is mainly located in exon 5 and the last part of the motif (…xxE) in exon 13. Also based on mouse FAH crystal structure, substrate binding sites are located in exon 5, 9 and 12 (Bateman et al. 2001, 2007; Timm et al. 1999). In addition to these features, phosphorylation of S309 (exon 11) and Y395 (exon 14) have been observed but the role of these post-translational modifications has not been investigated further (Bian et al. 2014). As can be seen on Fig. 3.3a, all these functional elements are spread all over the FAH C-terminal domain, which explains why even nonsense and deletions mutations located in *FAH* last exons are causing HT1. The listing of nonsense mutations causing HT1 is presented in Table 3.4 and the corresponding proteins are depicted in Fig. 3.3b, while the listing of deletion mutations causing frameshift is presented in Table 3.5 and proteins are depicted in Fig. 3.3c (see also Fig. 3.1 for localization of mutations in exons).

3.3.2.1 Missense HT1 Mutations Located in the FAH C-Terminal Domain

While the FAH C-terminal domain is ~3 times larger then the FAH N-terminal domain, it contains nearly eight times more disease causing mutations (69 versus 9) due to its importance for FAH function. As mentioned above, the two exons containing the most disease-causing mutations are exon 9 and 12 (Table 3.1 and Fig. 3.1). Based on the fact that it was recently

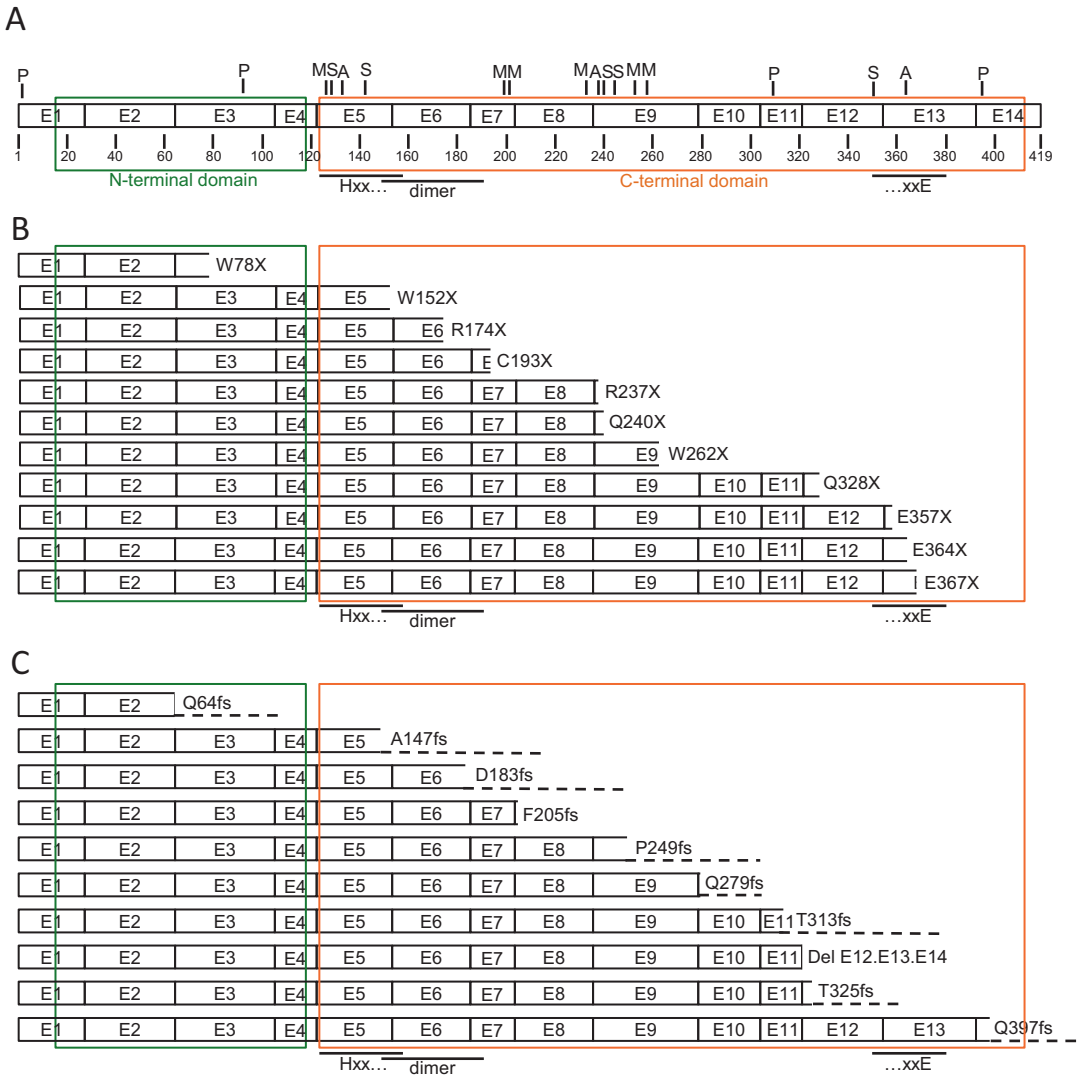

Fig. 3.3 FAH region of interest and effect of nonsense mutations as well as deletion mutations causing frameshift. (**a**) Scaled schematic representation of *FAH* exons. FAH N-terminal domain (Pfam: PF09298), FAH C-terminal doamin (PF01557), Hxx… and …xxE: conserved motif of the active site (Ran et al. 2013). *P* post-translational modifications (Bian et al. 2014; Huttlin et al. 2010; Vaca Jacome et al. 2015), *M* metal binding sites, *S* substrate binding sites, *A* active site (Bateman et al. 2001, 2007; Timm et al. 1999). Most of these features can also be found on the *Homo sapiens* FAH UniProt entry (P16930). (**b**) Schematic representation of the nonsense mutations causing HT1. The corresponding mutations can be found in Tables 3.3 and 3.4. (**c**) Schematic representation of the *FAH* deletion mutations causing frameshift. The corresponding mutations can be found in Tables 3.3 and 3.5

shown that 20–45% of pathogenic SNPs affect splicing (Wu and Hurst 2016), *FAH* missense mutations of the C-terminal domain were separated according to experimental data and to their potential effect on splicing as determined by the Human Splicing Finder website (Desmet et al. 2009). Nineteen mutations were found to potentially affect splicing (Table 3.6), while 29 mutations were not (Table 3.7). Not surprisingly all mutations affecting important residues for FAH activity are found in Table 3.7.

