



ORIGINAL ARTICLE

Mutational spectrum of Mexican patients with tyrosinemia type 1: In silico modeling and predicted pathogenic effect of a novel missense *FAH* variant

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Abstract

Background: Tyrosinemia type 1 (HT1, MIM#276700) is caused by a deficiency in fumarylacetoacetate hydrolase (FAH) and it is associated with severe liver and renal dysfunction. At present, the mutational *FAH* (15q25.1, MIM*613871) spectrum underlying HT1 in the Mexican population is unknown. The objective of this study was to determine the *FAH* genotypes in eight nonrelated Mexican patients with HT1, who were diagnosed clinically.

Methods: Sequencing of *FAH* and their exon–intron boundaries and in silico protein modeling based on the crystallographic structure of mouse FAH.

Results: We identified pathogenic variants in 15/16 studied alleles (93.8%). Nine different variants were found. The most commonly detected HT1-causing allele was NM_000137.2(*FAH*):c.3G > A or p.(?) [rs766882348] (25%, $n = 4/16$). We also identified a novel missense variant NM_000137.2(*FAH*):c.36C > A or p.(Phe12Leu) in a homozygous patient with an early and fatal acute form. The latter was classified as a likely pathogenic variant and in silico protein modeling showed that Phe-12 residue substitution for Leu, produces a repulsion in all possible Leu rotamers, which in turn would lead to a destabilization of the protein structure and possible loss-of-function.

Conclusion: HT1 patients had a heterogeneous mutational and clinical spectrum and no genotype–phenotype correlation could be established.

KEYWORDS

FAH genotype, fumarylacetoacetate hydrolase, hepatocellular carcinoma, heterogeneous mutational spectrum, inborn error of metabolism, nitisinone

Isabel Ibarra-González and Cynthia Fernández-Lainez are contributed equally.

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1 | INTRODUCTION

Tyrosinemia type I (HT1, MIM#276700) is an autosomal recessive disorder caused by deficiency of the hepatic enzyme fumarylacetoacetate hydrolase (FAH, EC 3.7.1.2), which is involved in the catabolism of tyrosine (Tyr) (Larochele and Tanguay 2017). The active form of FAH comprises a homodimer of 46 kDa subunits, which catalyzes the hydrolytic cleavage of fumarylacetoacetate to yield fumarate and acetoacetate (Timm, Mueller, Bhanumorthy, Harp, & Bunick, 1999). FAH deficiency results in an accumulation of fumarylacetoacetate and maleylacetoacetate, as well as the intermediate succinylacetone (SA), all of which are highly toxic to the liver, kidneys, and peripheral nerves (Larochele & Tanguay, 2017). Although approximately 98 *FAH* (15q25.1, MIM*613871) pathogenic variants have been reported (Morrow, Angileri, & Tanguay, 2017), the mutational spectrum in Mexican patients is virtually unknown to date, as only one heterozygous patient c.[1062 + 5G>A];[=] has been reported (Angileri et al., 2015).

Despite the fact that no clear correlation between genotype and phenotype has been established for HT1, analyses of the protein structure of pathogenic *FAH* alleles could facilitate a better understanding of their potential clinical effects (Morrow et al., 2017). Furthermore, establishing the causative *FAH* genotype would be useful for accurate genetic counseling (Mayorandan et al., 2014) and prenatal diagnosis in families that require it. Hence, the aim of the present study was to determine the mutational spectrum of the *FAH* in Mexican HT1 patients.

2 | MATERIAL AND METHODS

2.1 | Patients

In this study, we assessed the *FAH* genotype of eight (5 male/3 female) nonrelated Mexican HT1 patients, whose biochemical diagnosis was confirmed by quantitation of SA in blood by tandem mass spectrometry. We also analyzed the results of Tyr, phenylalanine (Phe), methionine (Met), alpha-fetoprotein (AFP), and liver function tests. Family history, including consanguinity and siblings with HT1, or suggestive symptoms were recorded. Nutritional management (Tyr and Phe restriction), nitisinone administration (1–2 mg kg⁻¹ day⁻¹), and liver transplant, or their combination, and clinical outcomes were also documented. The patients were classified into one of the following three clinical types proposed by Morrow and Tanguay in 2017, based on the age of symptom onset and clinical manifestations: (a) Acute form (onset before 2 months of age), (b) Subacute (symptoms appearing between 2 and 6 months), and (c) Chronic (symptoms present after 6 months of age)

(Morrow & Tanguay 2017). The study was approved by the Bioethics and Research Committees of the National Institute of Pediatrics (017/2011).

