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Diagnosis and the importance of early treatment of tyrosinemia type 1: A case report

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ABSTRACT

Tyrosinemia type 1 is an autosomal recessive aminoacidopathy caused by fumarylacetoacetate hydrolase (FAH) deficiency. Consequently, tyrosine and its metabolites accumulate, resulting in liver and kidney toxicity. Symptoms of the disease usually manifest after three weeks of life and include vomiting, failure to thrive, hepatomegaly, jaundice, bleeding diathesis, rickets and renal tubular dysfunction. Untreated, the disease eventually progresses to liver or kidney failure and generally results in a fatal outcome. Expedient diagnosis is critical because an early start of treatment can increase the likelihood of a positive outcome. Here, we report on a male newborn with a family history positive for tyrosinemia type 1 who was subjected to a metabolic work-up immediately after birth. Amino acids were quantified by tandem mass spectrometry coupled with ultra performance liquid chromatography. Urinary organic acids were analyzed on capillary gas chromatography coupled with mass spectrometry. DNA analysis of the FAH gene was performed by Sanger sequencing. On the first day of life, the patient's plasma amino acids showed an increased tyrosine concentration, while urine organic acids detected succinylacetone, a tyrosine metabolite specific for tyrosinemia type 1. The patient's DNA analysis revealed homozygosity of the c.554-1G > T mutation in the *FAH* gene, which was consistent with the diagnosis. Nitisinone treatment, combined with a dietary restriction of tyrosine and phenylalanine, was introduced immediately. Regular visits and measurement of amino acid concentrations, which enables therapy adjustment and treatment efficiency monitoring in patients with tyrosinemia type 1, has continued over the past 4+ years, and is expected to continue.

1. Introduction

Tyrosinemia type 1 (OMIM# 276700; TYRSN1) is an autosomal recessive aminoacidopathy caused by deficiency of fumarylacetoacetate hydrolase (FAH), an enzyme responsible for the final step of tyrosine breakdown. This deficiency results in accumulation of tyrosine metabolites, which leads to malfunction of the liver and kidney, in particular. Untreated, TYRSN1 eventually progresses to organ failure with a fatal outcome by early childhood. The majority of children present symptoms of the disease before six months of age. Patients who survive past early childhood have an increased risk of hepatocellular carcinoma [1]. Timely introduction of nitisinone (NTBC) therapy and a low protein (specifically, low tyrosine) diet can improve the liver and kidney function of TYRSN1 patients and increase their survival rate.

Abbreviations: BSTFA + 1%TCMS, N,O-Bis (trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane 99:1; CXP, collision cell exit potential; CE, collision energy; DBS, dried blood spot; DP, declustering potential; ESI, electrospray ionization; EP, entrance potential; FAA, fumarylacetoacetate; FAH, fumarylacetoacetate hydrolase; GC MS, capillary gas chromatography coupled with mass spectrometry; GS1, nebulizer gas; GS2, heater gas; HCl, hydrochloric acid; LC-MS/MS, tandem mass spectrometry coupled with ultra performance liquid chromatography; MRM, multiple reaction monitoring; NBS, newborn screening; NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione; PFBO, O-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine hydrochloride; Q1, first quadrupole; Q3, third quadrupole; RCF, Relative Centrifugal Force; RP, reverse phase; SUAC, succinylacetone; TIC, Total Ion Chromatogram; Tyr, tyrosine; Tyr_IS, tyrosine internal standard; TYRSN1, tyrosinemia type 1

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Over the past 15–20 years, tandem mass spectrometry coupled with ultra performance liquid chromatography (LC-MS/MS) has become widely implemented in clinical laboratories, particularly in newborn screening (NBS) programs. LC-MS/MS technology has brought new possibilities for newborn screening because it improves detection of many diseases by analyzing a series of metabolites from a single dried blood spot (DBS). Currently, newborns are screened for TYRSN1 in many countries [2,3] using elevated tyrosine levels as the primary marker. However, tyrosine alone is not sensitive enough to detect all cases and may results false negatives [2,5], and it is not specific enough to differentiate TYRSN1 from other tyrosine metabolism disorders [2,4]. Parallel analysis of succinylacetone (SUAC) from the same blood spot significantly improves TYRSN1 diagnostics [4,6] and has been demonstrated to be a valuable marker for recognition of TYRSN1 patients [6].