Table 3.3 HT1 causing mutations in the FAH N-terminal domain

	cDNA/protein/location	mRNA	Protein	HT1 alleles	References
1	c.1A>G/p.M1?[a]/ 1st nt of E1	No experimental data on mRNA	No experimental data on protein	20	Al-Shamsi et al. (2014), Georgouli et al. (2010), Imtiaz et al. (2011), and Mohamed et al. (2013), GQET
		Start loss, the next ATG is located in intron 1, 325 base pairs away from the end of exon 1			
2	c.47A>T/p.N16I/ within E1	Normal mRNA	Protein absent in liver extracts, inactive	1	Phaneuf et al. (1992) and Bergeron et al. (2001), GQET
			Recombinant protein: insoluble and inactive		
			N16 is conserved in 9/9 species[b]		
			N16I may cause general structural effects		
3	c.67T>C/p.S23P/ within E1	No experimental data on mRNA	No experimental data on protein	4	Heath et al. (2002) and Ijaz et al. (2016)
		Alteration of an ESE site predicted[c]	S23 is conserved in 8/9 species[b]		
			S23 is near the dimer interface		
4	c.103G>A/p.A35T/ within E2	Normal mRNA	Decreased level of protein expression and activity in fibroblasts and liver extracts	2	Cassiman et al. (2009)
			A35 is conserved in 9/9 species[b]		
5	c.185T>G/p.F62C/ end of E2	No experimental data on mRNA	No experimental data on protein	2	Awata et al. (1994) and Bergeron et al. (2001)
		Activation of an exonic cryptic donor site and alteration of an ESE site predicted[c]	Recombinant protein: insoluble and inactive		
			F62 is conserved in 8/9 species[b]		
6	c.191delA/p.Q64fs/ end of E2	No experimental data on mRNA	No experimental data on protein	2	Dursun et al. (2011)
		Alteration of the wild-type donor site, activation of an exonic cryptic donor site and alteration of an ESE site predicted[c]			

(continued)

Table 3.3 (continued)

	cDNA/protein/location	mRNA	Protein	HT1 alleles	References
7	c.192G>T[d]/p.Q64H/ E2/I2 junction	Decreased level of mRNA retention of 94 pb from intron 2 and PTC after 9 missense amino acids	Absence of protein	41	Rootwelt et al. (1994a, 1996), Angileri et al. (2015) and Ijaz et al. (2016)
8	c.233G>A/p.W78X within E3	Normal mRNA	No experimental data on protein	4	Arranz et al. (2002)
			Truncation in the middle of E3		
9	c.234G>A/p.W78X/ within E3	No experimental data on mRNA	No experimental data on protein	1	Couce et al. (2011)
			Truncation in the middle of E3		

GQET Groupe québécois d'étude de la tyrosinémie/Quebec HT1 study group
[a]*referred to p.M1V in* (Al-Shamsi et al. 2014; Angileri et al. 2015; Mohamed et al. 2013)
[b]based on Fig. 3.2
[c]*prediction made with Human Splicing Finder* (Desmet et al. 2009)
[d]also described in Table 3.2 (splicing mutations)

3.4 Most Frequent FAH Mutations and Their Geographical Localization

In total, more than 98 *FAH* mutations have been reported at this time to cause HT1. This number will likely increase, since SNPs causing the disease are found in SNPs database such as the ones from the Exome Aggregation Consortium (ExAC) (URL: http://exac.broadinstitute.org) and NHLBI Exome Sequencing project (http://evs.gs.washington.edu/EVS/). For example, a W234X SNP is reported on the ExAC website (last access: April 2016). This mutation is located at the end of exon 8 and based on experimental data from Table 3.4 is likely to cause HT1.

The worldwide incidence of HT1 is relatively low, with 1/100,000 affected individual (Hutchesson et al. 1996). The population that possesses the highest incidence of HT1 is the French Canadian population of the Saguenay-Lac-Saint-Jean (SLSJ) region in the province of Quebec (Canada). Not surprisingly, the most frequent *FAH* mutation in SLSJ region (~90% of all the disease causing alleles; c.1062 + 5G>A (IVS12 + 5G>A)) is also the most frequent worldwide (32.3% of the reported alleles) (Table 3.8) (Angileri et al. 2015). Since c.1062 + 5G>A accounts for the third of the HT1 reported allele and due to the fact that it is reported in a wide range of ethnic groups, it is likely to be a very old mutation (Angileri et al. 2015). The second most frequent HT1 mutation encountered worldwide is c.554-1G>T (IVS6-1G>T) with a frequency of 16.4% (Table 3.8). While this mutation is not associated to a specific cluster, it is more prevalent in the Mediterranean region and in southern Europe (Angileri et al. 2015).

Two other clusters of HT1 are found in the world. The first one is in the Finnish population of Pohjanmaa where the c.786G>A (p.W262X) represents ~88% of disease causing alleles (Angileri et al. 2015; Kvittingen 1991; Mustonen et al. 1997). The other cluster is in an immigrant population from Pakistan living in the United Kingdom (predominantly in Birmingham), and for which the c.192G>T (p.Q64H) mutation accounts for 42% of the alleles reported (Angileri et al. 2015; Hutchesson et al. 1998). These two mutations are also among the most frequent mutations worldwide, with frequencies of 5.6% and 4.3% (Table 3.8; third and fourth rank respectively).

Table 3.4 Nonsense mutations found in FAH C-terminal domain

	cDNA/protein	Location	Information regarding mRNA and protein	HT1 alleles	References
1	c.455G>A/p.W152X	End of E5	No experimental data on mRNA and protein	3	Dou et al. (2013) and Yang et al. (2012)
2	c.456G>A/p.W152X	Beginning of E6	No experimental data on mRNA and protein	1	GQET
3	c.520C>T/p.R174X	Middle of E6	No experimental data on mRNA and protein	4	Dursun et al. (2011), Heath et al. (2002), and Timmers and Grompe (1996)
4	c.579C>A/p.C193X	Middle of E7	No experimental data on mRNA and protein	2	Vondrackova et al. (2010)
5	c.709C>T/p.R237X	Beginning of E9	Reduced level of mRNA, the PTC is far from the last exon-exon junction and could therefore lead to NMD	39	Angileri et al. (2015), Cao et al. (2012), Dursun et al. (2011), Heath et al. (2002), Imtiaz et al. (2011), Jitraruch et al. (2011), la Marca et al. (2011), and Ploos van Amstel et al. (1996), GQET
6	c.718 C>T/p.Q240X	Beginning of E9	No experimental data on mRNA and protein	2	Imtiaz et al. (2011)
7	c.726G>A/p.W242X	Middle of E9	No experimental data on mRNA and protein	1	Angileri et al. (2015)
8	c.786G>A/p.W262X	Middle of E9	Drastic reduction of mRNA level No protein	53	Angileri et al. (2015), Mustonen et al. (1997), Rootwelt et al. (1994a, 1996), and St-Louis et al. (1994)
9	c.982C>T/p.Q328X	Beginning of E12	mRNA normal No experimental data on protein	2	Arranz et al. (2002)
10	c.1069G>T/p.E357X	Beginning of E13	From reduced to normal level of mRNA depending on the publication No protein	12	Angileri et al. (2015), Grompe and al-Dhalimy (1993), Heath et al. (2002), Ploos van Amstel et al. (1996), Rootwelt et al. (1994d, 1996), and St-Louis et al. (1995)
11	c.1090G>T/p.E364X	Middle of E13	From reduced to normal level of mRNA depending on the publication No protein	16	Bergman et al. (1998), Grompe and al-Dhalimy (1993), Grompe et al. (1994), Ploos van Amstel et al. (1996), Poudrier et al. (1999), Rootwelt et al. (1994d 1996), and Timmers and Grompe (1996)
12	c.1100G>A/pW367X	Middle of E13	No experimental data on mRNA and protein	2	Yang et al. (2012)