2.2 | *FAH* genotyping

Genomic DNA samples were obtained from dried blood spots using standard methods. Direct and bidirectional automated DNA sequencing was applied to the 14 coding exons of the *FAH* and their exon–intron boundaries (NG_012833.1 RefSeqGene, NM_000137.2). Details regarding the primers and PCR conditions used are available upon request. All missense *FAH* variants were assessed with respect to the dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), the Genome Aggregation Database (gnomAD, <http://gnomad.broadinstitute.org/>), and the NHLBI Exome Sequencing Project at the Exome Variant Server (EVS, <http://evs.gs.washington.edu/EVS/>), ClinVar Database (<https://www.ncbi.nlm.nih.gov/clinvar/>), as well as with reference to the literature. The novel *FAH* missense variants identified were assessed according to the pathogenicity/benignity scoring system recommended by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG-AMP) (Kleinberger, Maloney, Pollin, & Jeng, 2016).

2.3 | Protein in silico modeling

In order to predict the possible deleterious effects of the novel pathogenic variant we identified, we performed in silico modeling based on the crystallographic structure of mouse FAH, which has 89% sequence identity with human FAH at the amino acid level (PDB Code: 1QQJ). In silico mutagenesis was performed for p.(Phe12Leu) using Pymol (PDB code 1QQJ, <http://www.rcsb.org/pdb/home/home.do>).

3 | RESULTS

3.1 | Clinical and biochemical phenotypes

The main phenotypic characteristics of the eight studied patients are shown in Table 1, only one of whom was diagnosed through newborn screening (15 days old). This latter patient was treated at an early stage with nitisinone, and has been asymptomatic for 1 year, with normalization of the main biochemical parameters and normal development according to age. The remaining seven patients were diagnosed at ages ranging from 3 to 36 months of age, with a delay from 2 to 24 months since the initial appearance of symptoms until the definitive diagnosis. Chronic, acute, and subacute HT1 presentation forms were observed in four, two, and one patient(s), respectively.

3.2 | Pathogenic *FAH* genotypes

On the basis of Sanger sequencing methodology, we identified 15/16 *FAH* alleles (93.75%), including a previously unreported missense change located at exon 1 NM_000137.2(*FAH*):c.36C > A or p.(Phe12Leu). The other eight observed alleles have been reported previously. The most common pathogenic allele (25%, $n = 4/16$) was the “start-loss” variant NM_000137.2(*FAH*): c.3G > A or p.(?) [rs766882348] (Table 2).

FAH genotypes in five of the patients were homozygous and in two of these cases, parental consanguinity was documented. A compound heterozygous genotype was identified in two patients, whereas in the remaining patients, we identified one monoallelic genotype. This latter female patient had a classical biochemical phenotype characterized by high blood levels of SA (6.57 μM , reference value: <1 μM), Tyr (48 μM , reference value: 22–108 μM), Met (335 μM , reference value: 9–42 μM), and AFP (4,44,500 ng/ml, reference value: 0.5–5.0 ng/ml) with a subacute presentation. This patient had previously presented with hepatocellular carcinoma that required a liver transplantation (Table 1).

The novel missense variant was present in the homozygous state in patient number 4, with an acute HT1 form and fatal course (Table 1). This variant was predicted to be likely pathogenic (II) according to ACMG-AMP scoring (Kleinberger et al., 2016), as it met the following criteria: (a) its prevalence in affected individuals is significantly increased compared with the prevalence in controls (PS4-strong evidence), in accordance with its absence in the gnomAD and EVS databases (PM2-moderate evidence), (b) multiple lines of computational evidence support a deleterious effect on the gene product (PP3-supporting criteria; Table 2, Figure 2a and b) the patient's biochemical phenotype and his family history (i.e., parental consanguinity related to an autosomal recessive disorder) are highly specific for HT1 (PP4-supporting criteria). The homozygous p.(Phe12Leu) patient was a 5-month-old boy, for whom clinical data had been recorded from 18 days of age, and who showed progressive abdominal distension, pallor, fever, irritability, and hypotonia. At the age of 2 months, he was hospitalized, during which time hepatosplenomegaly, hyperbilirubinemia, anemia, prolonged clotting times, thrombocytopenia, and hypoglycemia were detected. A metabolic disease was suspected, and the patient was sent to our medical unit with aminoaciduria, glycosuria, and hypophosphatemic rickets (Fanconi syndrome). The clinical and biochemical HT1 diagnosis was established based on high blood Tyr levels (226 μM , reference value: 22–108) and elevated blood SA (2.36 μM , reference value: <1 μM) [Table 1]. Consequently, a low Tyr-Phe diet was started immediately, whereas nitisinone treatment commenced at a later stage (4 months old), owing to difficulties in obtaining this orphan drug in our country (Ibarra-González et al., 2017). The patient also began a liver transplantation protocol; however, there were

