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In silico analysis and the pathogenicity classification of *PTS* gene variants among Iranian population

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Abstract

Background: 6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency is an autosomal recessive disorder caused by *PTS* gene mutations. The aim of this study was to collect all *PTS* gene variants detected among Iranian patients with PTPS deficiency as well as in the Iranome project and classify them based on American College of Medical Genetics and Genomics (ACMG-AMP) guidelines.

Results: The number of *PTS* gene variants reported among Iranian PTPS patients and in the Iranome project were 19 and 36, respectively. Given that one variant was reported in both of our sources, the total number of variants was 54. These variants were classified as pathogenic ($n=11$), likely pathogenic ($n=7$), VUS ($n=23$), likely benign ($n=1$), and benign ($n=12$). Out of 19 variants reported among Iranian PTPS patients, c.155A>G (p.Asn52Ser, rs104894275) and c.317C>T (p.Thr106Met, rs200712908) were the most frequent ones, each with a frequency of 10%. c.84-3C>G (rs1230781262) (7.5%) and c.281A>T (p.Asp94Val) (5%) were in the next ranks of the list of variants.

Conclusions: The ACMG-AMP criteria need to be updated depending on the type of disease. In addition, to the best of our knowledge, no template has been described for classifying the variants identified in PTPS deficiency. Therefore, this study can be a good reference for future studies in this subject.

Keywords: PTPS deficiency, Variant interpretation, *PTS* gene, ACMG-AMP guidelines, Iran

Introduction

Phenylalanine hydroxylase (PAH) deficiency, with an autosomal recessive inheritance, is known to be the main cause of hyperphenylalaninemia (HPA). On the other hand, 6R-L-erythro-5,6,7,8-tetrahydrobiopterin (BH_4) is an essential cofactor for phenylalanine, tyrosine and tryptophan hydroxylases. There are two pathways for generating this cofactor, including de novo synthesis and recycling, in which different enzymes are involved in each pathway [1].

Patients with BH_4 deficiency may develop nervous system symptoms, including intellectual disability. It has been estimated that the frequency of BH_4 deficiency is about 1 to 2% of all patients with HPA [1, 2]. 6-Pyruvoyl-tetrahydropterin synthase (PTPS) deficiency (MIM #261640) is a main cause of BH_4 deficient HPA; out of 1161 cases with BH_4 disorders registered in BIODEF database (last update: September 13, 2019) (available at http://www.biopku.org), 735 cases had PTPS deficiency (63.3%). HPA, higher levels of neopterin and lower levels of biopterin are among the biochemical characteristics of patients with PTPS deficiency [1, 3]. As an autosomal recessive disorder, PTPS deficiency is caused by *PTS* gene mutations. This gene has been mapped on chromosome 11 (11q23.1) and comprises six exons [1]. According to PND database (available at http://www.biopku.org), 198

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variants have been recorded in this gene, most of them are missense variants.

Although the exact frequency of BH_4 deficiency among HPA patients from Iran is unknown, it seems that it has a higher frequency than the global rate. For example, based on a study performed in western Iran, its frequency was calculated to be 6.25% [4]. In another study performed on HPA patients from all over of the country, this rate was estimated to be 12.3% [5]. In addition, out of 123 patients with BH_4 deficiency, 60 cases had been reported with PTPS deficiency (48.8%) [5, 6].

The Iranome project (http://www.iranome.ir/) is a valuable recent project implemented in Iran. In the Iranome project, the genetics of 800 healthy Iranians with different ethnicities have been analyzed using the whole exome sequencing technique. The aim of this study was to collect all *PTS* gene variants detected among Iranian patients with PTPS deficiency as well as in the Iranome project, predict their effects using in silico predictive tools, and classify them based on American College of Medical Genetics and Genomics (ACMG-AMP) guidelines.

Methods

Our strategy in this study is as follows step by step.

Systematic collection of *PTS* gene variants

Two different sources including the Iranome project and scientific reports were used to extract all reported *PTS* gene variants in Iran.