GQET Groupe québécois d'étude de la tyrosinémie/Quebec HT1 study group

Table 3.5 Deletion mutations causing frameshift in FAH C-terminal domain

	cDNA/protein/location	Information regarding mRNA and protein	HT1 alleles	References
1	c.441_448del8/p.A147fs/end of E5	No experimental data on mRNA and protein	1	Dursun et al. (2011)
2	c.548_553+20del26/p.D183fs/end of E6	No experimental data on mRNA and protein	1	Arranz et al. (2002)
		(E6/I6del26)		
3	c.615delT/p.F205fs/beginning of E8	Frameshift changing F205 in L and the next triplet to a PTC	1	Bliksrud et al. (2012)
		(p.F205lLfsX2)		
4	c.744delG/p.P249fs/middle of E9	Frameshift changing P249 in H and leading to apparition of a PTC at the end of E10 (p.P249HfsX55)	7	Bliksrud et al. (2012)
5	c.835delC/p.Q279fs/end of E9	Frameshift changing Q279 in R and leading to apparition of a PTC at the end of E10	2	Bliksrud et al. (2012)
		(pQ279RfsX25)		
6	c.938delC/p.T313fs/middle of E11	Frameshift keeping a T in position 313 and introducing a PTC 60 amino acids downstream	1	Arranz et al. (2002)
		(p.T313TfsX60)		
7	c.960q1130_*1260q10539del18036/−/within I11	Large deletion beginning in intron 11 and ending in the intergenic region of FAH-ARNT2	4	Park et al. (2009)
		Absence of E12, E13 and E14		
8	c.974_976delCGAinsGC/p.T325fs/beginning of E12	No experimental data on mRNA and protein	6	Yang et al. (2012)
		Frameshit will change T325 in S but the has not been investigated further		
9	c.1190delA/pQ397fs/beginning of E14	Frameshift abolishing the wild-type stop codon and resulting in an abnormally prolonged protein with 41 extra amino acids	4	Imtiaz et al. (2011)

The geographical distribution of almost all of the *FAH* mutations has been the subject of a recent review (Angileri et al. 2015).

3.5 Correlation Between FAH Mutations and HT1 Phenotype

HT1 is classified in three different forms depending on the clinical phenotype of patients and the age of onset. The acute form presents before 2 months of age with acute liver failure, while the subacute form presents between 2 and 6 months of age with liver disease and the chronic form presents after 6 months of age with slowly progressive liver cirrhosis and hypophosphatemic rickets (Bergman et al. 1998; Mitchell et al. 2001; van Spronsen et al. 1994) (See Morrow and Tanguay, Chap. 2). However, despite multiple efforts, no clear genotype-phenotype relationships have been unveiled (Arranz et al. 2002; Bergman et al. 1998; Dursun et al. 2011; Rootwelt et al. 1996).

3.5.1 HT1 Pseudodeficiency

To date, one missense mutation (c.1021C>T, p. R341W) has been described as a pseudodeficiency

Table 3.6 FAH C-terminal domain missense mutations that could affect splicing

	cDNA/protein/location	mRNA	Protein	HT1 alleles	References
1	c.509G>T/p.G170V/within E6	No experimental data on mRNA	No experimental data on protein	4	Imtiaz et al. (2011)
		Creation of an ESE predicted[a]	V166 is conserved in 5/9 species[b]		
			V166 is near the active site and at the dimer interface		
2	c.536A>G/p.Q179R/within E6	No experimental data on mRNA	No experimental data on protein	1	Choi et al. (2014)
		Creation and alteration of an ESE predicted[a]	Q179 is conserved in 9/9 species[b]		
			Q179 is at the dimer interface		
3	c.648C>G/p.I216M/within E8	No experimental data on mRNA	No experimental data on protein	4	Sheth et al. (2012)
		Creation of a potential donor splice site suggested by the authors	I216 is conserved in 7/9 species[b]		
4	c.680G>C/p.G227A/within E8	No experimental data on mRNA	No experimental data on protein	2	Imtiaz et al. (2011)
		Alteration of an ESE predicted[a]	G227 is conserved in 9/9 species[b]		
5	c.696C>A/p.N232K/within E8	Alteration of an ESE predicted[a]	No experimental data on mRNA	2	Dursun et al. (2011)
			No experimental data on protein		
			N232 is conserved in 9/9 species[b]		
			N232 is located in the active site		
6	c.775G>C[c]/p.V259L within exon 9	Slightly affect splicing of exons 8 and 9 (del231 transcript)	Recombinant protein: same solubility and activity as wt, decreases activity when combined with G398E	1	Angileri et al. (2015) and Morrow et al. (submitted)
			V259 is conserved in 2/9 species[b]		
			V259 is at the dimer interface		
7	c.836A>G[c]/p.Q279R/E9/I9 junction	mRNA lacking exons 8 and 9 (del231 transcript)	No protein	2	Dreumont et al. (2001), Kim et al. (2000), and Perez-Carro et al. (2014)
			recombinant protein: same solubility and activity as wt		
			Q279 is conserved in 9/9 species[b]		
8	c.913G>C[c]/p.G305R E10/I10 junction	mRNA lacking exon 10	No experimental data on protein	1	Perez-Carro et al. (2014)
			G305 is conserved in 6/9 species[b]		
9	c.974C>T/p.T325M within E12	No experimental data on mRNA	No experimental data on protein	5	Angileri et al. (2015), Couce et al. (2011), and Heath et al. (2002), GQET
		Creation and alteration of an ESE predicted[a]	T325 is conserved in 7/9 species[b]		

10	c.1001 C>T/p.S334F/within E12	No experimental data on mRNA	No experimental data on protein	2	Imtiaz et al. (2011)
		Alteration of an ESE predicted[a]	S334 is conserved in 4/9 species[b]		
11	c.1009G>A[c,d]/p.G337S/middle of E12	3 distinct mRNA	No protein detected in patients	30	Bergman et al. (1998), Bliksrud et al. (2005, 2012), Haghighi-Kakhki et al. (2014), Prieto-Alamo and Laval (1998), Rootwelt et al. (1994b, d, 1996), and St-Louis et al. (1995)
		One lacking first 50 nucleotides of exon 12	G337 is conserved in 9/9 species[b]		
		One having all exon 12 plus 105 nucleotides from intron 12			
		One normal transcript			
12	c.1035_1037del[4] p.L345del/within E12	No experimental data on mRNA	No experimental data on protein	2	Mak et al. (2013)
		Alteration of an ESE site predicted[a]	L345 is conserved in 9/9 species[b]		
			L345 is near the xxE motif region		
13	c. 1061C>A/p.P354Q/end of E12	No experimental data on mRNA	No experimental data on protein	1	Bliksrud et al. (2005) and Bergman et al. (1998)
		Creation and alteration of an ESE predicted[a]	P354 is conserved in 6/9 species[b]		
			P354 is in the xxE motif region		
14	c.1097_1099delCGT[d]/p.S366del/within E13	No experimental data on mRNA	No experimental data on protein	2	
		Creation and alteration of an ESE sites predicted[a]	S366 is conserved in 7/9 species[b]		
			S366 is in the xxE motif region		
15	c.1141A>G/p.R381G/within E13	No experimental data on mRNA	No protein	6	St-Louis et al. (1995)
		Activation of an exonic cryptic donor site and alteration of an ESE site predicted[a]	R381 is conserved in 8/9 species[b]		
			R381 is near the xxE motif region		
16	c.1156G>C/p.D386H/within E13	No experimental data on mRNA	No experimental data on protein	2	Al-Shamsi et al. (2014)
		Activation of an exonic cryptic donor site and alteration of an ESE site predicted[a]	D386 is conserved in 9/9 species[b]		
17	c.1159G>A/p.G387R/within E13	No experimental data on mRNA	No experimental data on protein	4	Sheth et al. (2012)
		Activation of an exonic cryptic donor site, creation and alteration of an ESE site predicted[a]	G387 is conserved in 8/9 species[b]		

