Paraclinical Diagnosis of Tyrosinemia

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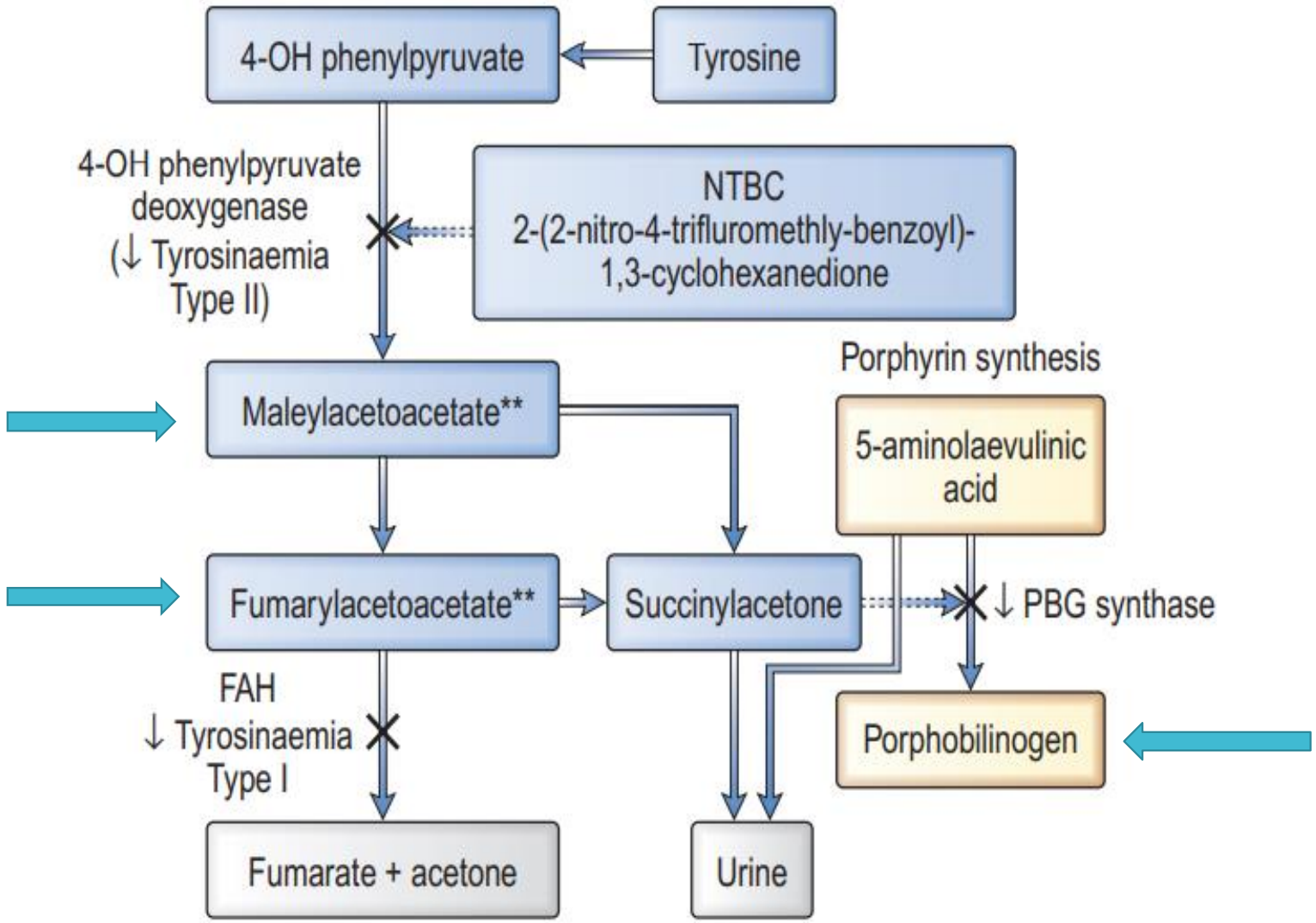
Tyrosinemia type I

Tyrosine metabolic pathway.
In tyrosinemia type 1, deficient fumarylacetoacetate hydrolase (FAH) leads to accumulation of highly reactive intermediate metabolites (**) and production of succinyl acetone, which is both excreted in urine (diagnostic test) and having an inhibitory effect on porphobilinogen (PBG) synthase (acute porphyria crisis and urinary excretion of 5-aminolaevulinic acid).
NTBC (nitisinone) therapy blocks the degradation pathway, preventing reactive metabolite formation.