Here, we report on the case of a male newborn with positive family history of TYRSN1 who was subjected to a metabolic examination immediately after birth and identified to have TYRSN1. We highlight the benefits of introducing a specific treatment for TYRSN1 as early as possible, and the need for continuous monitoring of plasma amino acid concentrations to enable treatment modifications that support the overall well-being of the patient. The significance of mass spectrometry for diagnosis and patient follow-up is emphasized. Considering the ongoing discussion on whether to introduce SUAC in newborn screening programs, this case report highlights the importance of SUAC as a reliable screening marker for TYRSN1.

2. Materials and methods

Biochemical and hematology analyses, quantitative analysis of amino acids, and urinary organic acid analysis were performed at the Division of Laboratory Diagnostics of Inherited Metabolic Diseases and Newborn Screening, University Hospital Center Zagreb, Croatia. Molecular genetic testing of the *FAH* gene was performed in Sahlgrenska University Hospital in Gothenburg, Sweden.

Informed consent was obtained from the patient's parents.

Biochemical and hematology analyses were performed using a Cobas c501/c311 (Roche Diagnostics®, Basel, Switzerland) for routine biochemical analyses and a UniCel® DxH 800 Coulter® analyser (Beckman Coulter Inc., Brea, CA, USA) for complete blood count.

Amino acids were quantified by LC-MS/MS (API 3200, *Sciex,* Framnigham, MA, USA; UPLC Nexera, *Shimadzu GmbH*, Zagreb, Croatia). The results were obtained using certified aTRAQ™ reagents from *Sciex* (Framingham, MA, USA) with commercial internal standards of known concentration for each amino acid. Patient samples were prepared according to the manufacturer's aTRAQ™ Reagents Application Kit protocol, designed for use with the LC/MS/MS System.

2.1. Sample preparation

40 μL of patient plasma were added together with commercial ClinChek® Plasma Controls for Amino Acids (*Recipe Chemicals + Instruments GmbH*, Munich, Germany) in Eppendorf tubes (*Eppendorf*, Hamburg, Germany), and sulfosalicylic acid (containing approximately 4000 pmol norleucine) was added to each tube for deproteinization. A short vortex and then spin at 16 000 RCF for two minutes was performed in a 5415 R microcentrifuge (*Eppendorf*, Hamburg, Germany). The supernatant was transferred to a clean tube and Labeling Buffer (containing approximately 800 pmol norvaline) was added to the aliquot. After a short vortex and spin, the supernatant was transferred to a clean tube and 5 μL of aTRAQ™ Reagent D8 solution for labeling samples was added. Samples were incubated at room temperature for 30 min and then hydroxylamine was added to reverse partial labeling of the phenolic hydroxyl group of tyrosine and quench any unreacted aTRAQ™ Reagent. Samples were incubated, once more, at room temperature for 15 min. 32 μL of Internal standard solution containing a mixture of D0-labeled amino acids was next added. The exact concentration of amino acids in the reconstituted standard was determined by the manufacturer for each lot and reported on the Certificate of Analysis. Sample volumes were reduced to approximately half of the initial volume using a nitrogen evaporator (*VLM GmbH,* Bielefeld, Germany) and 25 μL of ultra clean water (LiChrosolv® water for chromatography, LC-MS grade, *Merck,* Darmstadt, Germany*)* was added to each tube.

2.2. Chromatographic separation

Prepared samples were placed into 1.5 mL glass vials with polypropylene inserts (*Agilent Techonologies*, Santa Clara, CA, USA) and placed in the autosampler rack. Component separation of the samples was performed on a reverse phase (RP) analytical column, AAA C18 Column, 4,6 × 150 mm (*Sciex*, Framnigham, MA, USA) at a temperature of 50 °C using gradient elution. Mobile phases were made using two commercial modifiers from the aTRAQ™ reagent (Modifier A was 100% formic acid, Modifier B was 100% heptafluorobutiric acid), LiChrosolv® water for chromatography (LC-MS grade) and LiChrosolv® methanol hypergrade for LC-MS (both by *Merck,* Darmstadt, Germany). 300 mL of mobile phase A (water + 0.1% Modifier A + 0.01% Modifier B) and B (methanol + 0.1% Modifier A + 0.01% Modifier B) were prepared. The sample injection volume was 2 μL, and total run time was 18 min per sample with a flow rate of 0.8 mL/min.