(continued)

Table 3.6 (continued)

	cDNA/protein/location	mRNA	Protein	HT1 alleles	References
18	c.1195G>C/p.D399H[e]/within E14	No experimental data on mRNA	No experimental data on protein	2	Imtiaz et al. (2011)
		Alteration of an ESE site predicted[a]	D399 is conserved in 5/9 species[b]		
			D399 is between the N- and C-terminal domain (quaternary structure)		
19	c.1213_1214delTTinsCA/p.F405H/ within E14	No experimental data on mRNA	No experimental data on protein	1	Bergman et al. (1998)
		creation of an ESS site predicted[a]	F405 is conserved in 9/9 species[b]		
			F405 is between the N- and C-terminal domain (quaternary structure)		

GQET Groupe québécois d'étude de la tyrosinémie/Quebec HT1 study group
[a]Prediction made with Human Splicing Finder (Desmet et al. 2009)
[b]Based on Fig. 3.2.
[c]Proven splicing mutations, also presented in Table 3.2
[d]Also reported as c.961_1010del50 in (Angileri et al. 2015; Prieto-Alamo and Laval 1998; Rootwelt et al. 1994d)
[e]Also reported as p.N400H in (Angileri et al. 2015; Imtiaz et al. 2011)
[f]In frame deletion of one codon

Table 3.7 Missense mutations located in the FAH C-terminal domain and affecting protein function

	cDNA/protein/location	Information regarding mRNA and protein	HT1 alleles	References
1	c.374C>G/p.T125R/ exon 5	No experimental data on mRNA and protein	2	Imtiaz et al. (2011)
		T125 is conserved in 9/9 species[a]		
		T125 is in the active site (Hxx… motif region)		
2	c.398A>G/p.H133R/ exon 5	No experimental data on mRNA and protein	2	Heath et al. (2002)
		H133 is conserved in 9/9 species[a]		
		H133 is in the catalytic triad		
3	c.398A>T/p.H133L/ exon 5	No experimental data on mRNA and protein	2	Couce et al. (2011)
		H133 is conserved in 9/9 species[a]		
		H133 is in the catalytic triad		
4	c.401C>A/p.A134D/ exon 5	normal mRNA, low level of protein, no activity	3	Bergeron et al. (2001), Labelle et al. (1993), and Rootwelt et al. (1994c, 1996)
		Recombinant protein: same solubility as wild-type, inactive		
		A134 is conserved in 9/9 species[a]		
		A134D is in the active site, in the Hxx… motif		
5	c.424A>G/p.R142G/ Exon 5	No experimental data on mRNA and protein	1	GQET
		R142 is conserved in 9/9 species[a]		
		R142 is in the active site, is involved in substrate binding		
6	c.467C>A/p.P156Q/ exon 6	No experimental data on mRNA and protein	1	Heath et al. (2002)
		P156 is conserved in 9/9 species[a]		
		P156 is in the largest contiguous sequence of contacts between monomers		
7	c.473G>A/p.G158D/ exon 6	No experimental data on mRNA and protein	1	Bergman et al. (1998)
		G158 is conserved in 8/9 species[a]		
		G158 is in the largest contiguous sequence of contacts between monomers and beside a substrate interaction site		
8	c.497T>G/p.V166G/ exon 6	Normal mRNA, no experimental data on protein	11	Bergman et al. (1998), Dursun et al. (2011), Grompe and al-Dhalimy (1993), and Rootwelt et al. (1996)
		V166 is conserved in 5/9 species[a]		
		V166 is in the largest contiguous sequence of contacts between monomers		
9	c.577 T > C/p.C193R/ Exon 7	normal mRNA, no experimental data on protein	1	Bergeron et al. (2001) and Ploos van Amstel et al. (1996)
		Recombinant protein: insoluble and inactive		
		C193 is conserved in 4/9 species[a]		
		C193 is near the active site		

(continued)

Table 3.7 (continued)

	cDNA/protein/location	Information regarding mRNA and protein	HT1 alleles	References
10	c.620G>A/p.G207D/ exon 8	No experimental data on mRNA and protein	1	Timmers and Grompe (1996)
		G207 is conserved in 9/9 species[a]		
		G207 is near the active site		
11	c.680G>T/p.G227V/ exon 8	No experimental data on mRNA and protein	4	Vondrackova et al. (2010)
		G227 is conserved in 9/9 species[a]		
		G227 is beside F226 which is involved in metal binding		
12	c.698A>T/p.D233V/ exon 8	Normal mRNA, low level of protein, no activity	15	Dursun et al. (2011), Rootwelt et al. (1994a, 1996)
		Recombinant protein: same solubility as wild-type, inactive		
		D233 is conserved in 9/9 species[a]		
		D233 is involved in metal binding		
13	c.700T>G/p.W234G/ exon 8	Normal mRNA, inactive protein	1	Hahn et al. (1995), Rootwelt et al. (1996), and Bergeron et al. (2001)
		Recombinant protein: insoluble and inactive		
		W234 is conserved in 9/9 species[a]		
		W234 is within a metal cation pocket		
14	c.742G>A/p.G248R/ exon 9	No experimental data on mRNA and protein	2	GQET
		G248 is conserved in 9/9 species[a]		
		G248 is beside P249 which is involved in substrate binding		
15	c.745C>A/p.P249T/ exon 9	No experimental data on mRNA and protein	1	Timmers and Grompe (1996)
		P249 is conserved in 9/9 species[a]		
		P249 is involved in substrate binding and is located at the dimer interface		
16	c.776T>A/p.V259D/ exon 9	No experimental data on mRNA and protein	2	Dursun et al. (2011)
		V259 is conserved in 2/9 species[a]		
		V259 is in the active site, at the dimer interface		
17	c.782C>T/p.P261L/ exon 9	No experimental data on mRNA and protein	16	Bergman et al. (1998), Imtiaz et al. (2011), Elpeleg et al. (2002), and Angileri et al. (2015), GQET
		P261 is conserved in 9/9 species[a]		
		P261 occur between the N- and C-terminal domains (quaternary structure)		
18	c.787G>A/p.V263M/ exon 9	No experimental data on mRNA and protein	4	Imtiaz et al. (2011)
		V263 is conserved in 8/9 species[a]		
		V263 is near metal binding site		
19	c.880A>C/p.T294P/ exon 10	No experimental data on mRNA and protein	2	Bergman et al. (1998) and Timmers and Grompe (1996)
		T294 is conserved in 7/9 species[a]		