Tyrosinemia Type I

- Tyrosinemia type I results from deficiency of the enzyme fumarylacetoacetase (FAH):

- Increased succinyl acetone concentration in the blood and urine
- Elevated plasma concentrations of tyrosine, methionine, and phenylalanine
- Elevated urinary concentration of tyrosine metabolites and the compound δ-ALA
- Identification of biallelic pathogenic variants in FAH on molecular genetic testing

- Tyrosinemia type I should be suspected in individuals with the following newborn screening results, clinical features, and supportive laboratory findings

Newborn screening

- Elevated tyrosine or methionine concentration in the blood suggests liver disease, which can be from a variety of causes
- Infants with tyrosinemia type I may have only **modestly elevated or normal blood concentrations of tyrosine and methionine** when the first newborn screening sample is collected.
- Elevated tyrosine concentration on newborn screening can be the result of **transient tyrosinemia of the newborn**, tyrosinemia type II or III, or **other liver disease**.
- Elevated methionine concentration can indicate **liver dysfunction, defects in methionine metabolism, or homocystinuria**

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A Rapid Screening Test on Dried Blood for the Neonatal Diagnosis of Tyrosinemia Type I

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Table 1. Frequency of Diseases in the 100 Patients who Have Been Tested by Rapid Method

Disease	Number	Gender		Age, d	SA Test Positive[a]	SA Test Negative[a]
		Female	Male			
Tyrosinemia	53	23	30	3 - 45	51 (96.2)	2 (3.8)
Biliary atresia	20	10	10	0.5 - 54	6 (30)	14 (70)
Paucity of intrahepatic bile ducts	19	9	10	1 - 50	7 (36.8)	12 (63.2)
Glycogen storage disease	2	1	1	120 - 180	0	2 (100)
Lipid storage disease	2	1	1	90 - 150	0	2 (100)
CMV hepatitis	2	2	0	3 - 35	1 (50)	1 (50)
Galactosemia	2	2	0	0.5 - 32	0	2 (100)
Total	100	100		100	65 (65)	35 (35)

[a]Values are expressed as No. (%).

Newborn screening

- Diagnosis by low delta-ALA-dehydratase (PBG synthase, Porphobilinogen Synthase) enzyme activity.
- This is measured in the newborn screening program in Quebec, Canada.

Confirmation of Diagnosis

- **The diagnosis of tyrosinemia type I should be further evaluated by quantification of plasma or urinary succinyl acetone.**

- **Presence of succinyl acetone, measured directly from the newborn blood spot by tandem mass spectroscopy, is pathognomonic for tyrosinemia type 1.**

Note: (1) Increased excretion of succinyl acetone in the urine of a child with liver failure or severe renal disease is a pathognomonic sign of tyrosinemia type I. (2) Many laboratories require that measurement of succinyl acetone be specifically requested when ordering urine organic acids

Confirmation of Diagnosis

Elevated plasma concentration of tyrosine, methionine, and phenylalanine

Note: (1) Plasma tyrosine concentration in affected infants can be normal in cord blood and during the newborn period.
(2) Elevated plasma tyrosine concentration can also be a nonspecific indicator of liver damage or immaturity; for example, in infants taking a high-protein formula, including undiluted goat's milk.

Confirmation of Diagnosis

Elevated urinary concentration of tyrosine metabolites p-hydroxyphenyl pyruvate, p-hydroxyphenyl lactate, and p-hydroxyphenyl acetate detected on urine organic acid testing

Confirmation of Diagnosis

- Increased urinary excretion of the compound δ-ALA secondary to inhibition of the enzyme δ-ALA dehydratase by succinyl acetone in the liver and circulating red blood cells

Changes in liver function (in untreated tyrosinemia type 1)

- Markedly elevated serum concentration of alpha-fetoprotein (average 160,000 ng/mL)

(normal: <1,000 ng/mL for infants age 1-3 months; <12 ng/mL for children age 3 months to 18 years)

- Prolonged prothrombin and partial thromboplastin times

Note:

(1) Changes in serum concentration of alpha-fetoprotein (AFP) and prothrombin time / partial thromboplastin time (PT/PTT) are more severe in tyrosinemia type I than in nonspecific liver disease and are often the presenting findings in tyrosinemia type I.

(2) Transaminases and bilirubin are only modestly elevated, if at all.

