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A Nonsense Mutation (R237X) in the Fumarylacetoacetate Hydrolase Gene, Responsible for Hereditary Tyrosinemia Type 1 in the Southwest of Iran

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ABSTRACT

Background: Hereditary tyrosinemia type 1 is a rare genetic disorder caused by a mutation in the Fumarylacetoacetate Hydrolase (FAH) gene. The identification of the mutation type is critically important in cascade screening to prevent the development of the disease. This study was conducted to identify the causative mutation of Hereditary Tyrosinemia type 1 (HT1) in numerous patients in Kohgiluyeh and Boyer Ahmad province, southwest of Iran.

Methods: All exons and exon-intron boundaries of the FAH gene were amplified by the polymerase chain reaction method. Subsequently, the amplified segments underwent analysis for possible mutations by direct sanger sequencing method. Biochemical tests were used to assess the biochemical factors in patients' blood and urine.

Results: All patients revealed c. 709 C > T (*p*. 237 R > X) as a causative mutation of HT1. There was no relevance between the mutation type and disease form.

Conclusion: It is recommended that 709 C >T (*p*. 237 R > X) be used in a cascade screening of patients' relatives to prevent the development of the disease in the next generations.

Keywords: Fumarylacetoacetate hydrolase (FAH) gene; Hereditary tyrosinemia type 1 (HT1); Kohgiluyeh and Boyer Ahmad province; Mutation

INTRODUCTION

The tyrosinemia type 1 (HT1) is a rare genetic disorder of autosomal recessive inheritance. The HT1 is categorized into three forms of acute, subacute, or chronic based on the onset and severity of the disease. While the acute form occurs in the first 2 months of life with hepatic failure, the sub acute form appears in the first year of life with progressive liver damage. The chronic form of the disease is diagnosed after the first year of life with minor hepatic damage [1]. The prevalence of HT1 has been estimated to be 1 in 100,000 to 120,000 births worldwide. However, it is more frequent in some populations. For instance, this ratio is estimated to be 1 in 16,000 births in Quebec, Canada. To be more specific, the estimated prevalence in the Saguenay Lac Saint Jean region of Quebec is 1 in 1,850 births [2]. In Iran, the prevalence of this disease is generally near the global ratio, with some subpopulations more frequent [3].

The HT1 is caused by a mutation in the Fumarylacetoacetate Hydrolase (FAH) gene. Fumarylacetoacetate hydrolase is the last enzyme in the tyrosine catabolic pathway. The improper functioning of this enzyme results in the accumulation of fumarylacetoacetate substrate and its conversion to Succinylacetone (SA) as a subsidiary by product [4,5]. Since SA has a harmful effect on hepatic and renal cells, tyrosinemia leads to hepatic failure and its complications [6]. Although this disease is fatal by age 10, it can be managed successfully with early interventions. In this respect, using drugs, such as NTBC (2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione), and following a low tyrosine, low phenylalanine diet can effectively control the disease [7].

The mutations in the FAH gene (almost in all parts of the gene) have been reported to account for HT1. However, no relevance has been shown between mutations and the severity of the disease [8]. Recently about 26 mutations were show in FAH gene, that mutations was due to single base substitutions in 16 amino acid replacem, five nonsense codons, four splicing deficiency, and one silent mutation causing a splicing defect [9]. Some of these mutations are more frequent in some populations or ethnic groups; for instance, the IVS12+5G > A splice mutation is more common in French Canadian patients [10,11].

The recognition of these mutations and groups at high risk should help in the diagnosis of HT1. In our initial assessment, a noticeable incidence of HT1 has been observed in Kohgiluyeh and Boyer-Ahmad province, southwest of Iran. To the best of our knowledge, no specific research has been performed to investigate the mutations underlying HT1 in this population. The identification of the mutation type is vitally important in cascade screening to prevent the incidence of this disease. Therefore, this study was carried out to investigate mutations underlying the FAH gene in Kohgiluyeh and Boyer Ahmad population. To this end, direct sequencing of exons and exon-intron boundaries of the FAH gene was applied to assess mutations underlying HT1 in patients living in Kohgiluyeh and Boyer Ahmad.