Verification of variants

The GenBank entry NM_000317.3 and UniProtKB/SwissProt Q03393 were used to determine the genetic and protein variant positions, respectively. The unique properties associated with each variant, including HGVS nomenclature and reference SNP ID (RSID), were obtained from Varsome database (https://varsome.com); variants whose nucleotide or amino acid changes were incorrectly reported were excluded from the study. In the next step, all variants reported in the Iranome project were searched in various databases including BioPKU (http://www.biopku.org/home/pnddb.asp), Leiden Open Variation Database (LOVD) (https://www.lovd.nl/), the Human Gene Mutation Database (HGMD) (http://www.hgmd.cf.ac.uk/ac/index.php), dbSNP (https://www.ncbi.nlm.nih.gov/snp/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), Genome Aggregation Database (gnomAD) (available at https://varsome.com), 1000 Genomes Project (1KGP) (available at http://grch37.ensembl.org/Homo_sapiens/Info/Index), as well as the literature scientific reports to determine if they were novel or previously reported. gnomAD and 1KGP were used as the reference to obtain population data.

In silico analysis

To apply PP3/BP4 criteria in the classification of missense variants, 10 predictive tools including: CADD (https://cadd.gs.washington.edu/), Mutation Taster (http://www.mutationtaster.org/), Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/), I-Mutant disease (http://gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi), PROVEAN and SIFT (both available at: http://provean.jcvi.org/index.php), SNPs&GO (https://snps.biofold.org/snps-and-go/), FATHMM-XF (http://fathmm.biocompute.org.uk/fathmm-xf/), PhD-SNPg (https://snps.biofold.org/phd-snpg/#), and PANTHER PSEP (http://pantherdb.org/), were used. On the other hand, seven splicing predictive tools including VarSEAK and MaxEntScan (both available at: https://varseak.bio/), ASSP (http://wangcomputing.com/assp/index.html), NetGene2 (http://www.cbs.dtu.dk/services/NetGene2/), NNSplice (https://www.fruitfly.org/seq_tools/splice.html), CRYP-SKIP (https://cryp-skip.img.cas.cz/), and GENSCAN (http://pbil.univ-lyon1.fr/members/duret/cours/tmp/results/GenScan.html) were used to predict the splicing effects of all missense, synonymous and intronic variants outside the canonical splice acceptor (−1 and −2) and donor (+1 and +2) regions. In the next step, the outcome of variants was examined.

Application of ACMG-AMP guidelines

Each of the 28 ACMG-AMP criteria is shown with a code and each code is assigned a weight and direction [7]. Accordingly, the final classification of variants would be as: pathogenic (P), likely pathogenic (LP), variant of uncertain significance (VUS), likely benign (LB), or benign (B). To the best of our knowledge, no template has been defined for classifying the variants identified in PTPS deficiency based on ACMG-AMP guidelines. In the present study, while we used the recommendations defined by expert groups [7–10], some adjustments were made to classify variants.

To assign PP3/BP4 criteria, Ellard et al. study [9] was used as a template and adapted based on the type and number of tools used here. Accordingly, deleterious results of missense variants in ≥ 7 or ≤ 4 out of 10 tools was accepted as a supporting criterion of PP3 or BP4, respectively. Otherwise, none of them were considered. To accept the effect of a variant on splicing process, three situations were considered: (a) reduction in the score of acceptor or donor splice sites (ASS of DSS, respectively) by 15% or more relative to reference sequence, (b) creating a new ASS or DSS, or c) activation of a cryptic splice site. If ≥ 5 or ≤ 3 out of 7 tools showed each of these

situations, PP3 or BP4 was assigned, respectively. Otherwise, neither PP3 nor BP4 was triggered. For synonymous variants with no effect on splicing process, BP7 was used.

PM2 criterion was assigned when a variant was absent in population databases. To assign PM2, BS1, or BA1 for other variants, the results of Varsome database were used. Although PM3 has a moderate weight in its original form, it was upgraded (PM3_strong: PM3_S or PM3_very strong: PM3_VS) or downgraded (PM3_supporting: PM3_Su) based on Zastrow et al. study [8]. PP4 was defined as a supporting criterion when the biochemical phenotype of a patient was consistent with PTPS deficiency (higher neopterin and lower biopterin). With adaptation from Zastrow et al. study [8], PS3 criterion was assigned for variants with PTPS enzyme activity of < 50% or RT-PCR confirming mis-splicing due to non-canonical intronic variants. In addition, with adaptation from Ellard et al. study [9], if there were two or only one related references, PS3 was downgraded to PS3_moderate (PS3_M) or PS3_supporting (PS3_Su), respectively. Moreover, the PTPS enzyme activity of > 85% was used for assigning of BS3 criterion. In situations that PVS1 or PS3 were assigned, PP3 was not used [9]. Finally, BS2 was applied when a variant was observed in homozygous form in a healthy adult [8].