no compatible donors. The clinical outcome was poor, with rapid progression to cirrhosis and liver failure, and he died in the palliative care unit of our institution at 1 year and 8 months of age. No autopsy was performed.

3.3 | Protein in silico modeling of the p.(Phe12Leu) allele

Phe-12 is located at a distance of 28 Å from the active site of the *FAH* enzyme (Figure 1) and is in close contact with residues from β -sheet number 15 (Timm et al., 1999). This residue shows high phylogenetic conservation (from human to *Caenorhabditis elegans*). When Phe-12 residue is substituted by Leu, repulsion is produced in all possible leucine rotamers (Figure 2).

4 | DISCUSSION

To the best of our knowledge, this is the first genetic study performed in Mexican patients with HT1, each of whom was from a different geographic region of the country. In all cases, the biochemical phenotype was similar to that previously reported worldwide (Morrow et al., 2017), characterized by high blood levels of SA and AFP, prolonged coagulation times, and variable elevation of blood Tyr. However, the clinical presentations were heterogeneous, as has been reported previously in other series (Mayorandan et al., 2014). Although we observed a predominance of chronic presentation of the disease (4/8, 50%), we should not discount the possibility of acute forms, as these are not readily detected clinically, and patients may die without diagnosis. The high proportion of hepatocellular carcinoma observed in our patients (5/8, 62.5%) is within the incidence previously reported for this complication in nontreated or late-treated patients (14%–75%) (Khanna & Verma, 2018). A conclusive explanation for the hepatocellular carcinoma pathogenesis in HT1 has not been established, it is known that fumarylacetoacetate, maleylacetoacetate, and SA form glutathione adducts that can promote free radical damage of hepatocytes and susceptibility to genotoxicity (Chinsky et al., 2017). Furthermore, fumarylacetoacetate inhibits DNA glycosylases, which play a role in the repair of mutagenic oxidative base lesions in DNA, (Bliksrud, Ellingsen, & Bjørås, 2013) and explains the high incidence of hepatocellular carcinoma seen in HT1 patients. Early nitisinone treatment has been found to reduce the incidence of hepatocellular carcinoma (Khanna & Verma, 2018). In the present study, the patient in whom HT1 was detected at an early stage was promptly treated with nitisinone and showed a positive 1-year outcome, which is consistent with the successful experiences reported worldwide (Alvarez & Mitchell 2017). Nevertheless, the development of hepatocellular carcinoma remains a risk

TABLE 2 FAH pathogenic variants detected in the Mexican HTI patients

FAH gene location	cDNA Sequence variation (NM_000137.2)	Protein variant	Classification according to ACMG/ACP scoring	Associated phenotype	Frequency (present work)		World frequency ^a	Studied populations	Reference
					n	%			
Exon 1	c.1A>G	p.(?)	Pathogenic	Chronic	2	12.50	2.1	Emirates/Greece/Saudi Arabia	Georgouli 2010
Exon 1	c.3G>A	p.(?)	Pathogenic	Acute, chronic	4	25.00	Unknown	Present work	gnomAD
Exon 1	c.36C>A	p.(Phe12Leu)	Likely pathogenic	Acute	2	12.50	Unknown	Present work	Present work
Exon 6	c.548_553+20del	p.(?)	Likely pathogenic	Detected by NBS	1	6.25	0.1	Brno-Czech Rep	Arranz 2002
Intron 7	c.607-6T>G	p.(?)	Pathogenic	Chronic	1	6.25	Unknown	USA	Sniderman 2006
Exon 9	c.742G>A	p.(Gly248Arg)	Pathogenic	Chronic	2	12.50	0.2	Canada	GQET
Exon 12	c.1025C>T	p.(Pro342Leu)	Pathogenic	Chronic	1	6.25	0.5	Greece/Norway/USA	Bergman 1998
Intron 12	c.1062+5G>A	p.(?)	Pathogenic	Subacute	1	6.25	32.3	French Canadian/Spain	Angileri 2015, Couce 2011
Exon 14	c.1213_1214delinsCA	p.(Phe405His)	Pathogenic	Detected by NBS	1	6.25	0.1	Portugal	Bergman 1998