(continued)

Table 3.7 (continued)

	cDNA/protein/location	Information regarding mRNA and protein	HT1 alleles	References
20	c.1022G>C/p.R341P/ exon 12	No experimental data on mRNA and protein	2	Imtiaz et al. (2011)
		R341 is conserved in 9/9 species[a]		
		R341 is near the active site		
21	c.1025C>T/p.P342L/ exon 12	Normal mRNA, absence of protein	5	Bergman et al. (1998, 1994c) and Rootwelt et al. (1996)
		P342 is conserved in 8/9 species[a]		
		P342 is near the active site, between the N- and C-terminal domains (quaternary structure)		
22	c.1027G>T/p.G343W/ exon 12	Normal mRNA, no experimental data on protein	2	Arranz et al. (2002)
		G343 is conserved in 9/9 species[a]		
		G343 is near the active site		
23	c.1027G>C/p.G343R/ exon 12	No experimental data on mRNA and protein	9	Dou et al. (2013) and Imtiaz et al. (2011)
		G343 is conserved in 9/9 species[a]		
24	c.1043C>G/p.S348C[b]/ exon 12	No experimental data on mRNA and protein	?	Prieto-Alamo and Laval (1998)
		S348 is conserved in 7/9 species[a]		
		S348 is near the active site		
25	c.1056C>A/p.S352R/ exon 12	No experimental data on mRNA and protein	2	Heath et al. (2002)
		S352 is conserved in 9/9 species[a]		
		S352 is in the …xxE motif region		
26	c.1106G>T/p.G369V/ exon 12	Normal level of mRNA, no experimental data on protein	1	Ploos van Amstel et al. (1996)
		G369 is conserved in 8/9 species[a]		
		G369 is in the …xxE motif region		
27	c.1124T>C/p.L375P/ exon 12	Normal level of mRNA	2	Cao et al. (2012)
		L375 is conserved is 6/9 species[a]		
		L375 is in the …xxE motif region		
28	c.1193G>A/p.G398E/ exon 14	Normal mRNA, no protein	1	Morrow et al. (submitted)
		Recombinant protein: soluble, decreased activity		
		G398 is conserved in 7/9 species[a]		
29	c.1210G>A/p.G404S/ exon 14	No experimental data on mRNA and protein	2	Vondrackova et al. (2010)
		G404 is conserved in 9/9 species[a]		

GQET Groupe québécois d'étude de la tyrosinémie/Quebec HT1 study group
[a]Based on Fig. 3.2
[b]Reported as p.S348G in (Angileri et al. 2015; Prieto-Alamo and Laval 1998)

Table 3.8 HT1 most frequent mutations

cDNA/protein	Location	Type of mutation	HT1 alleles	Frequency[1] (%)
c.1062 + 5G>A (IVS12 + 5G>A)/−	E12/I12 junction	Splicing (Table 3.2)	305	32.3
c.554-1G>T (IVS6-1G>T)/−	I6/E7 junction	Splicing (Table 3.2)	155	16.4
c.786G>A/p.W262X	Exon 9	Nonsense (Table 3.4)	53	5.6
c.192G>T/p.Q64H	E2/I2 junction	Splicing (Table 3.2)	41	4.3
c.709C>T/p.R237X	Exon 9	Nonsense (Table 3.4)	39	4.1
c.1009G>A/p.G337S	Exon 12	Splicing (Table 3.2)	30	3.2
c.1A>G/p.M1?/	1st nt of E1	Start loss (Table 3.3)	20	2.1
c.707-1G>C (IVS8-1G>C)/−	I8/E9 junction	Splicing (Table 3.2)	16	1.7
c.782C>T/p.P261L/	Exon 9	Missense (Table 3.7)	16	1.7
c.1090G>T/p.E364X	Exon 13	Nonsense (Table 3.4)	16	1.7
c.698A>T/p.D233V	Exon 8	Missense (Table 3.7)	15	1.6
c.707-1G>A (IVS8-1G>A)/−	I8/E9 junction	Splicing (Table 3.2)	13	1.4
c.1069G>T/p.E357X	Exon 13	Nonsense (Table 3.4)	12	1.3
c.497T>G/p.V166G	Exon 6	Missense (Table 3.7)	11	1.2

[1]Frequency is calculated on the 944 alleles reported in this chapter

variant since individuals homozygous for this mutation are healthy due to residual FAH activity while compound heterozygotes with another *FAH* mutation develop HT1 (Rootwelt et al. 1994b). This mutation does not change the mRNA level nor its size but it results in decreased amount of FAH protein with less activity than the wild-type protein (Bergeron et al. 2001; Rootwelt et al. 1994b), suggesting that a minimal requirement of FAH activity is needed to prevent HT1 disease.

3.5.2 Reversion of FAH Mutation

A mosaic pattern of FAH expression in liver of HT1 patients has been reported for four splicing mutations; c.192G>T (p.Q64H), c.836A>G (p.Q279R), c.1009G>A (p.G337S) and c.1062 + 5G>A (IVS12 + 5G>A) (Demers et al. 2003; Dreumont et al. 2001; Kvittingen et al. 1993, 1994; Poudrier et al. 1998). The presence of FAH positive nodules was shown to be due to the reversion of the primary point mutation (Demers et al. 2003; Kvittingen et al. 1993, 1994) and favored by the selective advantage that the reversion would provide (Demers et al. 2003). Interestingly, it was also shown that the severity of the disease is directly correlated with the extent of HT1 mutation reversion in the liver of HT1 patients (Demers et al. 2003).

3.6 Concluding Remarks

This report summarizes the available information for each of the *FAH* mutations reported in the literature and places them back in their molecular context. While it does not provide explanations

for the effect of all mutations on *FAH* mRNA and protein, it does suggest new ways to look at them in addition to highlight the importance of splicing mutations in HT1.

Acknowledgements The work on HT1 in RMT's laboratory is supported by grants from the Canadian Institutes of Health Research (CIHR) and Fondation du Grand Défi Pierre Lavoie.