2.3. Triple quadrupole mass spectrometer analysis

After chromatographic separation of amino acid conjugates with aTRAQ reagent, samples were ionized using electrospray ionization (ESI) in positive ion mode, with ion source parameters as follows: curtain gas (N_2), 20 psi; ionspray voltage, 4500 V; temperature, 600 °C; GS1, nebulizer gas (N_2), 50 psi; GS2, heater gas, 50 psi; collision gas (N_2), 6 psi. The experiment was conducted using multiple reaction monitoring (MRM), where each amino acid (including corresponding internal standards) was characterized by specific quadrupole (Q1 and Q3) mass transitions, as well as transition-specific parameters (Table 1). The entrance potential (EP) and collision cell exit potential (CXP) were set to 10 and 3 V, respectively, for each analyte. The collision energy (CE) and declustering potential (DP) were both set to 30 V for all amino acids with the exception of ornitine, lysine and their corresponding internal standards, which were set at 50 V. On entering the mass spectrometer quadrupoles, precursor ions were fragmented in a collision cell, and fragment ions were filtered by mass-to-charge (m/z) ratio and detected. Two commercial software applications, Analyst®, version 1.5.2, and Cliquid®, version 3.2 (*Sciex,* Framingham, MA, USA) were used to collect and process data for quantification of up to 45 physiological amino acids per sample (Fig. 1. Total Ion Chromatogram-TIC of a TYRSN1 patient compared with the TIC of a healthy individual). The concentration of individual amino acids was automatically calculated by comparison of peak area (D8-labeled amino acids) to the corresponding internal standard (D0-labeled amino acids). The retention times of the D8- and D0-labeled peaks are the same, since they are identical amino acids, only with different number of isotopes.

Urinary organic acids were analyzed on a capillary gas chromatograph coupled with a mass spectrometer (GC–MS-QP2010Plus, *Shimadzu GmbH*, Zagreb, Croatia).

2.4. Sample preparation

The patient's urine, without preservatives, was randomly collected for analysis. The volume of urine used for extraction was adjusted for each sample so that it contained 1 μmol of creatinine. In cases where creatinine concentrations were less than 0.5 mmol/L, 2 mL of urine was used. 50 μL of each internal standard ($^2H_4$ 4-nitrophenol, 98%, *Cambridge Isotope Laboratories,* Dresden, Germany; 2-oxo caproic acid

Table 1
Q1 and Q3 mass transitions and transition-specific parameters for each analyte.

Analyte name	Q1 *m/z* (Da)	Q3 *m/z* (Da)	RT (min)	CEP(V)
Gly_IS	216.130	113.100	3.60	16.09
Gly	224.150	121.100	3.60	16.36
Sar/bAla/Ala_IS	230.150	113.100	4.38	16.56
Sar/bAla/Ala	238.160	121.100	4.38	16.83
GABA/bAib/Abu_IS	244.170	113.100	5.40	17.03
GABA/bAib/Abu	252.180	121.100	5.40	17.30
Ser_IS	246.150	113.100	3.40	17.10
Ser	254.160	121.100	3.40	17.37
Pro_IS	256.170	113.100	5.50	17.44
Pro	264.180	121.100	5.50	17.71
Val/NVal_IS	258.180	113.100	6.57	17.51
Val/NVal	266.200	121.100	6.57	17.78
Thr_IS	260.160	113.100	4.30	17.57
Thr	268.180	121.100	4.30	17.84
Tau_IS	266.120	113.100	2.71	17.77
Tau	274.130	121.100	2.71	18.04
Ile/Leu/NLeu_IS	272.200	113.100	7.75	17.98
Ile/Leu/NLeu	280.210	121.100	7.75	18.25
Asn_IS	273.160	113.100	3.40	18.01
Asn	281.170	121.100	3.40	18.28
Asp_IS	274.140	113.100	3.78	18.04
Asp	282.150	121.100	3.78	18.31
Gln_IS	287.170	113.100	3.70	18.48
Gln	295.190	121.100	3.70	18.75
Glu_IS	288.160	113.100	4.30	18.52
Glu	296.170	121.100	4.30	18.79
Met_IS	290.150	113.100	6.40	18.58
Met	298.170	121.100	6.40	18.85
His_IS	296.170	113.100	3.80	18.79
His	304.190	121.100	3.80	19.06
Phe_IS	306.200	113.100	7.70	19.12
Phe	314.200	121.100	7.70	19.39
Arg_IS	315.210	113.100	4.60	19.43
Arg	323.230	121.100	4.60	19.70
Cit_IS	316.200	113.100	4.20	19.46
Cit	324.210	121.100	4.20	19.73
Tyr_IS	322.180	113.100	6.70	19.66
Tyr	330.190	121.100	6.70	19.93
Trp_IS	345.190	113.100	8.30	20.44
Trp	353.210	121.100	8.30	20.71
Orn_IS	413.290	113.100	5.30	22.73
Orn	429.320	121.100	5.30	23.27
Lys_IS	427.300	113.100	5.70	23.20
Lys	443.330	121.100	5.70	23.74