Abbreviations: ACMG-AMP, American College of Medical Genetics and Genomics and the Association for Molecular Pathology; GQET, Groupe québécois d'étude de la tyrosinémie/Quebec HTI study group. gnomAD, Genome Aggregation Database (<http://gnomad.broadinstitute.org/>); NBS, Newborn screening.

The novel variant is in bold type, which was predicted either as "disease causing" (Mutation Taster; <http://www.mutationtaster.org/>), "damaging" (SIFT score: 0.00; <http://sift.bii.a-star.edu.sg/>), "probably damaging" (PolyPhen-2 score: 1.00, sensitivity 0.000, specificity: 1.00; <http://genetics.bwh.harvard.edu/pph2/>), or "deleterious" (PROVEAN; <http://provean.jcvi.org/index.php>).

^a According with Morrow, et al. (2017).

in all HT1 patients, thereby indicating the need for promising novel or complementary therapeutic strategies (Aktuglu-Zeybek, Kiykim, & Cansever, 2017; VanLith et al., 2018).

The delay in establishing a diagnosis of HT1 reflects the fact that, at least in Mexico, general physicians and pediatricians are poorly skilled at identifying the possibility of HT1, and thus it is necessary to increase the requisite training and to enhance the likelihood of early detection of the disease through newborn screening (Couce, Dalmau, Del Toro, Pintos-Morell, & Aldámiz-Echevarría, 2011; Ibarra-González et al., 2017; Mayorandan et al., 2014). Here, we documented seven different genotypes, with a predominance of homozygosity (5/8, 62.5%, Table 1), which is similar to that reported in Spain 66% (Moreno-Estrada et al., 2014) and Turkey 60% (Couce et al., 2011). The highly heterogeneous mutational spectrum identified in this study is consistent with that reported worldwide (Angileri et al., 2015; Couce et al., 2011; Morrow et al., 2017). We found that one-third (3/9) of the pathogenic *FAH* alleles were in exon 1, which differ from that reported by other authors, who describe exons 9 and 12 as harboring the largest clusters of disease-causing *FAH* variants (Morrow et al., 2017). The current Mexican population is characterized by considerable ethnic diversity (Arranz et al., 2002), and therefore it is expected that HT1 alleles previously reported in Asian and European populations were detected in the present study (Table 2).

In our study, the c.1062 + 5G>A variant, one of the most frequently identified worldwide (5.4%–32% in Barcelona to 90% in Quebec) (Bergman et al., 1998; Morrow et al., 2017), was detected only in a monoallelic genotype in patient 8 (Table 1). This 8-month-old female had a classical biochemical phenotype, characterized by high blood levels of SA, Tyr, Met, and AFP with subacute presentation. Despite the late diagnosis, nitisinone treatment was started; however,

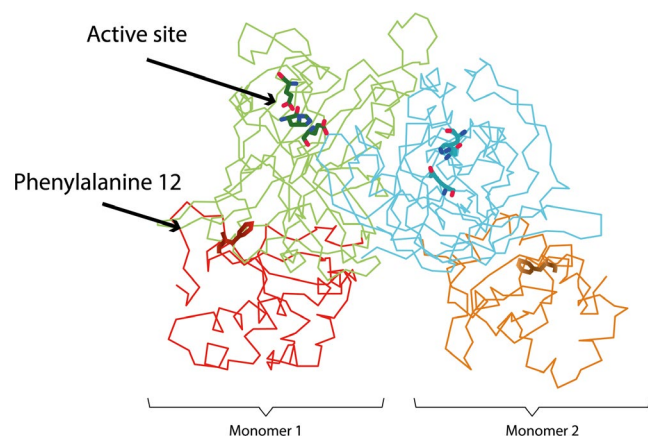


FIGURE 1 Ribbon scheme of the *FAH* dimer. The N-terminal end is colored red and orange; the C-terminal end is colored green and cyan. Phe-12 is located at a distance of 28 Å from the active site residues (His 133, Glu 199, Glu 364); (PDB: 1QQJ; the figure was prepared using Pymol)

she developed hepatocellular carcinoma that required liver transplant.

