Aminotrasferase (TAT) Gene Mutations Among Palestinian Tyrosinemia Type II Patients: An extended study

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Abstract

Tyrosinemia type II, known as Richner-Hanhart syndrome (RHS), is a rare autosomal recessive disorder caused by mutations in the tyrosine aminotransferase (TAT) gene characterized by painful palmoplantar hyperkeratosis, pseudo dendritic keratitis and variable mental retardation. Several various mutations have been reported so far in the gene. Although many clinical complications of patients from the Middle East were described, the molecular basis of the disease was limited to some Tunisian and Palestinian patients. Direct molecular analysis represents the optimum approach to identify new patients or carriers of mutations in prenatal diagnosis since TAT is not expressed in chorionic villi or amniocytes. In the present study, an expanded molecular analysis of mutations in the TAT gene among new seven Palestinian tyrosinemia type II patients from six unrelated families is described. After sequencing the entire 12 exons and exon-intron boundaries of the gene, two mutations could be identified: a nonsense mutation, p. R417X, in two patients and a splicing mutation, p.T408T, among the other five patients. Six polymorphisms in the gene were also detected;three previously described including IVS11+143a>g, IVS8+113t>c, and p.S103S and three additional ones including g→t @-17, IVS7+84c>g, and IVS9-73g>t are described here. The p.T408T splicing mutation seems specific to the Palestinian RHS families since this nucleotide transversion was not reported in patients from other populations. Mutation analysis in tyrosinemia is very beneficial to identify carriers among high risk groups and communities for premarital genetic counseling.

Keywords: *TAT, Tyrosine Aminotransferase, Tyrosinemia type II, Richner-Hanhart syndrome.*

Paper take home message. This investigation represents an expanded molecular genetics analysis on Tyrosine aminotransferase (TAT) gene in new identified Palestinian tyrosinemia type II patients. The results indicate some mutations are specific to the Palestinian population with newly identified polymorphisms in the gene.

Electronic database reference: Gene Bank (http://www.ncbi.nlm.nih.gov/BLAST).

Two sequence BLAST search program was used with the accession number gi37541544 assigned for the human TAT gene.

List of abbreviations: TAT: Tyrosineaminotransferase, **RHS**: Richner-Hanhart syndrome, **Kbp**: kilo base-pairs, **IVS**: Intervening sequence

Introduction

Richner – Hanhart syndrome (RHS) or type II oculocutaneous tyrosinemia is a rare metabolic disorder that results from autosomal recessive mutations in the tyrosine aminotransferase (TAT) gene (Rettenmeier et al., 1990) located on chromosome 16q22 (Barton et al., 1986 and Natt et al., 1986). It is a hereditary inborn error of tyrosine degradation pathway, that may be expressed in association with other clinical abnormalities including hyperthyroidism and liver failure of any cause (Mitchell et al., 2001, Kimura et al., 1998). Tyrosine aminotransferase or L-tyrosine: 2-Oxoglutarate amino transferase (EC 2.6.1.5) is a hepatic, cytosolic, pyridoxal phosphate – dependent enzyme that catalyzes the reversible transamination of tyrosine to *p*-hydroxyphenylpyruvate, an important step in tyrosine metabolism (Rettenmeier et al., 1990 and Sivaraman and Kirch, 2006). The purified enzyme is a dimer with a molecular mass of 95,000 daltons (Natt et al., 1986). It is composed of 454 amino acids encoded by a 3.0 kb mRNA (Rettenmeier et al., 1990). The human TAT gene was shown to be induced by glucocorticoids and cyclic AMP in organ cultures (Raiha and Schwartz, 1973). Tyrosinemia type II manifests as painful, palmoplantar hyperkeratosis and/or bilateral pseudodendritic keratitis associated with elevated plasma tyrosine concentrations (frequently more than 10-fold) (Buist et al., 1985, Macsai et al., 2001, Mitchell et al., 2001). Furthermore, approximately 50% of patients suffer from neurological complications including fine co-ordination and language deficits, microcephaly, self-mutilation and severe developmental delay (McDonald et al., 2004). Prenatal diagnosis of the disorder by biochemical assays is not possible, as tyrosine and its degradation products do not accumulate in amniotic fluid and tyrosine aminotransferase is not expressed in chorionic villi, amniocytes or fibroblasts (Maydan et al*.,* 2006). Tyrosine and phenylalanine restricted diet is effective to heal skin lesions and prevent long term ocular damage (corneal scarring and glaucoma) (Machino et al., 1983, Ney et al., 1983). However, its influence on neurological deficit is unclear, partly because of the great variability in neurological dysfunction among untreated patients (Paige et al., 1992). Prevention can be achieved by carrier detection and genetic counseling since the parents of an affected child are obligate heterozygotes (asymptomatic carriers) (Maydan et al., 2006).