References

Al-Shamsi A, Hertecant JL, Al-Hamad S, Souid AK, Al-Jasmi F (2014) Mutation spectrum and birth prevalence of inborn errors of metabolism among emiratis: a study from Tawam Hospital Metabolic Center, United Arab Emirates. Sultan Qaboos Univ Med J 14(1):e42–e49

Angileri F, Bergeron A, Morrow G, Lettre F, Gray G, Hutchin T, Ball S (2015) Geographical and ethnic distribution of mutations of the fumarylacetoacetate hydrolase gene in hereditary tyrosinemia type 1. JIMD Rep 19:45–58. doi:10.1007/8904_2014_363

Arranz JA, Pinol F, Kozak L, Perez-Cerda C, Cormand B, Ugarte M, Riudor E (2002) Splicing mutations, mainly IVS6-1(G>T), account for 70% of fumarylacetoacetate hydrolase (FAH) gene alterations, including 7 novel mutations, in a survey of 29 tyrosinemia type I patients. Hum Mutat 20(3):180–188. doi:10.1002/humu.10084

Awata H, Endo F, Tanoue A, Kitano A, Nakano Y, Matsuda I (1994) Structural organization and analysis of the human fumarylacetoacetate hydrolase gene in tyrosinemia type I. Biochim Biophys Acta 1226(2):168–172. doi:0925-4439(94)90025-6

Bateman RL, Bhanumoorthy P, Witte JF, McClard RW, Grompe M, Timm DE (2001) Mechanistic inferences from the crystal structure of fumarylacetoacetate hydrolase with a bound phosphorus-based inhibitor. J Biol Chem 276(18):15284–15291. doi:10.1074/jbc.M007621200

Bateman RL, Ashworth J, Witte JF, Baker LJ, Bhanumoorthy P, Timm DE, Hurley TD, Grompe M, McClard RW (2007) Slow-onset inhibition of fumarylacetoacetate hydrolase by phosphinate mimics of the tetrahedral intermediate: kinetics, crystal structure and pharmacokinetics. Biochem J 402(2):251–260. doi:10.1042/BJ20060961

Bergeron A, D'Astous M, Timm DE, Tanguay RM (2001) Structural and functional analysis of missense mutations in fumarylacetoacetate hydrolase, the gene deficient in hereditary tyrosinemia type 1. J Biol Chem 276(18):15225–15231. doi:10.1074/jbc.M009341200

Bergman AJ, van den Berg IE, Brink W, Poll-The BT, Ploos van Amstel JK, Berger R (1998) Spectrum of mutations in the fumarylacetoacetate hydrolase gene of tyrosinemia type 1 patients in northwestern Europe and Mediterranean countries. Hum Mutat 12(1):19–26. doi:10.1002/(SICI)1098-1004(1998)12:1<19::AID-HUMU3>3.0.CO;2-3

Bian Y, Song C, Cheng K, Dong M, Wang F, Huang J, Sun D, Wang L, Ye M, Zou H (2014) An enzyme assisted RP-RPLC approach for in-depth analysis of human liver phosphoproteome. J Proteome 96:253–262. doi:10.1016/j.jprot.2013.11.014

Bliksrud YT, Brodtkorb E, Andresen PA, van den Berg IE, Kvittingen EA (2005) Tyrosinaemia type I – de novo mutation in liver tissue suppressing an inborn splicing defect. J Mol Med (Berl) 83(5):406–410. doi:10.1007/s00109-005-0648-2

Bliksrud YT, Brodtkorb E, Backe PH, Woldseth B, Rootwelt H (2012) Hereditary tyrosinaemia type I in Norway: incidence and three novel small deletions in the fumarylacetoacetase gene. Scand J Clin Lab Invest 72(5):369–373. doi:10.3109/00365513.2012.676210

Cao YY, Zhang YL, Du J, Qu YJ, Zhong XM, Bai JL, Song F (2012) Compound mutations (R237X and L375P) in the fumarylacetoacetate hydrolase gene causing tyrosinemia type I in a Chinese patient. Chin Med J 125(12):2132–2136

Cassiman D, Zeevaert R, Holme E, Kvittingen EA, Jaeken J (2009) A novel mutation causing mild, atypical fumarylacetoacetase deficiency (Tyrosinemia type I): a case report. Orphanet J Rare Dis 4:28. doi:1750-1172-4-28

Choi HJ, Bang HI, Ki CS, Lee SY, Kim JW, Song J, Shin MR, Lee YW, Lee DH, Park HD (2014) Two novel FAH gene mutations in a patient with hereditary tyrosinemia type I. Ann Clin Lab Sci 44(3):317–323. doi:44/3/317

Couce ML, Dalmau J, del Toro M, Pintos-Morell G, Aldamiz-Echevarria L (2011) Tyrosinemia type 1 in Spain: mutational analysis, treatment and long-term outcome. Pediatr Int 53(6):985–989. doi:10.1111/j.1442-200X.2011.03427.x

Demers SI, Russo P, Lettre F, Tanguay RM (2003) Frequent mutation reversion inversely correlates with clinical severity in a genetic liver disease, hereditary tyrosinemia. Hum Pathol 34(12):1313–1320. doi:S0046817703004064

Desmet FO, Hamroun D, Lalande M, Collod-Beroud G, Claustres M, Beroud C (2009) Human splicing finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res 37(9):e67. doi:10.1093/nar/gkp215

Dou LM, Fang LJ, Wang XH, Lu W, Chen R, Li LT, Zhao J, Wang JS (2013) Mutation analysis of FAH gene in patients with tyrosinemia type 1. Zhonghua Er Ke Za Zhi 51(4):302–307

Dreumont N, Poudrier JA, Bergeron A, Levy HL, Baklouti F, Tanguay RM (2001) A missense mutation (Q279R) in the fumarylacetoacetate hydrolase gene, responsible for hereditary tyrosinemia, acts as a splicing mutation. BMC Genet 2:9

Dreumont N, Maresca A, Khandjian EW, Baklouti F, Tanguay RM (2004) Cytoplasmic nonsense-mediated mRNA decay for a nonsense (W262X) transcript of the gene responsible for hereditary tyrosinemia, fumarylacetoacetate hydrolase. Biochem Biophys Res Commun 324(1):186–192. doi:10.1016/j.bbrc.2004.09.041

Dreumont N, Maresca A, Boisclair-Lachance JF, Bergeron A, Tanguay RM (2005) A minor alternative transcript of the fumarylacetoacetate hydrolase gene produces a protein despite being likely subjected to nonsense-mediated mRNA decay. BMC Mol Biol 6:1. doi:1471-2199-6-1

Dursun A, Ozgul RK, Sivri S, Tokatli A, Guzel A, Mesci L, Kilic M, Aliefendioglu D, Ozcay F, Gunduz M, Coskun T (2011) Mutation spectrum of fumarylacetoacetase gene and clinical aspects of tyrosinemia type I disease. JIMD Rep 1:17–21. doi:10.1007/8904_2011_10

Elpeleg ON, Shaag A, Holme E, Zughayar G, Ronen S, Fisher D, Hurvitz H (2002) Mutation analysis of the FAH gene in Israeli patients with tyrosinemia type I. Hum Mutat 19(1):80–81. doi:10.1002/humu.9001

Fisher AL, Page KE, Lithgow GJ, Nash L (2008) The *Caenorhabditis elegans* K10C2.4 gene encodes a member of the fumarylacetoacetate hydrolase family: a *Caenorhabditis elegans* model of type I tyrosinemia. J Biol Chem 283(14):9127–9135. doi:10.1074/jbc.M708341200

Georgouli H, Schulpis KH, Michelakaki H, Kaltsa M, Sdogou T, Kossiva L (2010) Persistent coagulopathy during *Escherichia coli* sepsis in a previously healthy infant revealed undiagnosed tyrosinaemia type 1. BMJ Case Rep 2010. doi:2010/dec21_1/bcr0720103150