Using Sanger sequencing methodology, the mutation diagnostic rate for HT1 is close to 91%–100% (Bliksrud, Brodtkorb, Backe, Woldseth, & Rootwelt, 2012; Imtiaz et al., 2011; Park et al., 2009), and thus failure to identify another pathogenic allele is a possibility as noted in patient 8. To date, however, no variants other than point mutations or small deletions have been reported, which make us suspect that deep intron sequences, large deletions, duplications, gene inversions, or promoter defects could go undetected by Sanger sequencing, thereby highlighting the necessity to apply alternative techniques, such as multiplex ligation-dependent probe amplification or massive parallel sequencing of regions other than coding regions (Georgouli et al., 2010).

The c.3G > A variant was the most frequently detected variant in this study, and was identified in two patients with acute and chronic forms, respectively (Table 1). This is a very rare allele that has only twice been reported in a heterozygous state from individuals of Latin American and European descent, according to the gnomAD database (<http://gnomad.broadinstitute.org/variant/15-80445399-G-A>). In this case, although a founder effect might be suspected, it has been difficult to demonstrate because the number of studied families has been insufficient, and thus further population genetic studies are needed.

Start-loss variants have been described in approximately 2% of all known pathogenic *FAH* alleles (Morrow et al., 2017), and these were observed in three of our patients who carried one of two homozygous genotypes (c.1A > G, $n = 1$ and c.3G > A, $n = 2$). The former was found in one patient with a chronic presentation. Other authors have reported this variant in the homozygous state, although associated with subacute and acute forms (Bliksrud et al., 2012; Mohamed et al., 2013; Pomerantz et al., 2018). We were, however, unable to establish a phenotype–genotype correlation in our patients with start-loss variants (Table 2, patients 1–3). Similar types of start-loss variants have been identified in several human disorders, and have been suggested to lead to aberrant mRNA processing from the next downstream Met codon, and consequently to the generation of a shorter and hence partially functional protein product (Morrow et al., 2017; Touriol et al., 2003), or to the usage of an alternative Val start codon, which has been reported in COS cells (Sniderman King, Trahms, & Scott, 2017). Thus, in order to establish a genotype–phenotype correlation, the precise pathogenic effect of these variants in our patients would require functional and in vitro or in vivo expression studies.

The in silico predicted that pathogenicity of the c.36C > A of p.(Phe12Leu) novel variant was disease-causing in all the bioinformatic tools used (Table 2), which is consistent with the severe phenotype observed in our homozygous patient,