A variety of mutations have been reported in the TAT gene thus far including two major deletions (Natt et al., 1987, Legarda et al., 2011) : a dinucleotide deletion, single base insertion leading to frame shift and premature termination, nine missense and three nonsense point mutations, and three splicing mutations (Natt et al., 1992, Huhn et al., 1998, Maydan et

al., 2006, Charfeddine et al., 2006, Meissner et al., 2008, Culic et al., 2011). Recently, two new mutations have been reported in a Danish family including an insertion mutation in exon 4 resulting in premature stop codon due to a frame shift and a missence mutation in exon 5 (Pasternmack et al., 2009) and G duplication mutation (c.869dupG, p.Trp291Leufs 6) in a Tunisian family (Bouyacoub et al., 2013). In addition, four polymorphisms were also reported in the gene (Huhn et al., 1998, Maydan et al., 2006). No clear correlation could be established between variations in the clinical phenotype and any particular gene mutation (genotype) among tyrosinemia type II patients (Huhn et al., 1998). In the present investigation, we investigated the type of mutations in the TAT gene among seven new Palestinian tyrosinemia patients from six unrelated families. The results confirm previously identified mutations in the gene with emphasis on population specific mutations and new polymorphisms are also implicated within the gene.

Methods

Patients

The genetic analysis of the tyrosine aminotransferase gene was carried out for seven patients from six unrelated Palestinian families, who were diagnosed to have tyrosinemia type II syndrome based on their blood tyrosine level and clinical features. The parents of all patients were first or second cousins. The TAT gene of an eighth child with normal tyrosine and phenylalanine levels was also subjected to molecular analysis as control. The complete description and related information about the RHS patients are summarized in Table 1.

Table 1. Demographic and clinical features and plasma tyrosine levels of Richner –

Patients		**Related information about the patients at the age of diagnosis**					***Related information about the patients at the time of performing this study***				
Symbol	**Gender**	**Age**	**Ocular involvement**	**Cutaneous**	***Developmental**	**Plasma tyrosine before treatment**	**Age**	**Ocular involvement**	**Cutaneous**	**Developmental**	***Plasma tyrosine before treatment***
TYR1	**F**	3 m	-	-	-	757	9m	-	-	-	*761*
TYR2A	**M**	5y	+	+	+	1261	14y	?	+	+	*ND*
TYR2B	**M**	2y	+	+	+	ND	7y	?	+	+	*ND*
TYR3	**M**	2y	+	+	+	2015	7y	-	-	-	*1150*

TYR4	**M**	14 m	+	+	-	1109	3y	-	-	-	*1117*
TYR5	**M**	8 m	+	-	-	1825	4y	-	-	-	*1044*
TYR6	***M***	*7 m*	+	-	-	*1060*	*5y*	-	-	-	*952*

Hanhart Syndrome patients.

Note: Normal plasma tyrosine levels according to age are 40-110 µmol/L (0-6 months), 40-75 µmol/L (7 months-10 years), 45-90 µmol/L (11-17 years), 20-110 µmol/L (adults).

+: present, -:absent, ?:uncertain, ND :not done, y: years, m: months, F: female, M: male.