(3) An individual with liver disease and normal serum concentration of AFP and normal PT/PTT has a low probability of having tyrosinemia type I

Establishing the Diagnosis

- The diagnosis of tyrosinemia type I is established in a proband with characteristic biochemical findings
- Increased succinyl acetone concentration in the blood and urine
- Elevated plasma concentrations of tyrosine, methionine, and phenylalanine
- Elevated urinary concentration of tyrosine metabolites and the compound δ-ALA
- Identification of biallelic pathogenic variants in FAH (Fumarylacetoacetate Hydrolase)on molecular genetic testing

Gene [1]	Method	Proportion of Probands with Pathogenic Variants [2] Detectable by Method
FAH	Sequence analysis [3]	>95%
	Gene-targeted deletion/duplication analysis [4]	Unknown; one reported large deletion [5]

Molecular Genetic Testing

Single-gene testing

- ***Sequence analysis of FAH*** is performed first and followed by gene-targeted deletion/duplication analysis if only one or no pathogenic variant is found.
- Targeted analysis for the p.Pro261Leu pathogenic variant can be performed first in individuals of Ashkenzai Jewish ancestry; this variant accounts for more than 99% of the pathogenic variants in this population.
- The pathogenic variant c.1062+5G>A (IVS12+5 G>A) accounts for 87.9% of pathogenic variants in the French Canadian population
- The four common FAH pathogenic variants – c.1062+5G>A (IVS12+5 G>A), c.554-1G>T (IVS6-1 G>T), c.607-6T>G (IVS7-6 T>G), and p.Pro261Leu – account for approximately 60% of pathogenic variants in tyrosinemia type I in the general US population

Molecular Genetics

Information in the Molecular Genetics and OMIM tables may differ from that elsewhere in the GeneReview: tables may contain more recent information. —ED.

Table A. Tyrosinemia Type I: Genes and Databases

Gene	Chromosome Locus	Protein	Locus-Specific Databases	HGMD	ClinVar

Table A. continued from previous page.

FAH	15q25.1	Fumarylacetoacetase	FAH database	FAH	FAH

Data are compiled from the following standard references: gene from HGNC; chromosome locus from OMIM; protein from UniProt. For a description of databases (Locus Specific, HGMD, ClinVar) to which links are provided, click here.

Table B. OMIM Entries for Tyrosinemia Type I (View All in OMIM)

276700	TYROSINEMIA, TYPE I; TYRSN1

Gene structure. *FAH* is approximately 35 kbp in size and comprises 14 exons. For a detailed summary of gene and protein information, see Table A, **Gene**.

Benign variants. A single pseudodeficiency allele, c.1021C>T (p.Arg341Trp), leads to decreased FAH enzyme activity and very little immunoreactive protein, but adequate amounts of FAH mRNA [Rootwelt et al 1994].

Pathogenic variants. See Table 5. Pathogenic missense, nonsense, and splice site variants, as well as small deletions and indels of *FAH*, have been reported. Park et al [2009] reported a large deletion involving *FAH*.

The following population-specific pathogenic variants result from founder effects or genetic drift [Angileri et al 2015] (see Table 5):

- Ashkenazi Jewish: p.Pro261Leu
- Finnish: p.Trp262Ter
- French Canadian: c.1062+5G>A
- Pakistani: p.Gln64His
- Scandinavian: p.Gly337Ser
- Turkish: p.Asp233Val
- Northern European: c.1062+5G>A
- Southern European: c.554-1G>T

Variant Classification	DNA Nucleotide Change (Alias [1])	Predicted Protein Change	Reference Sequences
Pseudodeficiency	c.1021C>T	p.Arg341Trp	NM_000137.1 NP_000128.1
Pathogenic	c.103G>A	p.Ala35Thr	
	c.192G>T	p.Gln64His	
	c.424A>G	p.Arg142Gly	
	c.554-1G>T (IVS6-1G>T)	--	
	c.607-6T>G (IVS7-6T>G)	--	
	c.698A>T	p.Asp233Val	
	c.782C>T	p.Pro261Leu	
	c.786G>A	p.Trp262Ter	
	c.1009G>A	p.Gly337Ser	
	c.1062+5G>A (IVS12+5 G>A)	--	