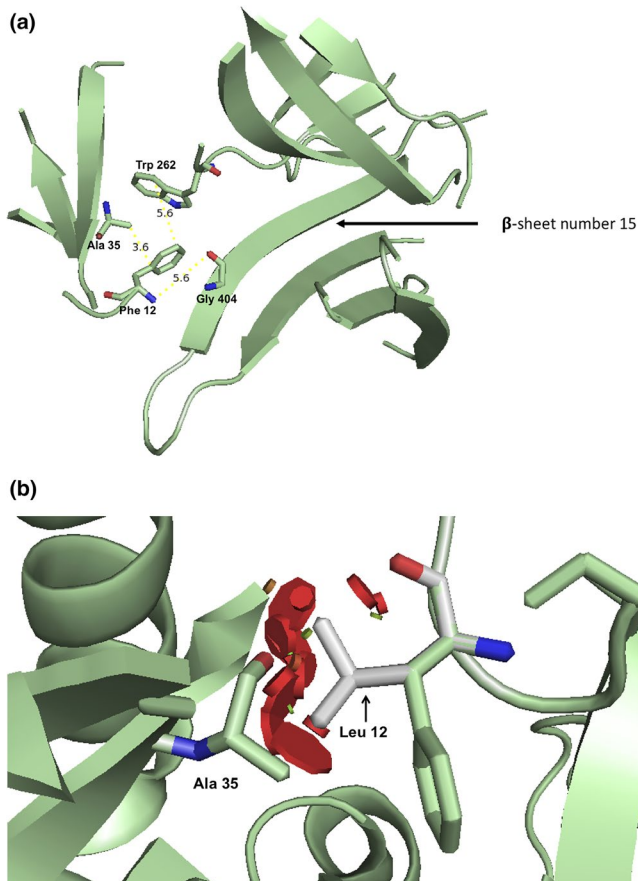


FIGURE 2 (a) Phe-12 is in close contact with residues from β -sheet number 15, which contributes to the structural cohesion among these structural motifs. (b) When Phe-12 is substituted by a leucine, a repulsion is produced in all possible leucine rotamers

who was affected by an acute and rapidly progressive form with fatal outcome. The structural *in silico* modeling revealed that when phe-12 is substituted by a leucine, a generalized repulsion is produced in all possible leucine rotamers (Figure 2). This in turn would promote a destabilization of the protein structure, which could eventually lead to a pronounced loss of FAH activity.

Although we were unable to establish a genotype–phenotype correlation, knowledge of a patient's genotype enables the provision of better genetic counseling to his/her family, including prenatal diagnosis, detection of carriers, and informed reproductive decisions (Mayorandan et al., 2014).

In conclusion, we found that clinical presentation of HT1 was heterogeneous thus, a clear genotype–phenotype correlation could not be established. Sanger automated sequencing enabled us to identify 93.8% ($n = 15/16$) of pathogenic *FAH* alleles in a sample of Mexican HT1 patients, among whom we detected a heterogeneous mutational spectrum and in one case identified a novel missense variant c.36C > A or p.(Phe12Leu). This latter variant was found to be associated with the fatal acute form of the disease,

and on the basis of protein modeling, we predicted that this mutation would cause a destabilization of FAH structure. However, further studies are required to establish the pathogenic effect of this mutation and to investigate its effect on the functional activity of the resulting FAH mutant enzyme.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest, financial, or otherwise.

AUTHOR CONTRIBUTION

IGI. Design of experimental procedures for biochemical analyses; interpretation of biochemical data; drafting and revision of the manuscript.

FLC. Performance of protein *in silico* modeling; analysis and interpretation of biochemical and molecular data; drafting and revision of the manuscript.

AOMA. Designed molecular assays, performed PCR and Sanger sequencing of *FAH* with subsequent annotation, and *in silico* damaging prediction of the genetic variants. Participated in the analysis and interpretation of the genotypic and clinical data, as well as the reviewing of the manuscript.

GAA. Provided genetic counseling to HT1 families, participated in the analysis and interpretation of the genotypic and clinical data, as well as the reviewing of the manuscript.

FHL. Designed molecular assays, performed PCR and Sanger sequencing of *FAH* with subsequent annotation, and *in silico* damaging prediction of the genetic variants. Provided genetic counseling to HT1 families. Participated in the analysis and interpretation of the genotypic and clinical data, as well as the reviewing of the manuscript.

GLS. Nutritional management of studied patients and critical revision of the manuscript.

BML. Medical care of studied patients; analysis of clinical data and critical revision of the manuscript.

LML. Nutritional management of studied patients and critical revision of the manuscript.

VFG. Liver transplant management of patients, critical revision of the manuscript.

VAM. Conception and design of the study; acquisition of funding, drafting, and revision of the manuscript. Approval of the final version of the manuscript to be published.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