* Development was assessed based on height, weight of these children in comparison to average normal values for children of matched ages.

PCR amplification

Genomic DNA of patients and their parents was isolated from whole EDTA venous blood samples using Master Pure^{TM} Genomic DNA Purification Kit (Epicenter Technologies, USA). All 12 TAT exons plus flanking intron sequences individually or in groups of two or three were amplified using commercially-synthesized oligonucleotide primers (invitrogen) previously reported by Huhn et al., (1998) except for the Ii-5-1 primer with the sequence TAGACACCATCACTTTCCAAG that was designed in this study (Integrated DNA Technologies, Inc. *IDT*®). This primer is complementary to the sequence starting at -94 nucleotides in the 5' flanking region of exon 1 and was used as for direct sequencing of exon 1 and flanking regions using the H+I+J purified PCR product as DNA template. All PCR amplifications were carried out using thermo cycler Gene Amp® PCR System 9700 in a total volume of 50µl containing 10X PCR buffer (TaKaRa) , 0.2 mM dNTP mixture (TaKaRa), 150 ng each primer (invitrogen), 1U T*aq*™ DNA polymerase (TaKaRa) (5U/µl), and 400 ng genomic DNA (0.2µg/µl). PCR amplification was carried out as described by Huhn et al., 1998 except the number of cycles were increased from 30 to 35 for the amplification of exons 1,2,3,8+9+10, and exons 11+12, and to 40 cycles for the amplification of exons 4,5+6,7 and 8+9.

DNA Sequence Analysis

Agarose gel electrophoresis was performed to verify the length and purity of the amplified DNA fragments and purified using the Wizard® SVGel and PCR Clean-Up System

(Promega). Purified PCR products were then subjected to direct sequencing using an automated sequencer (ABI PRISM^{TM} Model 310 Version 3.7).

BLAST Analysis

BLAST search at NCBI and Alignment, which is presentation of two compared sequences that show regions of greatest statistical similarity, was applied to identify homologies of DNA sequence of the purified PCR products with HomoSapiens TAT gene sequence stored in Gene Bank (http://www.ncbi.nlm.nih.gov/BLAST). Two sequence BLAST search program was used with the accession number NM_000353.2 (GI:169808381) assigned for the human TAT gene.

Restriction Fragment Length Polymorphism (RFLP) Analysis

Endonuclease restriction enzymes analysis was performed as previously reported by Maydan et al., 2006 to confirm the presence and segregation of the mutations that were identified by DNA sequencing. AlwN1 restriction enzyme (BioLabs Inc.) was used in a 15 µl reaction mixture containing 10 µl PCR product and 5 U AlwNI restriction enzyme (Biolabs Inc,). The mixture was incubated at 37°C for 1 hour and the digestion products were resolved on 2% agarose gel. Similar digestion was done using the DdeI restriction enzyme (Promega Corporation, USA).

Results

Direct DNA Sequencing and BLAST analysis

The amplified 12 exons and exon / intron boundaries of the TAT gene for all the indicated 7 patients and control subject were subjected to direct DNA sequence and BLAST analysis to identify mutations and/or other variations in the nucleotide sequence. The results showed complete match and normal sequence of exon 1 and flanking regions for all patients except for TYR2A and TYR2B, who are homozygous for a g→t polymorphism located at nucleotide -17 in the 5' flanking region of exon 1. A comparison of part of the DNA sequence in the region, where this polymorphism was identified, (Fig 1-A) shows that the two brothers from family 2, who have normal sequence of exon 1, are homozygous for this polymorphism, which was not previously reported. Sequence and BLAST results for exon 2 and flanking regions (data not shown) showed that all patients have normal sequence in this part of the gene; five patients have a normal sequence of exon 3 and the flanking regions, while TYR2A and TYR2B from family 2 were found to be homozygous for a silent mutation changing TCG