Grompe M, al-Dhalimy M (1993) Mutations of the fumarylacetoacetate hydrolase gene in four patients with tyrosinemia, type I. Hum Mutat 2(2):85–93. doi:10.1002/humu.1380020205

Grompe M, St-Louis M, Demers SI, al-Dhalimy M, Leclerc B, Tanguay RM (1994) A single mutation of the fumarylacetoacetate hydrolase gene in French Canadians with hereditary tyrosinemia type I. N Engl J Med 331(6):353–357. doi:10.1056/NEJM199408113310603

Haghighi-Kakhki H, Rezazadeh J, Ahmadi-Shadmehri A (2014) Identification of a combined missense/splice-site mutation in FAH causing tyrosinemia type 1. J Pediatr Endocrinol Metab. doi:10.1515/jpem-2013-0489

Hahn SH, Krasnewich D, Brantly M, Kvittingen EA, Gahl WA (1995) Heterozygosity for an exon 12 splicing mutation and a W234G missense mutation in an American child with chronic tyrosinemia type 1. Hum Mutat 6(1):66–73. doi:10.1002/humu.1380060113

Heath SK, Gray RG, McKiernan P, Au KM, Walker E, Green A (2002) Mutation screening for tyrosinaemia type I. J Inherit Metab Dis 25(6):523–524

Hutchesson AC, Hall SK, Preece MA, Green A (1996) Screening for tyrosinaemia type I. Arch Dis Child Fetal Neonatal Ed 74(3):F191–F194

Hutchesson AC, Bundey S, Preece MA, Hall SK, Green A (1998) A comparison of disease and gene frequencies of inborn errors of metabolism among different ethnic groups in the West Midlands, UK. J Med Genet 35(5):366–370

Huttlin EL, Jedrychowski MP, Elias JE, Goswami T, Rad R, Beausoleil SA, Villen J, Haas W, Sowa ME, Gygi SP (2010) A tissue-specific atlas of mouse protein phosphorylation and expression. Cell 143(7):1174–1189. doi:10.1016/j.cell.2010.12.001

Ijaz S, Zahoor MY, Imran M, Afzal S, Bhinder MA, Ullah I, Cheema HA, Ramzan K, Shehzad W (2016) Direct sequencing of FAH gene in Pakistani tyrosinemia type 1 families reveals a novel mutation. J Pediatr Endocrinol Metab 29(3):327–332. doi:10.1515/jpem-2015-0289

Imtiaz F, Rashed MS, Al-Mubarak B, Allam R, El-Karaksy H, Al-Hassnan Z, Al-Owain M, Al-Zaidan H, Rahbeeni Z, Qari A, Meyer BF, Al-Sayed M (2011) Identification of mutations causing hereditary tyrosinemia type I in patients of Middle Eastern origin. Mol Genet Metab 104(4):688–690. doi:S1096-7192(11)00206-X

Jitraruch S, Treepongkaruna S, Teeraratkul S, Wattanasirichaigoon D, Leelaudomlipi S, Sornmayura P, Viengteerawat S, Sriphojanart S (2011) Long-term outcome of living donor liver transplantation in a Thai boy with hereditary tyrosinemia type I: a case report. J Med Assoc Thail 94(10):1276–1280

Kim SZ, Kupke KG, Ierardi-Curto L, Holme E, Greter J, Tanguay RM, Poudrier J, D'Astous M, Lettre F, Hahn SH, Levy HL (2000) Hepatocellular carcinoma despite long-term survival in chronic tyrosinaemia I. J Inherit Metab Dis 23(8):791–804

Kvittingen EA (1991) Tyrosinaemia type I – an update. J Inherit Metab Dis 14(4):554–562

Kvittingen EA, Rootwelt H, Brandtzaeg P, Bergan A, Berger R (1993) Hereditary tyrosinemia type I. Self-induced correction of the fumarylacetoacetase defect. J Clin Invest 91(4):1816–1821. doi:10.1172/JCI116393

Kvittingen EA, Rootwelt H, Berger R, Brandtzaeg P (1994) Self-induced correction of the genetic defect in tyrosinemia type I. J Clin Invest 94(4):1657–1661. doi:10.1172/JCI117509

Labelle Y, Phaneuf D, Leclerc B, Tanguay RM (1993) Characterization of the human fumarylacetoacetate hydrolase gene and identification of a missense mutation abolishing enzymatic activity. Hum Mol Genet 2(7):941–946

Laszlo A, Rozsa M, Sallay E, Tiszlavicz L, Janovszky A, Varkonyi A, Karg E, Wittmann G, Turi S, Ugarte M (2013) The fate of tyrosinaemic Hungarian patients before the NTBC aera. Ideggyogy Sz 66(11–12):415–419

Mahuran DJ, Angus RH, Braun CV, Sim SS, Schmidt DE Jr (1977) Characterization and substrate specificity of fumarylacetoacetate fumarylhydrolase. Can J Biochem 55(1):1–8

Mak CM, Lam CW, Chim S, Siu TS, Ng KF, Tam S (2013) Biochemical and molecular diagnosis of tyrosinemia type I with two novel FAH mutations in a Hong Kong chinese patient: recommendation for expanded newborn screening in Hong Kong. Clin Biochem 46(1–2):155–159. doi:S0009-9120(12)00540-1

la Marca G, Malvagia S, Pasquini E, Cavicchi C, Morrone A, Ciani F, Funghini S, Villanelli F, Zammarchi E, Guerrini R (2011) Newborn screening for tyrosinemia type I: further evidence that succinylacetone determination on blood spot is essential. JIMD Rep 1:107–109. doi:10.1007/8904_2011_24

Mitchell GA, Grompe M, Lambert H, Tanguay RM (2001) Hypertyrosinemia. In: Scriver C, Beaudet A, Sly WSJ, Valle D (eds) The metabolic and molecular bases of inherited diseases, vol Volume II, 8th edn. McGrawHill, New York, pp 1777–1805

Mohamed S, Kambal MA, Al Jurayyan NA, Al-Nemri A, Babiker A, Hasanato R, Al-Jarallah AS (2013) Tyrosinemia type 1: a rare and forgotten cause of reversible hypertrophic cardiomyopathy in infancy. BMC Res Notes 6(1):362. doi:1756-0500-6-362

Mustonen A, Ploos van Amstel HK, Berger R, Salo MK, Viinikka L, Simola KO (1997) Mutation analysis for prenatal diagnosis of hereditary tyrosinaemia type 1. Prenat Diagn 17(10):964–966. doi:10.1002/(SICI)1097-0223(199710)17:10<964::AID-PD164>3.0.CO;2-6

Park HD, Lee DH, Choi TY, Lee YK, Kim JW, Ki CS, Lee YW (2009) Clinical, biochemical, and genetic analysis of a Korean neonate with hereditary tyrosinemia type 1. Clin Chem Lab Med 47(8):930–933. doi:10.1515/CCLM.2009.223

Perez-Carro R, Sanchez-Alcudia R, Perez B, Navarrete R, Perez-Cerda C, Ugarte M, Desviat L (2014) Functional analysis and in vitro correction of splicing FAH mutations causing tyrosinemia type I. Clin Genet 86(2):167–171. doi:10.1111/cge.12243