- Aktuglu-Zeybek, A. C., Kiykim, E., & Cansever, M. S. (2017). Hereditary Tyrosinemia type 1 in Turkey. In R. M. Tanguay (Ed.), *Hereditary Tyrosinemia: Pathogenesis, screening and management* (pp. 157–172). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_15
- Alvarez, F., & Mitchell, G. A. (2017). Tyrosinemia and liver transplantation: Experience at CHU Sainte-Justine. In R. M. Tanguay (Ed.), *Hereditary Tyrosinemia: Pathogenesis, screening and management* (pp. 67–73). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_5
- Angileri, F., Bergeron, A., Morrow, G., Lettre, F., Gray, G., Hutchin, T., & Tanguay, R. M. (2015). Geographical and ethnic distribution of mutations of the fumarylacetoacetate hydrolase gene in hereditary tyrosinemia type 1. In J. Zschocke, M. Baumgartner, E. Morava, M. Patterson, S. Rahman, & V. Peters (Eds.), *JIMD reports*, (Vol. 19, pp. 43–58). Berlin, Heidelberg: Springer, Berlin Heidelberg. https://doi.org/10.1007/8904_2014_363
- Arranz, J. A., Piñol, F., Kozak, L., Pérez-Cerdá, 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. *Human Mutation*, 20(3), 180–188. <https://doi.org/10.1002/humu.10084>
- Bergman, A. J., van den Berg, I. E., Brink, W., Poll-The, B. T., Ploos van Amstel, J. K., & Berger, R. (1998). Spectrum of mutations in the fumarylacetoacetate hydrolase gene of tyrosinemia type 1 patients in northwestern Europe and Mediterranean countries. *Human Mutation*, 12(1), 19–26. [https://doi.org/10.1002/\(SICI\)1098-1004\(1998\)12:1<19:AID-HUMU3>3.0.CO;2-3](https://doi.org/10.1002/(SICI)1098-1004(1998)12:1<19:AID-HUMU3>3.0.CO;2-3)
- Bliksrud, Y. T., Brodtkorb, E., Backe, P. H., Woldseth, B., & Rootwelt, H. (2012). Hereditary tyrosinaemia type i in Norway: Incidence and three novel small deletions in the fumarylacetoacetase gene. *Scandinavian Journal of Clinical and Laboratory Investigation*, 72(5), 369–373. <https://doi.org/10.3109/00365513.2012.676210>
- Bliksrud, Y. T., Ellingsen, A., & Bjørås, M. (2013). Fumarylacetoacetate inhibits the initial step of the base excision repair pathway: Implication for the pathogenesis of tyrosinemia type I. *Journal of Inherited Metabolic Disease*, 36(5), 773–778. <https://doi.org/10.1007/s10545-012-9556-0>
- Chinsky, J. M., Singh, R., Ficiocioglu, C., van Karnebeek, C. D. M., Grompe, M., Mitchell, G., ... Scott, C. R. (2017). Diagnosis and treatment of tyrosinemia type I: A US and Canadian consensus group review and recommendations. *Genetics in Medicine*, 19(12), 1–16. <https://doi.org/10.1038/gim.2017.101>
- Couce, M. L., Dalmau, J., Del Toro, M., Pintos-Morell, G., & Aldámiz-Echevarría, L. (2011). Tyrosinemia type 1 in Spain: Mutational analysis, treatment and long-term outcome. *Pediatrics International*, 53(6), 985–989. <https://doi.org/10.1111/j.1442-200X.2011.03427.x>
- Georgouli, H., Schulpis, K. H., 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. *Case Reports*, 2010, bcr0720103150. <https://doi.org/10.1136/bcr.07.2010.3150>
- Ibarra-González, I., Ridaura-Sanz, C., Fernández-Lainez, C., Guillén-López, S., Belmont-Martínez, L., & Vela-Amieva, M. (2017). Hepatorenal Tyrosinemia in Mexico: A call to action. In R. M. Tanguay (Ed.), *Hereditary Tyrosinemia: Pathogenesis, screening and management* (pp. 147–156). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_14
- Imtiaz, F., Rashed, M. S., Al-Mubarak, B., Allam, R., El-Karaksy, H., Al-Hassnan, Z., ... Al-Sayed, M. (2011). Identification of mutations causing hereditary tyrosinemia type I in patients of Middle Eastern origin. *Molecular Genetics and Metabolism*, 104(4), 688–690. <https://doi.org/10.1016/j.ymgme.2011.06.019>
- Khanna, R., & Verma, S. K. (2018). Pediatric hepatocellular carcinoma. *World Journal of Gastroenterology*, 24(35), 3980–3999. <https://doi.org/10.3748/wjg.v24.i35.3980>
- Kleinberger, J., Maloney, K. A., Pollin, T. I., & Jeng, L. J. (2016). An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. *Genetics in Medicine*, 18(11), 1165. <https://doi.org/10.1038/gim.2016.13>
- Larochelle, J. (2017). Discovery of hereditary tyrosinemia in Saguenay-Lac St-Jean. In R. M. Tanguay (Ed.), *Hereditary tyrosinemia: Pathogenesis, screening and management* (pp. 3–8). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_1
- Mayorandan, S., Meyer, U., Gokcay, G., Segarra, N. G., de Baulny, H. O., van Spronsen, F., ... Das, A. M. (2014). Cross-sectional study of 168 patients with hepatorenal tyrosinaemia and implications for clinical practice. *Orphanet Journal of Rare Diseases*, 9, 107. <https://doi.org/10.1186/s13023-014-0107-7>
- Mohamed, S., Kambal, M. A., Al Jurayyan, N. A., Al-Nemri, A., Babiker, A., Hasanato, R., & Al-Jarallah, A. S. (2013). Tyrosinemia type 1: A rare and forgotten cause of reversible hypertrophic cardiomyopathy in infancy. *BMC Research Notes*, 6(1), 1. <https://doi.org/10.1186/1756-0500-6-362>
- Moreno-Estrada, A., Gignoux, C. R., Fernandez-Lopez, J. C., Zakharia, F., Sikora, M., Contreras, A. V., ... Bustamante, C. D. (2014). Human genetics. The genetics of Mexico recapitulates Native American substructure and affects biomedical traits. *Science*, 344(6189), 1280–1285. <https://doi.org/10.1126/science.1251688>
- Morrow, G., Angileri, F., & Tanguay, R. M. (2017). Molecular aspects of the FAH mutations involved in HT1 disease. In R. M. Tanguay (Ed.), *Hereditary tyrosinemia: Pathogenesis, screening and management* (pp. 25–48). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_3