Results

Scientific reports and the Iranome project were our sources to collect all *PTS* gene variants in Iran. Our literature review revealed two studies in which the mutational spectrum of a total of 60 Iranian patients with PTPS deficiency had been examined [5, 6]. The ratio of male to female, the rate of consanguineous marriages among the patients' parents, and the age range at the time of diagnosis were 1.00 (30/30), 73.34%, and 13-days to 12-years, respectively. Out of 19 variants reported, six variants were novel at the time of these studies (Table 1A). On the other hand, a total of 36 variants had been recorded in the Iranome project (Table 1B). Given that one variant, c.373G>A (p.Gly125Arg), was reported in both of our sources, the total number of variants collected in this study was 54 (Fig. 1 and Table 1). Variants were classified as exonic (missense: $n=15$, synonymous: $n=4$, and nonsense: $n=1$), intronic ($n=32$), and untranslated region (UTR) ($n=2$) (Tables 2 and 3). Two variants were excluded from the study because they did not match to NM_000317.3 [5].

Missense and nonsense variants

A total of 15 missense variants were identified; all of them, except for c.351C>A (p.Asn117Lys), had deleterious effects in seven or more predictive tools (Table 2). The ACMG-AMP criteria assigned to each variant are shown in Table 4; accordingly, except for c.70C>G (p.His24Asp) and c.351C>A (p.Asn117Lys) with VUS classification, other missense variants were classified as P or LP (Table 4). On the other hand, only one nonsense variant, c.297C>A (p.Tyr99Ter, rs145882709), was identified; based on ACMG-AMP criteria, it was classified as P (Table 4).

Splicing variants

Using splicing predictive tools, all intronic and synonymous variants were evaluated to find their possible effects on splicing process. Out of 36 variants in this group (Table 3), only four variants including c.84-3C>G, c.163+2T>C, c.164-1G>C, and c.315-1G>A (rs776543880) showed significant effects on splicing process (Table 3); according to ACMG-AMP guidelines, they were classified as P (Table 4). BP4 or BP7 criteria were assigned to the remaining 32 variants and their final classification was B, LB, or VUS (Table 4).

Discussion

To collect all reported *PTS* gene variants reported in Iran and analyze their pathogenicity, a literature search was performed and a total of 54 variants were identified (Table 1). So far, only a small number of studies on the genetics of patients with PTPS deficiency have been performed in Iran, and only 800 healthy individuals have been studied in the Iranome project. On the other hand, Iran is a country with a population of about 85 million people as well as a high ethnic diversity [11–18]. In fact, recent studies have shown high mutational diversity of single-gene disorders in Iran [19–23]. From all the above issues, it is possible that the number of *PTS* gene variants in Iran increase in the future.

A total of 19 variants had been reported among Iranian patients with PTPS deficiency. c.155A>G (p.Asn52Ser) and c.317C>T (p.Thr106Met) were the most frequent variants, each with a frequency of 10% (Table 1). c.155A>G (p.Asn52Ser) has been known as a common variant in East Asia [1], especially in Taiwan [24]. In its homozygous form, c.155A>G (p.Asn52Ser) causes sever PTPS deficiency [25]. On the other hand, c.317C>T (p.Thr106Met) has a high frequency of 32% in Russia [26]. Both of these variants were reported repeatedly in combination with P/LP variants, as well as in the form of homozygous [5, 6, 24–31]. Therefore, the two ACMG-AMP criteria, PM3_VS and PP4, were assigned for these variants. Although no functional studies were found in the literature for these variants, in silico analysis revealed that both are deleterious in all 10 predictive tools, therefore, PP3 was met (Table 2). Finally, based on the assigned criteria, c.155A>G (p.Asn52Ser) and c.317C>T (p.Thr106Met) were classified as P (Table 4).