Phaneuf D, Labelle Y, Berube D, Arden K, Cavenee W, Gagne R, Tanguay RM (1991) Cloning and expression of the cDNA encoding human fumarylacetoacetate hydrolase, the enzyme deficient in hereditary tyrosinemia: assignment of the gene to chromosome 15. Am J Hum Genet 48(3):525–535

Phaneuf D, Lambert M, Laframboise R, Mitchell G, Lettre F, Tanguay RM (1992) Type 1 hereditary tyrosinemia. Evidence for molecular heterogeneity and identification of a causal mutation in a French Canadian patient. J Clin Invest 90(4):1185–1192. doi:10.1172/JCI115979

Ploos van Amstel JK, Bergman AJ, van Beurden EA, Roijers JF, Peelen T, van den Berg IE, Poll-The BT, Kvittingen EA, Berger R (1996) Hereditary tyrosinemia type 1: novel missense, nonsense and splice consensus mutations in the human fumarylacetoacetate hydrolase gene; variability of the genotype-phenotype relationship. Hum Genet 97(1):51–59

Poudrier J, St-Louis M, Lettre F, Gibson K, Prevost C, Larochelle J, Tanguay RM (1996) Frequency of the IVS12 + 5G–>A splice mutation of the fumarylacetoacetate hydrolase gene in carriers of hereditary tyrosinaemia in the French Canadian population of Saguenay-Lac-St-Jean. Prenat Diagn 16(1):59–64. doi:10.1002/(SICI)1097-0223(199601)16:1<59::AID-PD810>3.0.CO;2-D

Poudrier J, Lettre F, Scriver CR, Larochelle J, Tanguay RM (1998) Different clinical forms of hereditary tyrosinemia (type I) in patients with identical genotypes. Mol Genet Metab 64(2):119–125. doi:S1096-7192(98)92695-6

Poudrier J, Lettre F, St-Louis M, Tanguay RM (1999) Genotyping of a case of tyrosinaemia type I with normal level of succinylacetone in amniotic fluid. Prenat Diagn 19(1):61–63. doi:10.1002/(SICI)1097-0223(199901)19:1<61::AID-PD455>3.0.CO;2-#

Prieto-Alamo MJ, Laval F (1998) Deficient DNA-ligase activity in the metabolic disease tyrosinemia type I. Proc Natl Acad Sci U S A 95(21):12614–12618

Ran T, Gao Y, Marsh M, Zhu W, Wang M, Mao X, Xu L, Xu D, Wang W (2013) Crystal structures of Cg1458 reveal a catalytic lid domain and a common catalytic mechanism for the FAH family. Biochem J 449(1):51–60. doi:10.1042/BJ20120913

Rootwelt H, Berger R, Gray G, Kelly DA, Coskun T, Kvittingen EA (1994a) Novel splice, missense, and nonsense mutations in the fumarylacetoacetase gene causing tyrosinemia type 1. Am J Hum Genet 55(4):653–658

Rootwelt H, Brodtkorb E, Kvittingen EA (1994b) Identification of a frequent pseudodeficiency mutation in the fumarylacetoacetase gene, with implications for diagnosis of tyrosinemia type I. Am J Hum Genet 55(6):1122–1127

Rootwelt H, Chou J, Gahl WA, Berger R, Coskun T, Brodtkorb E, Kvittingen EA (1994c) Two missense mutations causing tyrosinemia type 1 with presence and absence of immunoreactive fumarylacetoacetase. Hum Genet 93(6):615–619

Rootwelt H, Kristensen T, Berger R, Hoie K, Kvittingen EA (1994d) Tyrosinemia type 1 – complex splicing defects and a missense mutation in the fumarylacetoacetase gene. Hum Genet 94(3):235–239

Rootwelt H, Hoie K, Berger R, Kvittingen EA (1996) Fumarylacetoacetase mutations in tyrosinaemia type I. Hum Mutat 7(3):239–243. doi:10.1002/(SICI)1098-1004(1996)7:3<239::AID-HUMU8>3.0.CO;2-5

Sheth JJ, Ankleshwaria CM, Pawar R, Sheth FJ (2012) Identification of novel mutations in FAH gene and prenatal diagnosis of tyrosinemia in Indian family. Case Rep Genet 2012:428075. doi:10.1155/2012/428075

Sniderman King L, Trahms C, Scott CR (2006) Tyrosinemia type 1. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT, Stephens K (eds). doi:http://www.ncbi.nlm.nih.gov/books/NBK1515/

van Spronsen FJ, Thomasse Y, Smit GP, Leonard JV, Clayton PT, Fidler V, Berger R, Heymans HS (1994) Hereditary tyrosinemia type I: a new clinical classification with difference in prognosis on

dietary treatment. Hepatology 20(5):1187–1191. doi:S027091399400340X

St-Louis M, Leclerc B, Laine J, Salo MK, Holmberg C, Tanguay RM (1994) Identification of a stop mutation in five Finnish patients suffering from hereditary tyrosinemia type I. Hum Mol Genet 3(1):69–72

St-Louis M, Poudrier J, Phaneuf D, Leclerc B, Laframboise R, Tanguay RM (1995) Two novel mutations involved in hereditary tyrosinemia type I. Hum Mol Genet 4(2):319–320

Timm DE, Mueller HA, Bhanumoorthy P, Harp JM, Bunick GJ (1999) Crystal structure and mechanism of a carbon-carbon bond hydrolase. Structure 7(9):1023–1033. doi:st7915

Timmers C, Grompe M (1996) Six novel mutations in the fumarylacetoacetate hydrolase gene of patients with hereditary tyrosinemia type I. Hum Mutat 7(4):367–369. doi:10.1002/(SICI)1098-1004(1996)7:4<367::AID-HUMU14>3.0.CO;2-0

Vaca Jacome AS, Rabilloud T, Schaeffer-Reiss C, Rompais M, Ayoub D, Lane L, Bairoch A, Van Dorsselaer A, Carapito C (2015) N-terminome analysis of the human mitochondrial proteome. Proteomics 15(14):2519–2524. doi:10.1002/pmic.201400617

Vondrackova A, Tesarova M, Magner M, Docekalova D, Chrastina P, Prochazkova D, Zeman J, Honzik T (2010) Clinical, biochemical and molecular characteristics in 11 Czech children with tyrosinemia type I. Cas Lek Cesk 149(9):411–416

Ward AJ, Cooper TA (2010) The pathobiology of splicing. J Pathol 220(2):152–163. doi:10.1002/path.2649

Wu X, Hurst LD (2016) Determinants of the usage of splice-associated cis-motifs predict the distribution of human pathogenic SNPs. Mol Biol Evol 33(2):518–529. doi:10.1093/molbev/msv251

Yang N, Han LS, Ye J, Qiu WJ, Zhang HW, Gong ZW, Zhang YF, Wang Y, Gu XF (2012) Analysis of clinical data and genetic mutations in three Chinese patients with tyrosinemia type I. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 29(6):648–652. doi:940629142