- Morrow, G., & Tanguay, R. M. (2017). Biochemical and clinical aspects of hereditary Tyrosinemia type 1. In R. M. Tanguay (Ed.), *Hereditary Tyrosinemia: Pathogenesis, screening and management* (pp. 9–21). Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-55780-9_2
- Park, H. D., Lee, D. H., Choi, T. Y., Lee, Y. K., Kim, J. W., Ki, C. S., & Lee, Y. W. (2009). Clinical, biochemical, and genetic analysis of a Korean neonate with hereditary Tyrosinemia type I. *Clinical Chemistry and Laboratory Medicine*, 47(8), 930–933. <https://doi.org/10.1515/CCLM.2009.223>
- Pomerantz, D. J., Ferdinandusse, S., Cogan, J., Cooper, D. N., Reimschisel, T., Robertson, A., ... Andrae, J. L. (2018). Clinical heterogeneity of mitochondrial NAD kinase deficiency caused by a NADK2 start loss variant. *American Journal of Medical Genetics Part A*, 176(3), 692–698. <https://doi.org/10.1002/ajmg.a.38602>
- Sniderman King, L., Trahms, C., & Scott, C. R. (2017). Tyrosinemia. Type, I. In M. P. Adam, H. H. Ardinger, & R. A. Pagon, et al. (Eds.), *GeneReviews®* [Internet]. Seattle, WA: University of Washington, Seattle. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK1515/>
- Timm, D. E., Mueller, H. A., Bhanumoorthy, P., Harp, J. M., & Bunick, G. J. (1999). Crystal structure and mechanism of a carbon-carbon bond hydrolase. *Structure*, 7(9), 1023–1033. [https://doi.org/10.1016/S0969-2126\(99\)80170-1](https://doi.org/10.1016/S0969-2126(99)80170-1)
- Touriol, C., Bornes, S., Bonnal, S., Audigier, S., Prats, H., Prats, A. C., & Vagner, S. (2003). Generation of protein isoform diversity by alternative initiation of translation at non-AUG codons. *Biology of the Cell*, 95(3–4), 169–178. [https://doi.org/10.1016/S0248-4900\(03\)00033-9](https://doi.org/10.1016/S0248-4900(03)00033-9)
- VanLith, C., Guthman, R., Nicolas, C. T., Allen, K., Du, Z., Joo, D. J., ... Hickey, R. D. (2018). Curative ex vivo hepatocyte-directed gene editing in a mouse model of hereditary Tyrosinemia type 1. *Human Gene Therapy*, 29(11), 1315–1326. <https://doi.org/10.1089/hum.2017.252>

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