to a TCA codon, both encoding for serine at position S103S (Fig 1-B). This substitution represents a polymorphism in the gene. Furthermore, sequence and BLAST analysis results demonstrated that all patients have normal sequence of exons 4, 5, 6, and their flanking regions (data not shown). However, all seven patients have a c→g homozygous transversion in intron 7, located 84 nucleotides downstream from the 3' end of exon 7 (IVS7 +84c>g) in the TAT gene (data not shown). This polymorphism was also detected in the control subject included in our investigation. Four patients, TYR1, TYR3, TYR4, and TYR6 were found to be homozygous for a known polymorphism in intron 8, IVS8 +113t>c (Fig 1-C). The sequencing results of exon 10 and its intron boundaries revealed a C to A mismatch in intron 9 located 73 nucleotides upstream from the 5' end of the exon in all patients (IVS9-73g>t). The included control subject was heterozygous for this polymorphism (Fig 1-D). The BLAST analysis of exons 11+12 revealed a homozygous G to T substitution in the last nucleotide of the last codon of exon 11 for patients TYR1, TYR3, TYR4, TYR5, and TYR6 (Fig 1-E). This is a silent substitution changing codon 408 from ACG to ACT, both encode for threonine (T408T). The BLAST results showed that all parents were heterozygous for the mutation and the polymorphism, except for the mother of TYR6, who was homozygous for the polymorphism and showed no clinical signs of the disease. All patients were homozygous for a polymorphism in intron 11 IVS11+143a>g (Fig 1-F). Furthermore, The BLAST results showed that patients TYR2A and TYR2B were homozygous for a C to T substitution in exon 12 (Fig 1-G). Table 2 summarizes the list of mutations and polymorphisms identified in the TAT gene among the seven patients in the present study.

Restriction Analysis

Restriction analysis with AlwNI and DdeI enzymes was used in our investigation to confirm the presence and segregation of exon 11 mis-splicing (G→T) and exon 12 nonsense (C→T) mutations respectively. The mis-splicing mutation creates an additional AlwNI restriction site in exon 11, while the nonsense mutation creates an additional DdeI restriction site in exon 12. The restriction analysis revealed that the affected patients were homozygous, while their parents were heterozygous for these mutations (data not shown).

Discussion

Seven patients from six non-related Palestinian families were diagnosed with tyrosinemia type II. The TAT gene sequence, except for exon 1, for all patients were analyzed to reveal the molecular basis of the disease among these patients. No clear picture is evident

from previous studies in respect to genotype/phenotype correlations. Although many clinical descriptions of tyrosinemia type II patients from the Middle East were reported, the molecular basis of the disease is limited only to Tunisian (Charfeddine et al., 2006) and some Palestinian patients (Maydan et al., 2006). This investigation represents an expended molecular analysis of tyrosinemia type II among Palestinian patients.

Previously reported mutations are not unique to a single family including the p. T408T, and the p. R57X nonsense mutation that was detected in one Scottish and three Italian families (Huhn et al., 1998, Maydan et al., 2006). Interestingly, the homozygous p.R417X nonsense mutation identified in our study was previously described in a French tyrosinemia type II patient who had severe mental retardation and no apparent TAT activity in a liver biopsy (Lemonnier et al., 1979). This patient was found to be compound heterozygous for the p. R417X mutation and a splice mutation IVS8 +2t>g (Natt et al., 1992). Functional transfection of the chimeric TAT genes constructed from normal and mutant alleles carrying these mutations showed that each mutation was sufficient for incomplete inactivation of the gene. The mutation in codon 417 indicates the carboxy terminal 38 amino acid residues are essential for the TAT activity (Hargrove et al., 1989). This finding is different from the amino terminus end of the enzyme where the first 64 amino acid residues can be removed from the protein without apparent loss in the enzyme activity (Rettenmeier et al., 1990). Evidently, the amino-terminal 64 residues in the human and rat TAT protein differ at 18 positions, while the carboxy terminal 38 residues are identical and conserved between the two species and required for the enzyme activity (Rettenmeier et al., 1990).