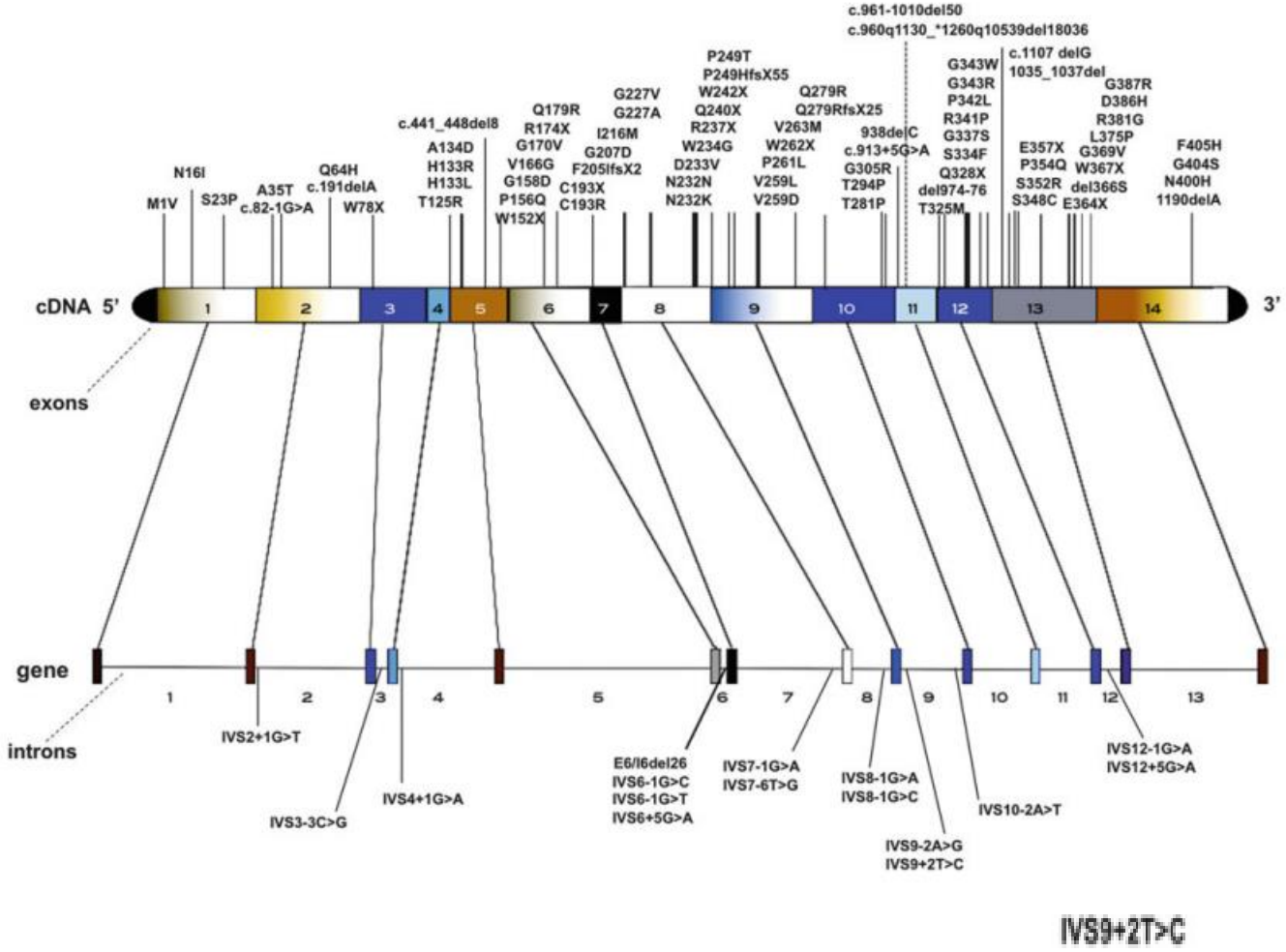

Fig. 1 Location of the 95 mutations identified on the *fah* gene. Among the known HT1 alleles causing mutations, 45 are missense mutations, 23 are splicing mutations, 13 are nonsense mutations, 10 are deletions and 4 are frameshift. Intronic mutations are illustrated at the *bottom* of the figure