MATERIALS AND METHODS

Study population

This cross sectional study was conducted on nine patients from Kohgiluyeh and Boyer Ahmad Province, affiliated to Yasuj University of Medical Sciences, Iran, during 2017 to 2020 The study protocol was reviewed and approved by Ethics Committees of Yasuj University of Medical Sciences (Number: IR.YUMS.REC.1398.093).

Children were confirmed of HT1 based clinical signs were included in the study. The disease type of each patient's was determine. Because these patients have high succinyl acetone in the urine, the patient was confirmed by pre-medication urinary succinylacetone test followed by hepatic and renal failure. The result of biochemical findings (Tyr Levels (mg/dl) and AFP IU/ µL) was reordered for each patient.

DNA extraction and PCR

Three ml of blood samples were obtained from each patients and their parents. Genomic DNA was extracted using a commercial kit (Gene All Biotechnology Co. Ltd., Seoul, South Korea).

The primers for FAH gene, used for normalization, were designed based on the sequence contained in the NCBI Genome Database and using the Primer3 program, which was available online (Table 1). For the preparation of primers, they were lyophilized, and then prepared at a concentration of 50 pmol/ml.

The amplification of the target sequences was carried out in iCycler 5 thermal cycler (Bio-Rad, Hercules, CA, USA) using 25 µL reaction volumes containing 50 ng DNA, 1×PCR buffer, 2.5 mM MgSO4, 200 µM of each dNTP, 10 ρmol of each forward and reverse primer, and 5U Taq DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA). The thermal profile included 5 min initial

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denaturation at 95°C, followed by 35 cycles of 30s denaturation at 94°C, 30s annealing at 56-62°C and 2 min extension at 72°C.

Direct DNA sequencing was performed using forward and reverse primer of each exon by an automatic sequencer (ABI Prism 377, Perkin-Elmer) using the instructions of Big Dye Terminator v.3.1 Cycle Sequencing Core kit (PE Applied Biosystems, Foster City, CA, USA).

RESULTS

This study was conducted on a total of nine patients (5 boys and 4 girls) with HT1, the mean of age of diagnosis was 6.2 month. The results show that only one patient (11.2%) had non consanguineous parents and the other eight (88.8%) had related consanguineous parents. In one and seven cases, the parents were first and second cousins, respectively.

Among the patients, one (11.1%), two (22.2%), and six (66.6%) cases had chronic, acute, and sub acute forms of HT1, respectively. The clinical manifestation of this disease, including liver failure, emerged in the first month of the patient's life with the acute form (Table 2).

Patients with a sub acute form of HT1 were diagnosed between 6 and 12 months of life with such symptoms as acute hepatic insufficiency, sepsis, and hepatomegaly. The symptoms of patient with chronic form were hepatomegaly and rickets diagnosed after the sixteenth month of life.

Biochemical tests summarized in table 2. In all cases, the mean of plasma tyrosine was 8.85 mg/dl and the mean of Alpha Fetoprotein (AFP) was 685.11 IU/ µL. The results show that the plasma Tyrosine, Phenylalanine, and AFP level were high in all cases that confirmed the diagnosis of HT1.

The results of FAH gene and the direct DNA sequencing show that in all patients the G nucleotide was replaced with A nucleotide in DNA genome and lead to the Arg codon change to the stop codon in amino acid chain, therefore show the C > T homozygous in 709 position. This nonsense mutation alters 237th codon in 9th exon from arginine to stop codon (Figure 1).

Table 1: Sequencing of the primers of FAH gene.