The homozygous p.R417X nonsense mutation was described before in two Palestinian brothers (Maydan et al., 2006); the oldest brother suffered from painful bilateral palmoplantar hyperkeratosis for 6 years before he was diagnosed with tyrosinemia II, while the younger brother was asymptomatic at the time of diagnosis but subsequently developed skin manifestations. This mutation occurs at a CG dinucleotide, a well-recognized mutational hotspot which results in a stop codon. In our study, the p. R417X nonsense mutation was identified in two brothers (TYR2A and TYR2B) who were homozygous ,while both their parents were carriers of the mutation. The p.T408T splicing mutation was previously described in two unrelated Palestinian families (Maydan et al., 2006). In the present study, this mutation was detected in 5 additional patients (TYR1, TYR3, TYR4, TYR5, and TYR6) from different unrelated families.

The consensus sequence at the 5' donor splice site is [C/A]AG**gt**[a/g]agt (exon and intron nucleotides are represented in capital and lowercase, respectively) (Shapiro and Senapathy, 1987). Mutations in the almost invariant **g** (IVS+1) and **t** (IVS+2) nucleotides at the beginning of the intron are very likely causing mis-splicing. The most frequent nucleotide found at the end of IVS-1position is a G. Replacement of this guanine at the last base of the preceding exon is predicted to significantly reduce base pairing stability between the splice site and the complementary region of the U1 smaller nuclear RNA. However, the G at this position is not invariant, being found in 78% of the wild-type donor splice sites, while a T is found in 8% of cases (Lerner et al.,1980, Rogers and Wall, 1980, Zhuang and Weiner, 1986). The majority of mutations listed in the Human Gene Mutation Database affecting a G at the donor site of IVS-1 position are G to A (Stenson et al., 2003). However, there are 47 recorded instances (as of May 2005) of a G to T substitution at this position. The most likely consequence of such a mutation is skipping of that same exon. For example, the c.662G>T mutation of the last nucleotide of exon 8 in ATM (ataxia telangectasis) gene results in exon 8 skipping and a frame shift with premature termination (Laake et al., 2000). Alternatively, activation of an illegitimate splice site may also occur, particularly if this cryptic site is located in the direct vicinity and bears sufficient resemblance to the consensus sequence. For example, the c.1701G>T transversion, the last nucleotide in exon 10 of factor V gene, leads to activation of an upstream cryptic splice site in exon 10 and deletion of the last 35 bases of exon 10, resulting in frame-shift and premature termination (Schrijver et al., 2002). Some G to T mutations affecting the last nucleotide of an exon produce a change in the amino acid codon and might be misclassified as simple missense mutations if RNA studies are not performed. For example, the c.995G>T mutation at the last base of exon 5 in the SPG4 gene causing autosomal dominant spastic paraplegia appears to produce an amino acid substitution K290N but more seriously results in complete exon 5 skipping (Svenson et al., 2001).

A conclusive evidence that p.T408T mutation results in missplicig was confirmed using a mini-gene splicing functional assay, which was the most direct approach to confirm missplicing and directly confirm the drastic effect of this mutation to express the disease (Maydan et al., 2006). However, since the expression of the human TAT is strictly limited to hepatocytes cytoplasm (Hargrove and Mackin, 1984), this would necessitate obtaining a liver biopsy from patients with this mutation for enzyme activity evaluation. Skipping of exon 11 of the TAT gene leads to an in-frame deletion of 99 nucleotides (33 amino acids). The mRNA stability should therefore not be affected by the nonsense-mediated decay mechanism. Two

other TAT gene splicing mutations were previously reported: the first; an A to G transversion in the intron that creates a new splice acceptor site near the 5' wild-type splice site of intron 2 and preferentially used, leading to frame-shift and premature termination (Natt et al., 1992); the second, IVS+2t>g, changes the invariant gt splice donor sequence to gg, however, missplicing was not experimentally confirmed (Natt et al., 1992).