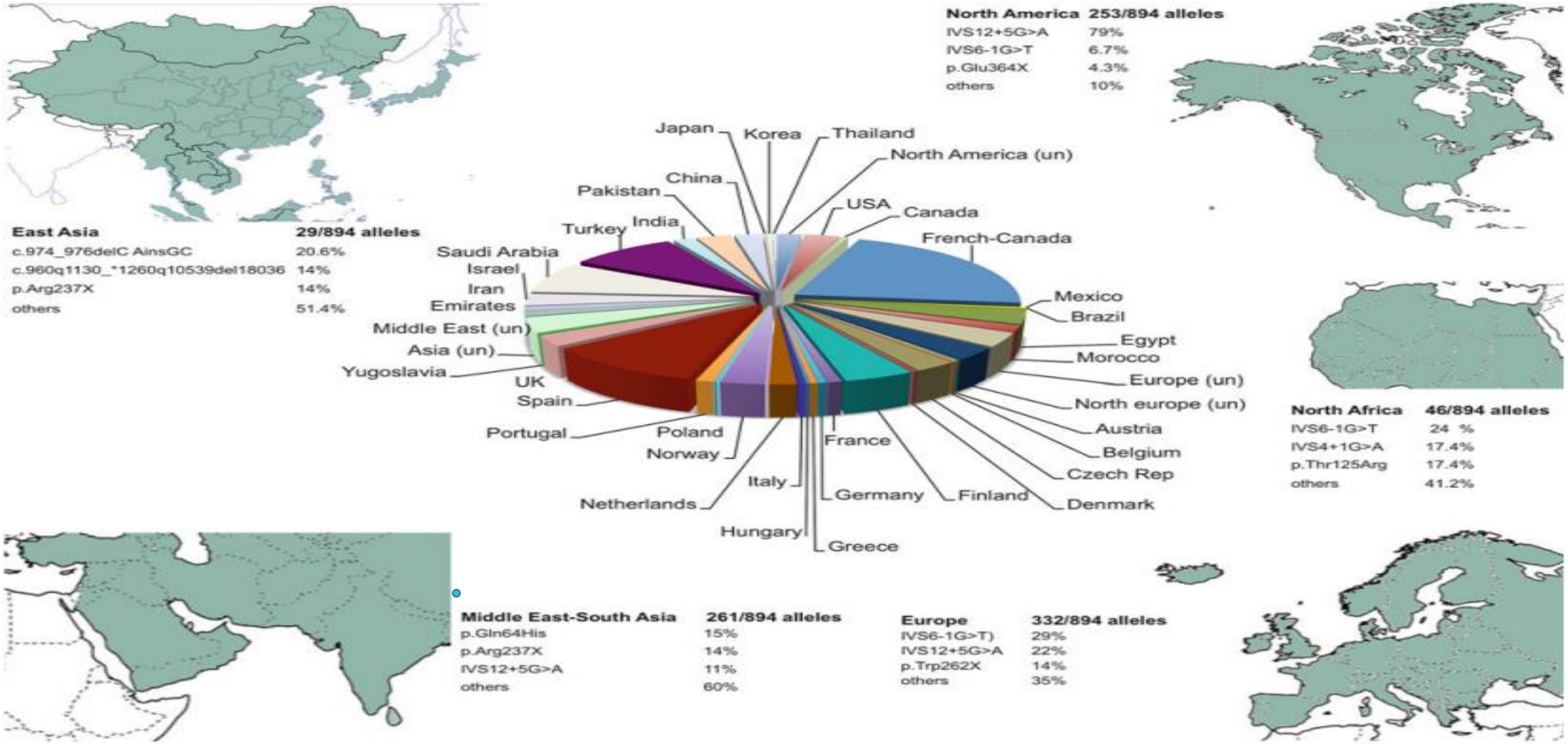

Fig. 2 Geographical distribution of the most common HT1 alleles causing mutations worldwide. *Pie chart* representing distribution of ethnic groups in HT1 alleles. Where the patient provenance was not clear, the mutation is included in the continent of origin, i.e. undefined (un) on graphic. The top three mutations and the total number of alleles for each continent are reported. There are more than 894 HT1 alleles reported worldwide. The most frequent HT1 mutation encountered is the IVS12+5G>A splice mutation, which accounts for 33.7% of all HT1 alleles, followed by the IVS6-1G>T mutation (16.4%). The French Canadian population alone accounts for as much as a third of all HT1 alleles reported. Both mutations are the most reported globally

Genotype-Phenotype Correlations

- In general, no correlation is observed between clinical presentation and genotype.
- Acute and chronic forms have been seen in the same families, as well as in unrelated individuals with the same genotype

Genotype-Phenotype Correlations

- One mechanism that explains this clinical variation is gene reversion. Hepatic nodules removed from livers of individuals with the chronic form of tyrosinemia type I have been shown to have cells that are immunologically positive for FAH protein and to have enzymatic activity for.
- These seemingly "normal" cells appear to have arisen by gene reversion – that is, the spontaneous self-correction (i.e.,reversion or "back-mutation") of the germline pathogenic variant to the normal gene sequence during somatic cell division

Genotype-Phenotype Correlations

- Spontaneous somatic variants that suppress the effects of the pathogenic variants and allow for normal or near normal gene expression in these cells have also been reported . This is a true reversion of the mutated sequence and not the result of maternal cell colonization or maternal cell fusion.