Q1 and Q3 – filtering quadrupoles of mass spectrometer; RT – retention time; CEP – collision cell entrance potential; Gly_IS – glycine internal standard; Gly – glycine; Sar/bAla/Ala_IS – L-sarcosine/β-alanine/L-alanine internal standard; Sar/bAla/Ala – L-sarcosine/β-alanine/L-alanine; GABA/bAib/Abu_IS – y-amino-n-butyric acid/D,L-β-aminoisobutyric acid/L-α-amino-n-butyric acid internal standard; GABA/bAib/Abu – y-amino-n-butyric acid/D,L-β-aminoisobutyric acid/L-α-amino-n-butyric acid; Ser_IS – L-serine internal standard; Ser – L-serine; Pro_IS – L-proline internal standard; Pro – L-proline; Val/NVal_IS – L-valine/L-norvaline internal standard; Val/NVal – L-valine/L-norvaline; Thr_IS – L-threonine internal standard; Thr – L-threonine; Tau_IS – taurine internal standard; Tau – taurine; Ile/Leu/NLeu_IS – L-isoleucine/L-leucine/L-norleucine internal standard; Ile/Leu/NLeu – L-isoleucine/L-leucine/L-norleucine; Asn_IS – L-asparagine internal standard; Asn – L-asparagine; Asp_IS – L-aspartic acid internal standard; Asp – L-aspartic acid; Gln_IS – L-glutamine internal standard; Gln – L-glutamine; Glu_IS – L-glutamic acid internal standard; Glu – L-glutamic acid; Met_IS – L-methionine internal standard; Met – L-methionine; His_IS – L-histidine internal standard; His – L-histidine; Phe_IS – L-phenylalanine internal standard; Phe – L-phenylalanine; Arg_IS – L-arginine internal standard; Arg – L-arginine; Cit_IS – citrulline internal standard; Cit – citrulline; Tyr_IS – L-tyrosine internal standard; Tyr – L-tyrosine; Trp_IS – L-tryptophan internal standard; Trp – L-tryptophan; Orn_IS – L-ornithine internal standard; Orn – L-ornithine; Lys_IS – L-lysine internal standard; Lys – L-lysine.

≥99%, *Sigma-Aldrich,* Taufkirchen, Germany; O-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride, PFBO, *Sigma-Aldrich,* Taufkirchen, Germany) were pipeted into sample tubes. In order to form stable oxims (2-keto acids), 200 µL of 0.5 N H_2SO_4 (Sulfuric acid 95–97%, for analysis, *Merck,* Darmstadt, Germany) was then added to each sample and incubated in closed tubes at room temperature for one hour. After acidifying samples with 6 N HCl (Hydrochloric acid fuming 37%, for analysis, *Merck,* Darmstadt, Germany), extraction with ethyl-acetate (Ethyl acetate for spectroscopy, Uvasol®, *Merck,* Darmstadt, Germany) was performed. The organic layer was separated and evaporated under a gentle nitrogen flow in a nitrogen evaporator (*VLM GmbH,* Bielefeld, Germany). Organic acids were transformed into stable trimethylsilyl derivates by addition of BSTFA + 1%TCMS (N,O-Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane 99:1, for GC derivatization, *Sigma-Aldrich,* Taufkirchen, Germany).