Table 1 *PTS* gene variants reported among Iranian population

Row	Variant	SNP ID	Exon/Intron	Allele frequency (%), ethnicity	Row	Variant	SNP ID	Exon/ Intron	Allele frequency (%)
A: Variants reported among Iranian patients with PTPS deficiency (based on Khatami et al., 2017 and Khani et al., 2021 studies). †:also reported in the Iranome project, ‡: first reported from Iran, NR: not reported									
1	c.155A>G (p.Asn52Ser)	rs104894275	E-2	12 (10.00), Persian	11	c.412A>C (p.Asn138His)	rs1329239489	E-6	2 (1.67), NR
2	c.317C>T (p.Thr106Met)	rs200712908	E-6	12 (10.00), Persian, Azeri	12	c.293C>T (p.Pro98Leu)	rs748040027	E-5	2 (1.67), NR
3	c.84-3C>G	rs1230781262	I-1	9 (7.50), Persian, Kurd	13	c.70C>G (p.His24Asp)‡	NR	E-1	2 (1.67), NR
4	c.281A>T (p.Asp94Val)‡	NR	E-5	6 (5.00), Kurd	14	c.407A>T (p.Asp136Val)	NR	E-6	2 (1.67), NR
5	c.331G>A (p.Ala111Thr)	rs1367077861	E-6	4 (3.33), Azeri	15	c.164-36A>G‡	NR	I-2	2 (1.67), Arab
6	c.351C>A (p.Asn117Lys)‡	NR	E-6	4 (3.33), Afghan	16	c.297C>A (p.Tyr99Ter)	rs145882709	E-5	1 (0.83), Persian
7	c.400G>A (p.Glu134Lys)‡	rs779681799	E-6	2 (1.67), Azeri	17	c.163+2T>C‡	NR	I-2	1 (0.83), Persian
8	c.259C>T (p.Pro87Ser)	rs104894276	E-5	2 (1.67), Persian	18	c.200C>T (p.Thr67Met)	rs370340361	E-4	1 (0.83), NR
9	c.286G>A (p.Asp96Asn)	rs104894280	E-5	2 (1.67), NR	19	c.373G>A (p.Gly125Arg)†	NR	E-6	1 (0.83), NR
10	c.367C>T (p.Pro123Ser)	rs141163668	E-6	2 (1.67), NR					
Detected mutated alleles									69 (57.5)
Total alleles									120 (100.00)
B: Variants reported among Iranian healthy individuals (based on the Iranome preoject). ‡: first reported from Iran, NR: not reported, PGI: Persian Gulf Islander									
20	c.163+14T>C	rs3819331	I-2	255:1600 (0.1594), Multiple	38	c.243+136A>G‡	NR	I-4	2:1588 (0.001259), Persian
21	c.84-7del	rs752347328	I-1	101:1464 (0.06899), Multiple	39	c.84-97_84-96del	rs137920358	I-1	1:1146 (0.000873), Arab
22	c.243+154C>T	rs111330439	I-4	51:1520 (0.03355), Multiple	40	c.315-94C>T	rs969821728	I-5	1:1534 (0.000652), Kurd
23	c.84-7dup	rs368800549	I-1	24:1387 (0.0173), Multiple	41	c.318G>A (p.Thr106=)	rs568878711	E-6	1:1584 (0.000631), Baloch
24	c.84-6G>T	rs778736284	I-1	19:1334 (0.01424), Multiple	42	c.243+119G>A‡	NR	I-4	1:1596 (0.000627), Azeri
25	c.243+94T>A	rs76465815	I-4	21:1600 (0.01312), Multiple	43	c.*16T>C	rs760097954	3´UTR	1:1600 (0.000625), Arab
26	c.405T>C (p.Thr135=)	rs59731976	E-6	20:1598 (0.01252), Multiple	44	c.-19C>T	rs1307475547	5´UTR	1:1600 (0.000625), Persian
27	c.186+184dup	rs530214261	I-3	14:1600 (0.00875), Multiple	45	c.163+92C>T‡	NR	I-2	1:1600 (0.000625), Azeri
28	c.84-5G>T	rs61900919	I-1	11:1336 (0.008234), Multiple	46	c.164-92C>G‡	NR	I-2	1:1600 (0.000625), Baloch

Table 1 (continued)