- The "normal" (i.e., reverted) cells have a selective growth advantage because they are no longer at risk for apoptosis from the accumulation of FAA. These foci of revertant "normal" cell colonies comprise many of the liver nodules in untreated individuals with chronic tyrosinemia type I who have a milder biochemical and clinical phenotype. However, the continued production of FAA by the non-revertant mutated cells places the individual at continued risk for hepatocellular carcinoma.

Genotype-Phenotype Correlations (an example)

- A rare and atypical form of tyrosinemia type I has been reported in a four-month-old Belgian infant with severe liver disease.
- Liver function studies were abnormal with markedly elevated alpha-fetoprotein, prolonged PT and PTT, and undetectable succinyl acetone in urine.
- Fumarylacetoacetase (FAH) protein and activity was decreased, but not absent.
- Homozygosity for a unique pathogenic variant, c.103G>A (p.Ala35Thr), was identified.
- Similarly, three sibs who developed chronic liver disease and HCC without detectable blood or urine succinyl acetone had deficient FAH activity. The family was of Middle Eastern background and each child was homozygous for c.424A>G in FAH.

Tyrosinemia type II

Tyrosinemia type II is caused by a **defect in tyrosine aminotransferase (TAT)**

- Establishing the diagnosis of tyrosinemia type II relies on the following:

- Plasma tyrosine concentration typically greater than 500 µmol/L that may exceed 1,000 µmol/L (The concentration of other amino acids is normal.)
- Increased excretion of p-hydroxy phenylpyruvate, p-hydroxyphenyl lactate, and p-hydroxy phenylacetate
- The presence of small quantities of N-acetyl tyrosine and 4-tyramine on urine organic acid analysis

Tyrosinemia type III

The rarest of the tyrosine disorders, is caused by a deficiency of **p-hydroxyphenyl pyruvic acid dioxygenase**.

- Plasma concentration of tyrosine ranges from 350 to 650 µmol/L.
- Excretion of 4-hydroxyphenylpyruvic acid, 4-hydroxyphenyl lactate, and 4-hydroxyphenylacetate is increased. The precise quantities vary with protein intake.
- Few individuals with the disorder have been identified, and the clinical phenotype remains ill defined.
- These individuals, like those with tyrosinemia type II, have no liver involvement but have skin or ocular changes.

Management

Evaluations Following Initial Diagnosis :

- To establish the extent of disease and needs of a child diagnosed with tyrosinemia type I based on newborn screening, the following evaluations are recommended:
- CBC with platelet count
- Serum concentration of electrolytes
- Assessment of liver function (PT, PTT, AST, ALT, GGT, serum bilirubin concentration, alkaline phosphatase, and serum AFP).

Management

Evaluation		Initiation of Therapy (Baseline)	First 6 Mos:		After 6 Mos of Rx: Every 6-12 Mos	After 2 Yrs of Rx: Every 6-12 Mos	As Clinically Indicated
			1x/Mo	Every 3 Mos			
Tyrosinemia type I markers	Plasma concentration of methionine, phenylalanine, tyrosine	X	x		X	X	or X
	Blood / urine succinylacetone	X	X (urine)			X	or X
	Blood nitisinone concentration		X		X	X	or X
CBC	Hemoglobin, hematocrit, WBC, platelet count	X	X		X	X	or X
Liver evaluation	Serum AFP concentration	X	X		X	X	X
	PT/PTT	X	X (until normal)				
	Bilirubin	X					
	ALT/AST/GGT	X		X (until normal)	X		
	Alkaline phosphatase	X		X (until normal)	X		X
	CT or MRI [1]						X
Renal studies	BUN / creatinine						X
	Urine: PO4, Ca, Prot/Cr ratio						X
Skeletal evaluation	X-ray of wrist (for rickets)						X

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Bookshelf URL: https://www.ncbi.nlm.nih.gov/books/

Tyrosinemia Type I

Synonyms: FAH Deficiency, Fumarylacetoacetase Deficiency, Fumarylacetoacetate Hydrolase Deficiency, Hepatorenal Tyrosinemia

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Created: July 24, 2006; Updated: May 25, 2017.