Six polymorphisms were identified within the TAT gene in our study. They include IVS11+143a>g, IVS8+113t>c, S103S (TCG→TCA), IVS7+84c>g, IVS9-73g>t, and a g→t polymorphism at position -17 in the promoter region. Three of these polymorphisms were previously identified and the polymorphism in intron 11, IVS11+143a>g (dbSNP:1799881) that was was detected in 5 patients here (homozygous for this polymorphism) were previously reported in Palestinian patients (Maydan et al., 2006) and therefore might be a "founder mutation. Another polymorphism in intron 8, IVS8+113t>c (dbSNP: 2303226) was also detected in four of our patients , who were homozygous for this polymorphism. This polymorphism was similarly identified before in two unrelated Palestinian patients (Maydan et al., 2006). The silent homozygous substitution S103S (TCG→TCA) detected in two patients here was previously reported in a patient from USA (Huhn et al., 1998).

Three additional polymorphisms were described in our study and were not previously reported. These include a homozygous g→t polymorphism in the promoter region of the TAT gene at position -17 identified in patients TYR2A and TYR2B. Interestingly, deletion of a CT dinucleotide at positions -8 and -7 in the TAT gene promoter region was previously described by Natt et al., 1992 who showed that this deletion mutation had no severe consequences on enzyme activity, however, since the site of this nucleotide transversion is critical, further investigation should be done to evaluate the effect of this transversion on the rate of TAT gene transcription. The second polymorphism, IVS7+84c>g, was detected in all RHS patients in our study in addition to the control gene sequence. All subjects were found to be homozygous for this C→G transversion. A third polymorphism, IVS9-73g>t, was similarly detected in all RHS patients who were found to be homozygous for the transversion, while the control subject was found to be heterozygous for this polymorphism.

The G→T splicing mutation, as well as the newly identified polymorphisms appear to be specific to the Palestinian RHS families as none of these nucleotide transversions were reported in other populations. These results provide a leading approach when screening for mutations in all suspected RHS patients. The identification of different mutations in the TAT gene among Palestinian tyrosinemia type II patients indicates that this disorder is genetically

heterogeneous in the population rather than having a pan-ethnic molecular basis. The goal of early screening and diagnoses provides strong bases for early intervention to avoid the development of the severe clinical complications of the disease. Furthermore, mutation screening of potential carriers within inflicted families is important for premarital genetic counseling especially, where consanguineous marriage is a wide spread common practice. Moreover, molecular genetic testing is the only option for prenatal diagnosis using chorionic villus sampling (CVS) at about 10-12 weeks of gestation. If the fetus proves positive for the tyrosinemia type II syndrome, the parents should be aware of the clinical progression of the disease and the rewarding clinical response and prevention of complications by use of dietary therapy very early to avoid further clinical consequences. Families with individuals who are carriers of type II tyrosinemia mutations should be provided with adequate information on the nature, inheritance, and implications of the disease to help them make informed personal decisions concerning their choice of partners. These practices will eliminate or greatly reduce the risk of having affected children.