Row	Variant	SNP ID	Exon/Intron	Allele frequency (%), ethnicity	Row	Variant	SNP ID	Exon/ Intron	Allele frequency (%)
29	c.243+11G>A	rs377396089	I-4	9:1600 (0.005625), Multiple	47	c.164-1G>C	NR	I-2	1:1600 (0.000625), Arab
30	c.84-3C>T	rs1230781262	I-1	8:1526 (0.005242), Multiple	48	c.186+122T>C	rs1322225548	I-3	1:1600 (0.000625), Arab
31	c.187-81A>G	rs74585386	I-3	5:1600 (0.003125), Multiple	49	c.186+132G>T‡	NR	I-3	1:1600 (0.000625), Azeri
32	c.164-97T>C	rs12291603	I-2	5:1600 (0.003125), PGI, Azeri	50	c.186+166A>G	rs899692941	I-3	1:1600 (0.000625), Lur
33	c.83+84A>T	rs12291869	I-1	3:1564 (0.001918), PGI, Azeri	51	c.187-52G>A	rs116660982	I-3	1:1600 (0.000625), Persian
34	c.312G>A (p.Val104=)	rs148185068	E-5	3:1600 (0.001875), Multiple	52	c.243+97C>T	rs1027779552	I-4	1:1600 (0.000625), Baloch
35	c.186+169G>C	rs545888598	I-3	3:1600 (0.001875), PGI, Lur	53	c.314+20G>A	rs566427241	I-5	1:1600 (0.000625), Arab
36	c.163+136G>T‡	NR	I-2	3:1600 (0.001875), Kurd, Lur	54	c.420G>T (p.Val140=)	rs146364246	E-6	1:1600 (0.000625), PGI
37	c.315-1G>A	rs776543880	I-5	2:1574 (0.001271), PGI					

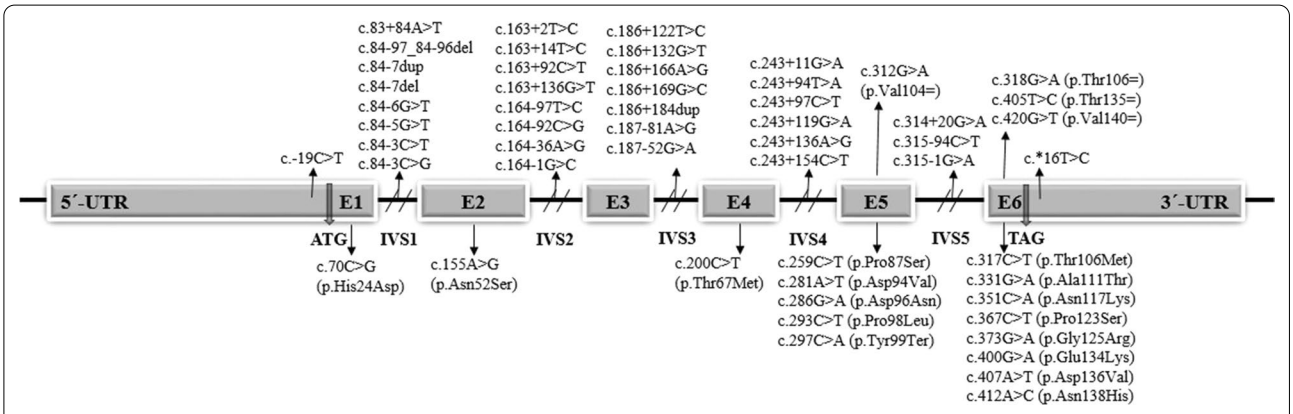

Fig. 1 Position of *PTS* gene variants reported among Iranian population (the genomic structure of *PTS* gene was adapted from Himmelreich et al., 2021)

c.84-3C>G, with a frequency of 7.5%, was the third most frequent variant in Iranian patients with PTPS deficiency (Table 1). It seems that this is the highest frequency reported to date for this variant. c.84-3C>G was reported for the first time in 1997 [32]. Subsequently, it was detected with low frequencies in homozygous form or in combination with P/LP variants in at least five other studies (consistent with PM3_VS and PP4 criteria) [5, 6, 26, 31, 33]. In silico analysis of this variant showed loss of function or a strong decrease of score for authentic ASS; accordingly, exon skipping was predicted to be the final outcome (Fig. 2 and Table 3). However, according