Exon of FAH gene	PCR primer	Sequence 5´→3´	Product size (base pairs)
Exon 1	Forward	GGGGTGTTCACGGTGAGACCA	???
	Reverse	GAGCCCATAGGCACTCTCACG	
Exon 2	Forward	GAACATGGTTCTTCAGTCCGCT	???
	Reverse	CCTCTGAGACCTTCGATGGCA	
Exon 3 and 4	Forward	TGAGAGTTTGAGTTAGCGGGA	???
	Reverse	GAGCGGGTAACAGCCCCAGAG	
Exon 5	Forward	TGCATTTGGTGCTGCCATTGC	???
	Reverse	CACAGCAAGTCCTAAGCCAAGC	
Exon 6 and 7	Forward	TGCATTTGGTGCTGCCATTGC	???
	Reverse	CTGCCGATGTGGCTGAAGAGG	
Exon 8 and 9	Forward	AGTCCTGGTCCATGGCTGGAG	???
	Reverse	GGAACTCCCAGGCACTCTGCTG	
Exon 10	Forward	CTGCCTAGGGGAGCAGGT	???
	Reverse	CTGTAGCACGTGCCCTCAC	
Exon 11	Forward	CACAGACTCTAAGTGAAATCATGTTG	???
	Reverse	CACAGGCTGACCCATCATC	
Exon 12	Forward	AAGGCGGTCGTGAGCAGGGCAG	???
	Reverse	GTTGTGAGATACACCCACCTCG	
Exon 13	Forward	CAGTGATCCCACCAAGGCCGA	???
	Reverse	CAGATGGCTTCATGGCCAGGT	

PCR: Polymerase Chain Reaction

DISCUSSION

A mutation of R237X in FAH gene down regulates the gene expression and main role in HT1 pateints. In this study evaluation the nonsense mutation R237X in FAH gene as responsible for HT1 in the southwest of Iran. The results of study show that C. 709 C > T (*p*. 237 R > X) nonsense mutation of FAH gen was the main cause of HT1 in the all samples.

HT1 is a serious disease with harmful effects on patients and their family lives. The identification of HT1 carriers (i.e., heterozygotes) in cascade screening is considered crucially important to prevent the disease. However, the carriers cannot be diagnosed merely by biochemical tests. One way to detect such carriers is through a molecular genetic test that finds mutations in the FAH gene [1]. The first R237X mutation was reported in a Turkish proband with HT1 with homozygous R237X mutation [12]. The further 10 acute forms of HT1 patients recognized with homozygous R237X mutation in seven different families in Saudi Arabia [13]. More than 40 pathogenic mutations have been found in the FAH gene so far, which are found throughout parts of the gene but are most commonly found in exons 8 to 13 [14]. These mutations are categorized into three types

Table 2: Disease type and biochemical findings of the patients.

Patient No	Age of Diag. (mo)	Disease Type	symptoms	Tyr Levels (mg/dl)	AFP IU/mla	SA	outcome	Parents relationship
1	3	S-A	HM	8.8	566	+	Liver. Tx	FC
2	7	S-A	HM-Con	4	96	+	well	N
3	1	A	Diarrhea-HM	6	245	+	well	FC
4	7	S-A	HM	8.7	421	+	well	SC
5	1	A	Rickets	16	59	+	well	FC
6	16	CH	Diarrhea-HM	9.5	109	+	well	FC
7	8	S-A	HM	11	116	+	well	FC
8	4	S-A	HM-Con	5.5	318	+	well	FC
9	9	S-A	HM	10.2	4236	+	Liver .Tx	FC

Mo: Month; A: Acute; S-A: Sub-Acute; CH: Chronic; HM: Hepatomegaly; Tx: Transplantation

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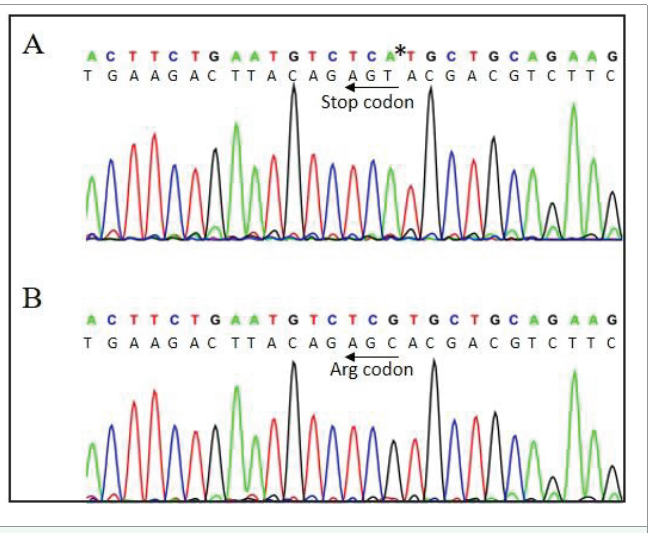