2.5. GC–MS analysis

Extracted samples were transferred into glass screw-cap vials and placed into an autosampler rack. Helium was used as a carrier gas with a flow rate of 0.8 mL/min. Analysis was performed on DB 5 M capillary column (30 m; 0.25 mm ID) in splitless mode. Samples were ionized using electron impact ionization with an accelerated electron energy of 70 eV, and transferred into a quadrupole analyzer where sample ions were separated based on their *m/z*. The scan range for detection was 40–650 amu, and the total run time was 60 min per sample. Identification of individual organic acids was based on retention time, methylene subunits and comparison of spectrums with commercial online libraries of organic compounds. Integration and peak identification were manually reviewed by labeling all relevant peaks for each sample chromatogram and noting any abnormalities in identification.

2.6. DNA analysis

Genomic DNA was extracted from 1 mL whole blood using the FlexiGene DNA Kit (Qiagen, Crawley, UK). *FAH* gene screening was performed by Sanger sequencing [7], specifically targeted for the variant *FAH*: c.554-1G > T, since a sister of the patient, who also had tyrosinemia type I, was previously found to be homozygous for that variant.

3. Results

The patient's laboratory results revealed certain abnormalities in total blood count and some serum metabolites (Table 2).

The patient's *plasma amino acid* analysis showed an increased concentration of tyrosine (169 µmol/L; reference range: 42–135 µmol/L). Concentrations of all other amino acids were within the reference range. Due to technical reasons (i.e., short stability of methionine in a specific lot of internal standard mixture after reconstitution), it was not possible to measure the concentration of methionine in this sample.

Urine organic acid analysis detected SUAC, a tyrosine metabolite specific for tyrosinemia type 1. No SUAC excretion was observed in any subsequent control analyses. However, extremely elevated excretions of 4-hydroxyphenyl-acetate, 4-hydroxyphenyl-lactate and 4- hydroxyphenyl-piruvate were present. In some control analyses, mild excretion of N-acetyltyrosin was present, as well.

Molecular genetic testing of the *FAH* gene revealed an apparent homozygosity for the known pathogenic mutation c.554-1G > T.

4. Discussion

TYRSN1 is an inherited metabolic disorder caused by FAH deficiency. FAH catalyzes the conversion of fumarylacetoacetate (FAA) to fumarate and acetoacetate, and the conversion of SUAC to succinate and acetoacetate in the catabolism of tyrosine [8]. Mutations in the *FAH* gene result in loss of FAH enzyme activity. Consequently, accumulation of FAA, which is converted into succinylacetoacetate and SUAC, results in liver and renal toxicity [9]. TYRSN1 is a rapidly progressive disease