Prenatal Testing and Preimplantation Genetic Diagnosis

- **Molecular genetic testing**. Once the FAH pathogenic variants have been identified in an affected family member, prenatal testing for a pregnancy at increased risk and preimplantation genetic diagnosis for tyrosinemia type I are possible.
- **Biochemical testing**. Prenatal diagnosis for pregnancies at 25% risk is possible by detection of succinyl acetone in amniotic fluid obtained by amniocentesis usually performed at approximately 15 to 18 weeks' gestation.
- Although detection of succinyl acetone in amniotic fluid is diagnostic, false negative results have been reported; thus, this method should only be used by laboratories consistently able to identify succinyl acetone at low levels by stable isotope detection.
- Because of these issues with biochemical testing, molecular genetic testing is the preferred method of prenatal diagnosis

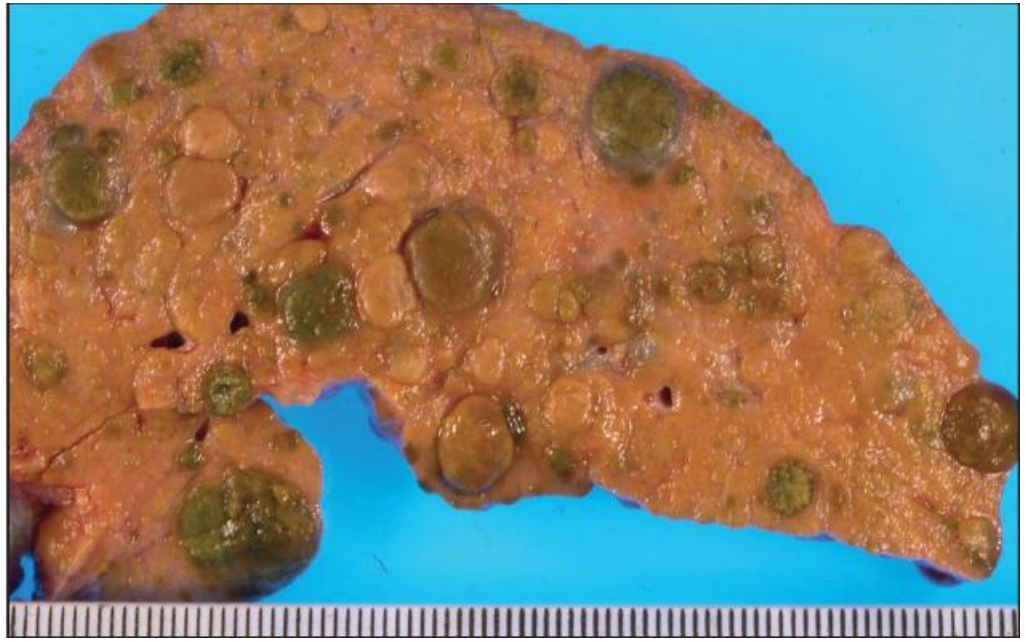
High Risk of HCC after 2 years of age

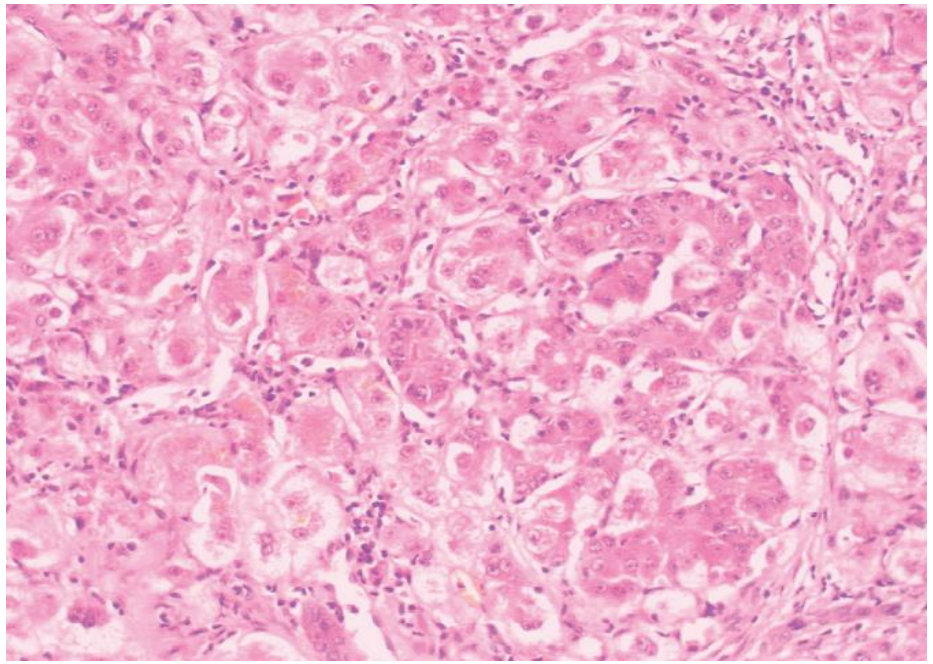
- Accumulated fumarylacetoacetate is mutagenic and carries with it a high risk for HCC in the children with HT-
- The reported incidence of HCC in these patients ranges 15% up to 50%.
- Early treatment with NTBC [2-(2-nitro-4-trifluoromethylbenzoil)-1,3 cyclohxanedione] before the age of 2 years has been suggested to prevent HCC.
- An early diagnosis, and the advent of a liver transplant, have been curative for these children. Fear of occurrence of HCC has pushed surgeons to perform a liver transplant in small children.