Table 2 Pathogenicity prediction of *PTS* gene missense variants

Row	Variant	PhD- SNPg	PANTHER PSEP	SNPs & GO	FATHMM-XF	I-Mutant Disease	PolyPhen -2	PROVEAN	Mutation Taster	CADD	SIFT	# of tools with pathogenic prediction
1	c.70C>G (p.His24Asp)	P	Pro Da	D	P	D	Pro Da	De	D	31	Da	10:10
2	c.155A>G (p.Asn52Ser)	P	Pro Da	D	P	D	Pro Da	De	D	25.5	Da	10:10
3	c.200C>T (p.Thr67Met)	P	Pos Da	N	P	D	Pro Da	De	D	31	Da	9:10
4	c.259C>T (p.Pro87Ser)	P	Pro Da	N	P	D	Pos Da	De	D	23.8	T	8:10
5	c.281A>T (p.Asp94Val)	P	Pro Da	N	P	D	Pro Da	De	D	31	Da	9:10
6	c.286G>A (p.Asp96Asn)	P	Pro Da	D	P	D	Pro Da	De	D	31	Da	10:10
7	c.293C>T (p.Pro98Leu)	P	Pro Da	N	P	D	Pos Da	De	D	27.2	Da	9:10
8	c.317C>T (p.Thr106Met)	P	Pro Da	D	P	D	Pro Da	De	D	26.2	Da	10:10
9	c.331G>A (p.Ala111Thr)	P	Pro Da	D	P	D	B	De	D	23.8	T	9:10
10	c.351C>A (p.Asn117Lys)	P	Pro Da	N	P	D	B	N	D	18.26	T	5:10
11	c.367C>T (p.Pro123Ser)	P	Pro Da	N	P	D	Pos Da	De	D	22.8	T	8:10
12	c.373G>A (p.Gly125Arg)	P	Pro Da	N	B	D	B	De	D	23.9	Da	7:10
13	c.400G>A (p.Glu134Lys)	P	Pro Da	D	P	D	Pro Da	De	D	31	Da	10:10
14	c.407A>T (p.Asp136Val)	P	Pro Da	N	P	D	Pos Da	De	D	28.6	Da	9:10
15	c.412A>C (p.Asn138His)	P	Pro Da	N	P	D	Pro Da	De	D	26	Da	9:10

P: pathogenic, Pro Da: probably damaging, Pos Da: possibly damaging, D: disease causing, N: neutral, B: benign, De: deleterious, T: tolerated

Table 3 Pathogenicity prediction of *PTS* gene intronic and synonymous variants. AASS: authentic acceptor splice site, ADSS: authentic donor splice site

Row	Variant	VarSeak	MaxEntScan	NNSplice	NetGene2	ASSP	cryp-skip	GENSCAN	# of tools with pathogenic prediction
1	c.84-5G>T	No effect	No effect	No effect	No effect	No effect	No effect	No effect	0:7
2	c.84-3C>G	AASS score ↓	AASS score ↓	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	7:7
3	c.84-3C>T	No effect	No effect	No effect	AASS loss	AASS score ↓	No effect	No effect	1:7
4	c.163+2T>C	ADSS loss	ADSS loss	ADSS loss	ADSS loss	ADSS loss	ADSS loss	ADSS loss	7:7
5	c.164-36A>G	No effect	No effect	No effect	No effect	No effect	No effect	No effect	0:7
6	c.164-1G>C	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	7:7
7	c.315-1G>A	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	AASS loss	7:7
Other variants									
8–36	None of the other variants, including c.83+84A>T, c.84-97_84-96del, c.84-7dup, c.84-7del, c.84-6G>T, c.163+14T>C, c.163+92C>T, c.163+136G>T, c.164-97T>C, c.164-92C>G, c.186+122T>C, c.186+132G>T, c.186+166A>G, c.186+169G>C, c.186+184dup, c.187-81A>G, c.187-52G>A, c.243+11G>A, c.243+94T>A, c.243+97C>T, c.243+119G>A, c.243+136A>G, c.243+154C>T, c.312G>A (p.Val104=), c.314+20G>A, c.315-94C>T, c.318G>A (p.Thr106=), c.405T>C (p.Thr135=), c.420G>T (p.Val140=), had an effect on the splicing process								

to Oppliger et al. functional analysis on this variant [32], the use of a cryptic splicing site would result in a 12 nucleotides deletion at the beginning of exon 2 and finally, a deletion of four amino acids (p.Lys29_Ser32del) (Fig. 2). These authors also reported a complete inactivity of PTPS enzyme encoded by this allele (consistent with PS3_Su criterion). Due to the assigning of PS3, PP3 was not applied for c.84-3C>G [9]. As shown in Table 4, this variant was classified as P according to ACMG-AMP guidelines.