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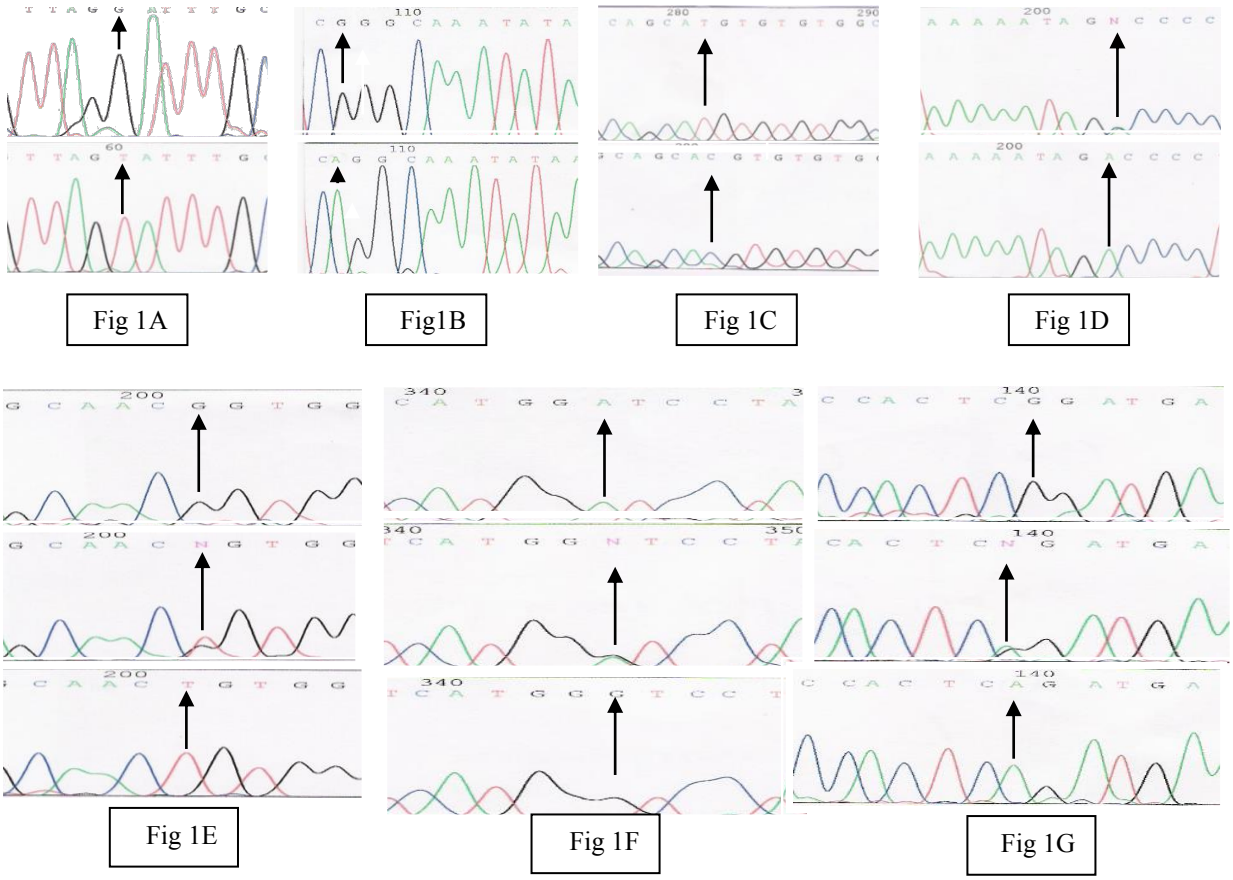

Figure 1. TAT mutations and polymorphisms identified in RHS patients. DNA sequence analysis in different regions of the TAT gene for the -17 G→T, S103S, IVS+113t>c, IVS-73g>t (c>a on the noncoding strand) polymorphisms, T408T splicing mutation, IVS+143a>g polymorphism, and R417X nonsense mutation (A-G) respectively. The regions representing the normal sequences are shown in the upper lanes (A-G). The same regions are shown in the middle lane for a carrier individual (E-G), and in the lower lane for a homozygous patient (A-G). Vertical arrows indicate the positions of nucleotide substitution

Table 2. Summary of mutations and polymorphisms identified in the TAT gene among patients in the present study. All patients are homozygous for the indicated mutations.