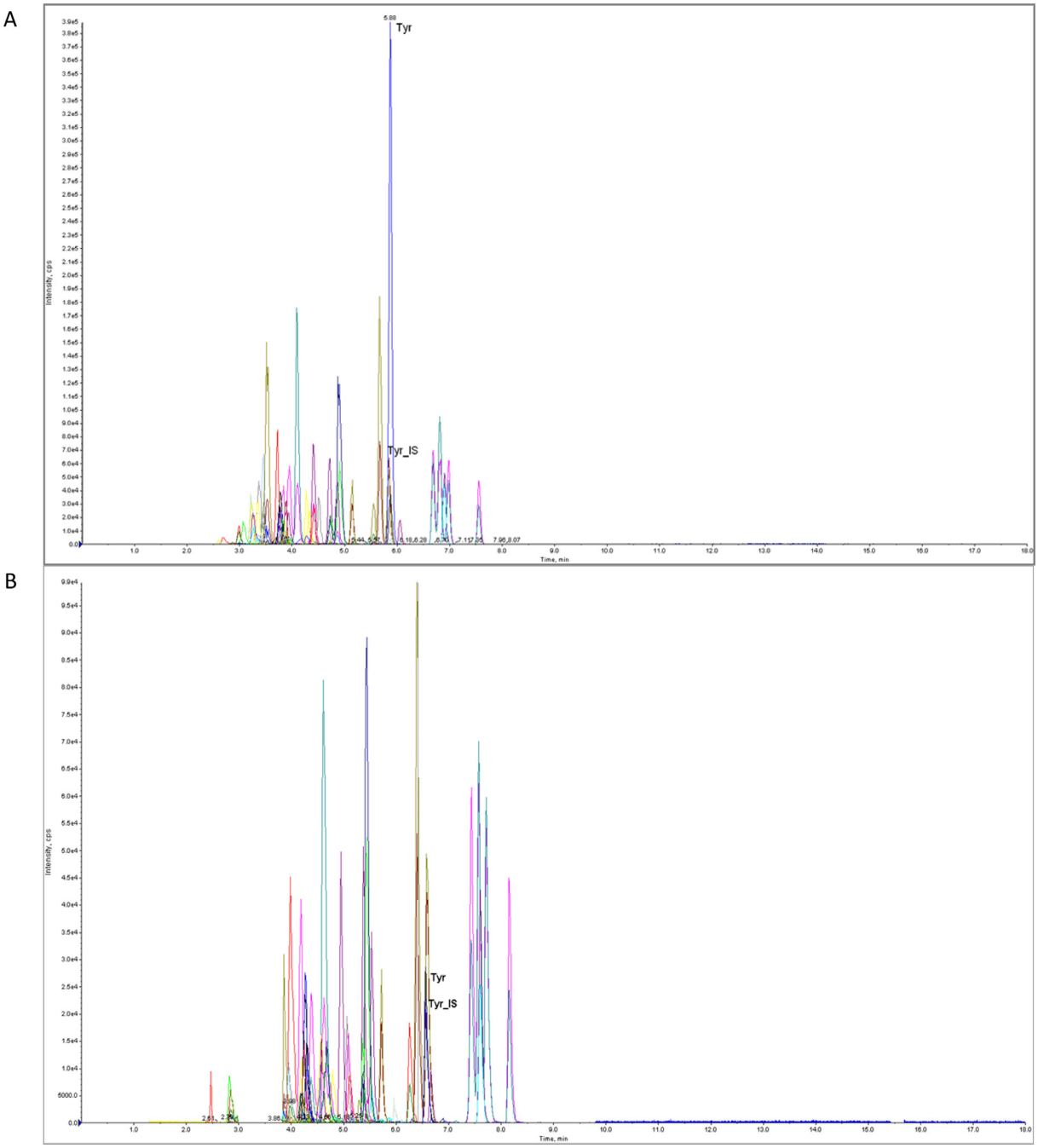

Fig. 1. Total Ion Chromatogram-TIC of a TYRSN1 patient compared with the TIC of a healthy individual. Plasma amino acid chromatograms show a high intensity peak of tyrosine (Tyr) in the TYRSN1 patient (A) compared to the chromatogram of a healthy individual (B).

that, if untreated, can cause liver failure in the first years of life. Common symptoms include vomiting, failure to thrive, hepatomegaly, jaundice, bleeding diathesis, peripheral neuropathy, abdominal pain, rickets and renal tubular dysfunction. Neurologic crises accompanied by pain, muscle hypertonia and weakness, hypertension and hyponatremia may occur at any age and can lead to respiratory failure and death [10].

To diagnose TYRSN1 at an asymptomatic stage, detection through NBS would be optimal [11]. However, using tyrosine as a primary screening marker for TYRSN1 has proved to have poor sensitivity and specificity, as it is usually elevated in benign transient tyrosinemia of newborns [6]. In many countries, SUAC has been used as a primary marker for TYRSN1 in NBS programs because it significantly improves the specificity of TYRSN1 screening [5]. In countries without TYRSN1 as part of their newborn screening program, diagnosis of TYRSN1 is usually established by identification of clinical symptoms in combination with biochemical findings (e.g., increased plasma concentrations of tyrosine and elevated excretion of SUAC in the urine), and identification of pathogenic variation in the *FAH* gene [8]. Unfortunately, by the time of diagnosis, many patients have already experienced severe organ damage and encephalopatic crises and it is probably too late to reverse damage caused by this disease.

The human *FAH* gene is located on the q arm of chromosome 15 (q23-q25). It is approximately 35 kbp in size and consists of 14 exons [5]. Population-specific pathogenic variants of *FAH* (e.g., missense, nonsense, and splice site variants, as well as small deletions and indels) have been reported. The IVS6-1G > T transversion confirmed in the homozygous state in our patient (c.554-1G > T) is the most common

Table 2
Patient's total blood count and biochemistry test results on the first day of life.