Figure 1: Sequence of the FAH gene in the mutation loci in HT1 patients (A) and healthy control group (B) [*Mutated nucleotide].

of missense, nonsense, and splice site mutations based on the age at which symptoms appear. Some of these mutations appear to be related to a specific racial group [15].

Specific FAH mutations are more common in some populations. For instance, c. 1062+5G > A (IVS 12+5 G >A) mutation is more common in French Canadians and Northern Europeans [16]. The previously demonstrated that the various IVS 12 þ 5G > A (c. 1062 þ 5G > A) in HT1 patients from a wide range of ethnic groups geographically distributed throughout the world [16]. The distribution of c. 1062+5G > A among different populations indicates that this mutation might be an ancient one [13].

Imtiaz et al. investigated FAH mutations in 43 HT1 patients in Saudi Arabia, Egypt, and Iran [13]. This variant was reported in six HT1 patients of Pakistani origin in the 1990s [17], and later in nine other HT1 patients of Pakistani descent in the UK [16]. Based on the results of their study, c. 718 C > T (*p*. Q > X) and c. 1062 + 5G > A homozygote mutations were found in patients. Moreover, IVS6-1G > T mutation has been found as a common mutation in the Mediterranean region [18]. The mutation of FAH gene has also been found in Iranian patients. Haghighi et al. studied the mutation of the FAH gene in a subacute case of HT1 which led to finding a compound heterozygote of c. 1062+5G > A and c. 1009 G > A (*p*. G337S). It was found out c. 1009 G > A was common in Finish HT1 patients [3]. Keshtkari, et al. reported that mutations among Iranian patients included c. 1009 G > A (*p*. G337S) and c. 718 C > T (*p*. Q > X) [19]. In this study, 9 patients carried the c. 709 C > T (*p*. 237 R > X) mutation in the homozygous state. This type of mutation has not been reported in HT1 Iranian patients before, although it has been reported in Turkish patients [12]. One possibility of this similarity of mutation between Iranian and Turkish patients might be related to their common ancestors. This hypothesis can be assessed by the evaluation of molecular markers such as Short Tandem Repeats (STRs) in the vicinity of this mutation. The same haplotype of STRs in this genomic region of patients confirms having common ancestors. However, considering the geographical distance between these two groups of patients, the possibility of inheriting this from a common ancestor seems extremely unlikely.

The HT1 is equally common in girls and boys. Each person has two genes that command the production of enzymes [20]. Neither of these two genes works properly in HT1 patients. These patients inherited each of the defective genes from one parent. In the parents of these children, a disorder is rarely observed. But each parent has a single defective gene for HT1. And there is a 25% chance of having a baby with HT1 in every pregnancy [21]. This possibility increases in consanguineous parents. The results of study show that the majority of patients were c. 709 C > T (*p*. 237 R > X) homozygous state had no close relationship; in one case parents were not even close relatives. According this results and other study the position of FAH gene mutation depended the specific racial and geographical distance of pentints and it can have its own genetic pattern based on race and region. This large body of evidence, along with the numerous patients, confirm the founder effect of this mutation in the Kohgiluyeh and Boyer Ahmad population. Since the c. 709 C > T mutation has led to one of three forms of HT1.

The results of this study are in line with those of other ones indicating the no relationship exists between phenotype and mutation type. Since genetic pattern of FAH gene mutation different based on race and region of people, suggested to identification of candidate genetic markers in each region, that would provide the families with an opportunity for carrier and prenatal testing, preimplantation genetic diagnosis. Therefore, by identifying couples carrying this disease, it is possible to prevent the birth of sick people by performing prenatal diagnosis or pre implantation diagnosis.