Pt. / Ef.	TYR1	TYR2A and TYR2B	TYR3	TYR4	TYR5	TYR6	Cont
A	WT	g→t @-17 polymor-phhism	WT	WT	WT	WT	WT
B	WT	WT	WT	WT	WT	WT	WT
C	WT	G→A (S103S) Polymor-phism	WT	WT	WT	WT	WT
D	WT	WT	WT	WT	WT	WT	WT
E+F	WT	WT	WT	WT	WT	WT	WT
G	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism	IVS7 +84c>g Polymor-phism
H	IVS8 +113t>c Polymor-phism	WT	IVS8 +113t>c polymor-phism	IVS8 +113t>c Polymor-phism	WT	IVS8 +113t>c Polymor-phism	WT
I	ND	ND	ND	ND	ND	ND	WT
J	IVS9 -37g>t Polymor-phism	IVS9 -37g>t Polymorphism	IVS9 -37g>t Polymor-phism	IVS9 -37g>t Polymor-phism	IVS9 -37g>t Polymor-phism	IVS9 -37g>t Polymor-phism	IVS9 -37g>t Polymorphism
K	G→T(T408T) Splicing mutation IVS11 +143a>g Polymor-phism	WT	G→T(T408T) Splicing mutation IVS11 +143a>g Polymor-phism	G→T(T408T) Splicing mutation IVS11 +143a>g Polymor-phism	G→T(T408T) Splicing mutation IVS11 +143a>g Polymor-phism	G→T(T408T) Splicing mutation IVS11 +143a>g Polymor-phism	WT
L	WT	C→T (R417X) Nonsense mutation	WT	WT	WT	WT	WT

Note: WT: wild type, ND: not done, Pt: Patient, Ef: amplified exon and flanking region.

الطفرات الجينية في مورثة انزيم أمينوتر أسفيريز لدى المرضى الفلسطينيين المصابين بمرض خلل في ايض الحمض الاميني تيروسين من النوع الثاني : دراسة ممتدة

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الملخص

*مرض أيض الحامض الأميني تيروسين من النوع الثاني والمعروف أيضاً بمتلازمة رشنر- ردنهارت من النوع النادر المتنحي وينشأ بسبب وجود طفرات في مورثة أنزيم تيروسين أمينوترانس فيريز. يعاني المريض بهذا المرض من آلام ناتجة عن خشونة في الجلد مع بياض في قزحية العين إضافة الى درجة متفاوتة من التخلف العقلي. تم سابقاً تحديد بعض الطفرات في هذه المورثة في عدد محدود من المرضى في تونس وفلسطين. ويشكل الفحص الجيني الطريقة الأمثل للتشخيص الدقيق بهذا المرض وتحديد حاملي الطفرات في هذه المورثة وتشخيص الجنين المصاب في الأسابيع الأولى من الحمل عن طريق أخذ عينة من الخلايا. في هذه الدراسة تم إجراء فحص جيني موسع للمورثة المذكورة لستة مرضى فلسطينيين من عائلات مختلفة تم تشخيصهم بهذا المرض للتعرف إلى الطفرات الوراثية التي يعانون منها بمساعدة عائلاتهم مستقبلاً. وقد تم إجراء مسح كامل لأجزاء المورثة المذكور كافة للتعرف إلى تسلسل النيوكليدات، وتم تحديد طفرة أولى تؤدي إلى اختلال في عملية نسخ البروتين **(R417X)** وطفرة ثانية تؤدي إلى خلل أساسي في عملية نضوج الحامض النووي الرسول **(p.T478T)** . بالإضافة إلى ذلك تم تحديد ستة نيوكليدات في هذه المورثة، تشكل تنوعاً طبيعياً في تسلسل النيوكليدات، فيها ثلاثة منهم تم تحديدهم سابقاً وثلاثة آخرون تم تحديدهم للمرة الأولى في هذه المورثة. وتشكل الطفرة **p.T478T** خاصة للمرض في فلسطين،*

حيث لم يتم الكشف عنها في أيٍ من المرضى في أماكن أخرى من العالم. وتُعد تحليل الطفرات في هذه المورثة عاملاً مساعداً مهماً لتحديد حاملي هذه الطفرات في عائلات المرضى لتوجيه النصح والإرشاد الطبي الجيني قبل الزواج لمنع ولادة أطفال مصابين بهذا المرض.

الكلمات الدالة: *مرض تيروسينيميا النوع الثاني، مورثة تيروسين أمينوتر أسفيريز, متلازمة رشنر-هاينهارت.*