Analyte	Value	Flag	Unit	Reference range
RBC	5.84	H	$x10^{12}$/L	3.90–5.50
Hb	199		g/L	136–199
Hct	0.572		L/L	0.391–0.585
MCV	97.9		fL	93.1–115.4
MCH	34.1		pg	31.1–37.8
MCHC	348		g/L	300–350
RDW	17.1		%	14.2–18.3
Erythroblasts	0.2		$\times 10^9$/L	< 0.6
Erythroblasts	1		/1000 Lkc	< 3
WBC	23.8	H	$\times 10^9$/L	6.2–17.8
Segmented Neutrophils	57		%	32–68
Band Neutrophils	3		%	0–9
Lymphocytes	19	L	%	22–41
Monocytes	13	H	%	6–12
Eosinophils	6		%	0–6
Myelocytes	1		%	
Promyelocytes	1		%	
PLT	238		$\times 10^9$/L	150–450
Glucose	0.9	L	mmol/L	3.9–7.0
Total bilirubin	69		µmol/L	
Urea	5.3		mmol/L	1.0–7.5
Creatinine	103	H	µmol/L	27–81
ALP	236		U/L	25–500
AST	47		U/L	26–75
ALT	10	L	U/L	11–46
Y-GT	78		U/L	15–132
K	5,4		mmol/L	3.0–7.0
Na	140		mmol/L	134–145
Cl	104		mmol/L	96–111
Ca	2.09	L	mmol/L	2.15–2.80
P	1.71		mmol/L	1.25–2.50
Mg	0.86		mmol/L	0.65–1.03
Total protein	52		g/L	41–67
CRP	< 0.3		mg/L	0.1–4.1
NH_3	64.5		µmol/L	30–144
Alpha-fetoprotein	> 60 500		µg/L	7900–170000
Creatin kinase	409	H	U/L	60–305

RBC – Red Blood Cell Count; Hb – Hemoglobin; Hct – Hematocrit; RDW – Red Cell Distribution Width; MCV – Mean Corpuscular Volume; MCH – Mean Corpuscular Hemoglobin; MCHC – Mean Corpuscular Hemoglobin concentration; Retc – Reticulocytes; IRF – Immature Reticulocyte Fraction; WBC – White Blood Cell Count; MPV – Mean Platelet Volume; PLT – Platelet count; ALP – alkaline phosphatase; AST – aspartate aminotransferase; ALT – alanine aminotransferase; y-GT – y-glutamyl transferase; K – Potassium; Na – Sodium; Cl – Chloride; Ca – Calcium; P – Inorganic phosphates; Mg – Magnesium; CRP – C-reactive protein; NH_3 – Ammonia.

mutation in the Southern European population [12]. This mutation occurs in the last nucleotide of exon 6 of *FAH* and is often referred to as a splice acceptor variant. It changes the 2nd base region at the 3′ end of an intron affecting the splicing process and the result is production of a non-functional enzyme. There have been reports of TYRSN1 patients with a IVS6-1G > T mutation displaying phenotypes from acute to chronic forms, but no clear genotype-phenotype correlation has been established [13].

The incidence of TYRSN1 in the general population is estimated to be 1:100 000 live births [1], but it varies among populations. For example, French Canadians in the province of Quebec, Canada, have a TYRSN1 incidence of approximately 1:1846 live births [14]. The incidence of TYRSN1 in Croatia is unknown.

Our patient is the fourth child born from young and healthy parents with no reported consanguinity. The first two children are healthy and unaffected. The third child was a girl born after a normal pregnancy, but unfortunately died at 4.5 months due to hepatic failure. Molecular analysis of her genomic DNA confirmed the diagnosis of TYRSN1. It was for this reason that our patient was subjected to a complete metabolic examination for TYRSN1 within several hours of delivery. *FAH* gene analysis showed that our patient carried the same mutation in a homozygous state. Significant laboratory findings that contributed to the diagnosis were a high tyrosine concentration in plasma and detectable SUAC in urine. Although plasma tyrosine elevation can be non-specific in newborns, SUAC is a pathognomonic metabolite for TYRSN1. These findings led to the immediate start of nitisinone (NTBC) therapy with a low-tyrosine diet.