CONCLUSION

The tyrosinemia type 1 (HT1) is a serious genetic disease, which the early and precise recognition of HT1 will allow provision of timely and appropriate disease management strategies. The results of this study show that the c. 709 C > T (*p*. 237 R > X) mutation in the homozygous state as a causative mutation of HT1. It is recommended that 709 C > T (*p*. 237 R > X) be used in a cascade screening of patients relatives to prevent the development of the disease in the next generations.

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REFERENCES

1. de Laet C, Dionisi-Vici C, Leonard JV, McKiernan P, Mitchell G, Monti L, de Baulny HO, Pintos-Morell G, Spiekerkötter U. Recommendations for the management of tyrosinaemia type 1. Orphanet J Rare Dis. 2013 Jan 11;8:8. doi: 10.1186/1750-1172-8-8. PMID: 23311542; PMCID: PMC3558375.
2. Larochelle J. Discovery of Hereditary Tyrosinemia in Saguenay- Lac St-Jean. Adv Exp Med Biol. 2017;959:3-8. doi: 10.1007/978-3-319-55780-9_1. PMID: 28755180.
3. Haghighi-Kakhki H, Rezazadeh J, Ahmadi-Shadmehri A. Identification of a combined missense/splice-site mutation in FAH causing tyrosinemia type 1. J Pediatr Endocrinol Metab. 2014 Jul;27(7-8):795-8. doi: 10.1515/jpem-2013-0489. PMID: 24756054.
4. Baydakova GV, Ivanova TA, Mikhaylova SV, Saydaeva DK, Dzhudinova LL, Akhlakova AI, Gamzatova AI, Bychkov IO, Zakharova EY. The Unique Spectrum of Mutations in Patients with Hereditary Tyrosinemia Type 1 in Different Regions of the Russian Federation. JIMD Rep. 2019;45:89-93. doi: 10.1007/8904_2018_144. Epub 2018 Nov 11. PMID: 30414057; PMCID: PMC6336551.
5. van Ginkel WG, Rodenburg IL, Harding CO, Hollak CEM, Heiner-Fokkema MR, van Spronsen FJ. Long-Term Outcomes and Practical Considerations

DOI: https://dx.doi.org/10.37871/ajrddt.id24

in the Pharmacological Management of Tyrosinemia Type 1. Paediatr Drugs. 2019 Dec;21(6):413-426. doi: 10.1007/s40272-019-00364-4. PMID: 31667718; PMCID: PMC6885500.

6. Saudubray JM, Berghe G, Walter JH. Inborn metabolic diseases. Springer; 2018.

7. van Ginkel WG, van Reemst HE, Kienstra NS, Daly A, Rodenburg IL, MacDonald A, Burgerhof JGM, de Blaauw P, van de Krogt J, Santra S, Heiner-Fokkema MR, van Spronsen FJ. The Effect of Various Doses of Phenylalanine Supplementation on Blood Phenylalanine and Tyrosine Concentrations in Tyrosinemia Type 1 Patients. Nutrients. 2019 Nov 18;11(11):2816. doi: 10.3390/nu11112816. PMID: 31752110; PMCID: PMC6893509.

8. Morrow G, Angileri F, Tanguay RM. Molecular Aspects of the FAH Mutations Involved in HT1 Disease. Adv Exp Med Biol. 2017;959:25-48. doi: 10.1007/978-3-319-55780-9_3. PMID: 28755182.

9. Morrow G, Dreumont N, Bourrelle-Langlois M, Roy V, Tanguay RM. Presence of three mutations in the fumarylacetoacetate hydrolase gene in a patient with atypical symptoms of hereditary tyrosinemia type I. Mol Genet Metab. 2019 May;127(1):58-63. doi: 10.1016/j.ymgme.2019.01.019. Epub 2019 Feb 7. PMID: 30954369.

10. Scalet D, Sacchetto C, Bernardi F, Pinotti M, van de Graaf SFJ, Balestra D. The somatic FAH C.1061C>A change counteracts the frequent FAH c.1062+5G>A mutation and permits U1snRNA-based splicing correction. J Hum Genet. 2018 May;63(5):683-686. doi: 10.1038/s10038-018-0427-x. Epub 2018 Mar 1. PMID: 29497141; PMCID: PMC5919117.