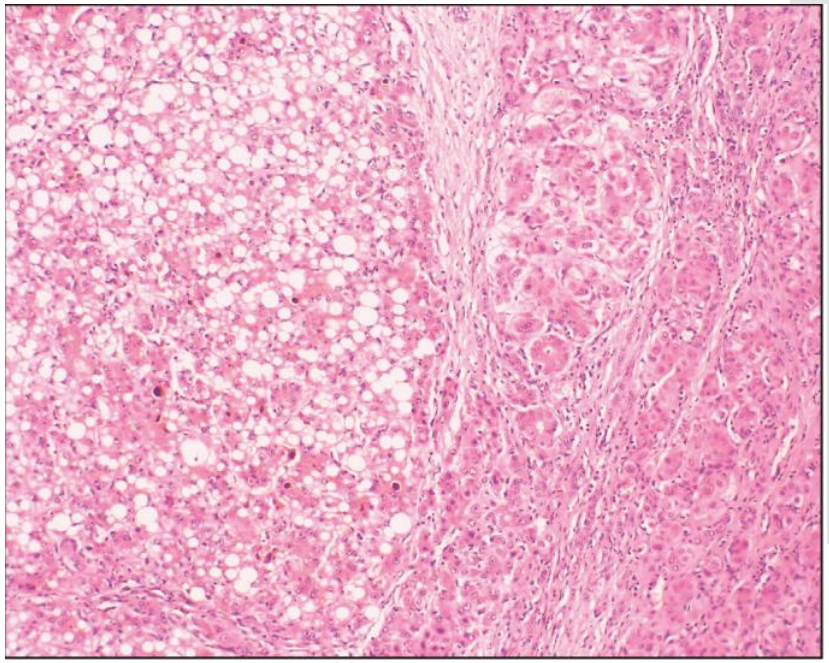
Gross

- The liver, at autopsy or removed at transplantation, is slightly to moderately enlarged, yellow, firm and nodular
- The microscopic features include fatty change, cholestasis, pseudoacinar transformation of hepatic plates, pericellular and periportal fibrosis, variable hemosiderosis, extramedullary hematopoiesis and varying sized foci of nodular regeneration, some qualifying as macro-regenerative nodules .
- The regenerating nodules, which appear as high-attenuation foci on CT scans, are difficult to differentiate from multifocal HCC.

Pathology







Liver Transplant for Children With Hepatocellular Carcinoma and Hereditary Tyrosinemia Type 1

Ali Bahador,[1] Seyed Mohsen Dehghani,[2] Bita Geramizadeh,[2] Saman Nikeghbalian,[1] Mohsen Bahador,[1] Seyed Ali Malekhosseini,[1] Kurosh Kazemi,[1] Heshmatolah Salahi[1]

Table 1. Demographics and Clinical Findings of Patients with HT-1 and HCC (n=5)

Age of the Patient (y)	AFP Level (µg/L)	No. of the HCC Nodules	Largest Size of HCC Nodules (mm)	Prior NTBC Treatment	Dietary Restriction
2	980	1	35	Yes	Relative
4	989	1	15	Yes	Relative
4	234	1	20	Yes	Relative
7	635	1	35	Yes	Relative
21	413	2	15	Yes	Relative

Abbreviations: AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; HT-1, hereditary tyrosinemia type 1; NTBC, nitisinone

Microscopy

- The nodules often show more fat accumulation than the adjacent liver , and some may exhibit dysplastic changes.
- Periportal ductular reaction, although present, is usually not striking.
- Cirrhosis may be micronodular, macronodular or mixed with the prominence and variegated colors of the macronodules (yellow, tan, green)
- Transition from a micronodular to a macronodular cirrhosis has been documented.
- Liver cell dysplasia, both the large and small cell varieties, is frequently observed, and the distinction between dysplastic nodules and HCC may be difficult, if not impossible

Dysplasia