NTBC therapy, together with a low-tyrosine diet, can increase the survival rate for TYRSN1 patients up to 90% [8]. It can also improve liver function, prevent cirrhosis and renal tubular acidosis and improve secondary rickets [15]. Therefore, prompt diagnosis is extremely important because an early start of treatment has the potential to significantly increase the well-being of the patient and reduce the chance of a fatal outcome. NTBC prevents formation of toxic tyrosine metabolites, but, unfortunately, also causes even larger tyrosine accumulation, which can lead to keratopathy, hyperkeratosis of palms and soles and neurological symptoms. For this reason it is necessary to combine NTBC therapy with dietary restriction of tyrosine and phenylalanine [11].

As soon as the diagnosis was determined, Orfadin (NTBC, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, Swedish Orphan Biovitrum International AB, Stockholm, Sweden), combined with a restrictive diet, was introduced orally to our patient daily. Based on the patient's clinical status, this therapy was continued with adjustment to the daily dosage of NTBC at about 1 mg per kg of body weight. Following the initiation of treatment, repeated organic acid analyses showed no trace of SUAC in the patient's urine. The child accepted the therapy and diet well and thrived. His clinical status was satisfying and he was soon released home. This case supports the thesis that if patients with TYRSN1 are started on NTBC therapy before clinical symptoms occur, potentially fatal complications of the disease can be minimized [11]. At the time of this report, we have routinely monitored the patient and his amino acid concentrations for over 4 years (Supplementary Table S1).

5. Conclusions

A male newborn was diagnosed with TYRSN1 immediately after birth by measurement of plasma amino acids and urinary organic acid analysis. After introduction of NTBC treatment with a restrictive diet, the patient's overall condition was good. The patient, a 5-year old boy, comes several times per year to the Department of Pediatrics, University Hospital Center Zagreb, Croatia, for a regular metabolic check-up. He has responded well to the therapeutic regimen and has not yet presented with any disease-related complications. Regular measurement of plasma amino acid concentrations, especially tyrosine levels, are regularly conducted since they are necessary for diet adjustment and to monitor treatment efficacy. This case presents one data point supporting the immediate introduction of a therapeutic regiment comprising NTBC treatment and a reduced tyrosine diet for newborns diagnosed with TYRSN1.

It should be noted that we only performed FAH genetic screening for the two affected children in the family; parental DNA was unavailable. In such cases, we advise screening any older siblings to identify potential mutation carriers, opening up the option for future genetic counseling and family planning.

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Conflict of interest

None of the authors has any conflicts of interest to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clinms.2019.01.005.

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Glossary

Tyrosinemia type 1: a disorder of amino acid metabolism caused by a deficiency of the enzyme fumarylacetoacetase. Accumulation of tyrosine and its metabolites results in liver and kidney toxicity and can end fatally if not treated.

Tandem mass spectrometry: a mass spectrometry which uses two connected mass analyzers (MS/MS). The first mass analyzer transfers selected ions ionized in the MS ionization source into collision cell. In the collision cell, selected ions are randomly fragmented into product ions which are then transferred through the second mass analyzer.

Ultra performance liquid chromatography (UPLC): chromatographic technique with liquid mobile phase which is characterized by high pressures and high separation performance.

Electrospray ionization: ionization technique in MS ionization source performed at atmospheric pressure. An electric field ionizes samples dissolved in a liquid mobile phase passing through a capillary. It is suitable for large polar biomolecules.

Multiple Reaction Monitoring (MRM): scan type in which every analyte is characterized by specific quadrupole mass transitions.

Total Ion Chromatogram (TIC): a chromatogram that sums up all mass peaks (*m/z* transitions) belonging to the same scan (same sample).

Capillary gas chromatography coupled with mass spectrometry (GC–MS): a mass spectrometry combined with gas chromatography for separation and analysis of volatile organic compounds.

Succinylacetone (SUAC): tyrosine metabolite specific for tyrosinemia type 1 which causes liver and renal toxicity.

Newborn screening (NBS): laboratory testing for certain metabolic disorders in whole newborn population. The most common screening method is the one using blood samples on a filter card paper. The number of diseases included in screening program differs from country to country. A screening test is not a diagnostic tool and positive screening result should be confirmed by another, more specific laboratory test.