11. Dweikat I, Qawasmi N, Najeeb A, Radwan M. Phenotype, genotype, and outcome of 25 Palestinian patients with hereditary tyrosinemia type 1. Metabol Open. 2021 Jan 28;9:100083. doi: 10.1016/j.metop.2021.100083. PMID: 33598652; PMCID: PMC7868710.

12. Ploos van Amstel JK, Bergman AJ, van Beurden EA, Roijers JF, Peelen T, van den Berg IE, Poll-The BT, Kvittingen EA, Berger R. 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. 1996 Jan;97(1):51-9. doi: 10.1007/BF00218833. PMID: 8557261.

13. 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. Identification of mutations causing hereditary tyrosinemia type I in patients of Middle Eastern origin. Mol Genet Metab. 2011 Dec;104(4):688-90. doi: 10.1016/j.ymgme.2011.06.019. Epub 2011 Jun 30. PMID: 21764616.

14. Maksimova NR, Gurinova EE, Sukhomyasova AL, Danilova AL, Kaimonov VS, Savvina MT, Yakovleva AE, Alekseeva EI. A novel homozygous mutation causing hereditary tyrosinemia type I in yakut patient in russia: case report. Wiad Lek. 2016;69(2 Pt 2):295-8. PMID: 27487552.

15. Giguère Y, Berthier MT. Newborn Screening for Hereditary Tyrosinemia Type I in Québec: Update. Adv Exp Med Biol. 2017;959:139-146. doi: 10.1007/978-3-319-55780-9_13. PMID: 28755192.

16. Angileri F, Bergeron A, Morrow G, Lettre F, Gray G, Hutchin T, Ball S, Tanguay RM. Geographical and Ethnic Distribution of Mutations of the Fumarylacetoacetate Hydrolase Gene in Hereditary Tyrosinemia Type 1. JIMD Rep. 2015;19:43-58. doi: 10.1007/8904_2014_363. Epub 2015 Feb 15. PMID: 25681080; PMCID: PMC4501228.

17. Rootwelt H, Høie K, Berger R, Kvittingen EA. Fumarylacetoacetase mutations in tyrosinaemia type I. Hum Mutat. 1996;7(3):239-43. doi: 10.1002/(SICI)1098-1004(1996)7:3<239::AID-HUMU8>3.0.CO;2-5. PMID: 8829657.

18. Dursun A, Ozgül RK, Sivri S, Tokatlı A, Güzel A, Mesci L, Kılıç M, Aliefendioglu D, Ozçay F, Gündüz M, Coşkun T. Mutation spectrum of fumarylacetoacetase gene and clinical aspects of tyrosinemia type I disease. JIMD Rep. 2011;1:17-21. doi: 10.1007/8904_2011_10. Epub 2011 Jun 22. PMID: 23430822; PMCID: PMC3509809.

19. Keshtkari A, Samanpour S, Hosseini S, Hassanzadeh S. Design of Polymerase Chain Reaction Initiators and Their Function to Identify FAH Mutations in Type I Tyrosinemia. Armaghane danesh. 2019;24(5): 820-829.

20. Schultz MJ, Netzel BC, Singh RH, Pino GB, Gavrilov DK, Oglesbee D, Raymond KM, Rinaldo P, Tortorelli S, Smith WE, Matern D. Laboratory monitoring of patients with hereditary tyrosinemia type I. Mol Genet Metab. 2020 Aug;130(4):247-254. doi: 10.1016/j.ymgme.2020.06.001. Epub 2020 Jun 6. PMID: 32546364.

21. Yasir Zahoor M, Cheema HA, Ijaz S, Fayyaz Z. Genetic Analysis of Tyrosinemia Type 1 and Fructose-1, 6 Bisphosphatase Deficiency Affected in Pakistani Cohorts. Fetal Pediatr Pathol. 2020 Oct;39(5):430-440. doi: 10.1080/15513815.2019.1672224. Epub 2019 Oct 4. PMID: 31584309.

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