c.281A>T (p.Asp94Val), c.331G>A (p.Ala111Thr, rs1367077861), and c.351C>A (p.Asn117Lys) had the allele frequencies of 5, 3.3, and 3.3% among Iranian PTPS patients. These variants were only reported in homozygous form and to the best of our knowledge, there is no report of their combination with P/LP variants in the literature [5, 6, 24, 34]; accordingly, along with PP4, PM3_Su was also applied [8]. Moreover, none of them were reported in gnomAD and 1KGP databases (consistent with PM2 criterion). While PP3 criterion was assigned to c.281A>T (p.Asp94Val) and c.331G>A (p.Ala111Thr) variants, c.351C>A (p.Asp117Lys) failed to reach the threshold defined by us in the Methods section (neither PP3 nor BP4 was used) (Table 2). Finally, according to ACMG-AMP guidelines, while the first two variants were classified as LP, the last one was classified as VUS (Table 4).

Nine other variants, each with a frequency of 1.67%, including c.400G>A (p.Glu134Lys, rs779681799), c.259C>T (p.Pro87Ser, rs104894276), c.286G>A (p.Asp96Asn, rs104894280), c.367C>T (p.Pro123Ser, rs141163668), c.412A>C (p.Asn138His, rs1329239489), c.293C>T (p.Pro98Leu, rs748040027), c.70C>G (p.His24Asp), c.407A>T (p.Asp136Val), and c.164-36A>G were also reported among Iranian PTPS patients. c.400G>A (p.Glu134Lys) was first identified in 2017 in an Iranian patient [5], and has subsequently been reported in Omani [30] and Russian [26] patients. Although c.259C>T (p.Pro87Ser) is a common variant in East Asia [27, 29, 35–38], it also has been detected in Iran [5] and Russia [26]. Similarly, c.286G>A (p.Asp96Asn) is common in East Asian populations [29, 35–37]. According to Imamura et al. study [35], the PTPS enzyme activities of p.Pro87Ser and p.Asp96Asn proteins in COS cells was 52 and 10%, respectively; therefore, PS3_Su was only applied for c.286G>A (p.Asp96Asn) (Table 4).

Oppliger et al. reported that the overexpression of c.407A>T (p.Asp136Val) in COS-1 cells would reduce PTPS activity to 31% compared to the wild-type enzyme (consistent with PS3 criterion) [32]. This variant has been found in Italian, Polish, Turkish, German, and Iranian patients so far [6, 32, 33, 39, 40]. Khatami et al. reported c.164-36A>G in homozygous form in an Iranian patient with PTPS deficiency [5] and to the best of our knowledge, there is no other report for this variant in the literature. Our analysis showed that this variant has no effect on splicing process (Table 3) and was classified as a VUS variant (Table 4). Four variants of c.200C>T (p.Thr67Met, rs370340361), c.297C>A (p.Tyr99Ter), c.163+2T>C, and c.373G>A (p.Gly125Arg) had the lowest frequencies in this study (Table 1). With the exception of c.200C>T (p.Thr67Met) [6, 32, 36, 41, 42], the other three variants have been reported in only a few studies [5, 6].

Out of 36 variants identified in the Iranome project, only three variants including c.164-1G>C, c.315-1G>A, and c.373G>A (p.Gly125Arg) were classified in the category of P/LP. c.373G>A (p.Gly125Arg) had also been reported among Iranian PTPS patients (the above paragraph)

Table 4 ACMG-AMP classification of *PTS* gene variants reported among Iranian population

Row	Variant	ACMG-AMP criteria	Classification	Reference/s used
1	c.70C>G (p.His24Asp)	PM2, PP2, PP3, PP4	VUS	Khani et al., 2021
2	c.155A>G (p.Asn52Ser)	PM3_VS, PM2, PP2, PP3, PP4	P	Khatami et al., 2017 & Khani et al., 2021 & Almannai et al., 2019 & Leuzzi et al., 2010 & Hong et al., 2015 & Wang et al., 2019 & Gundorova et al., 2021 & Han et al., 2015
3	c.200C>T (p.Thr67Met)	PM3_VS, PM2, PP2, PP3, PP4	P	Oppliger et al., 1997 & Khani et al., 2021 & Liu et al., 2001 & Pangkanon et al., 2006 & Chaiyasap et al., 2017
4	c.259C>T (p.Pro87Ser)	PM3_VS, PM2, PP2, PP3, PP4	P	Khatami et al., 2017 & Imamura et al., 1999 & Cao et al., 2014 & Wang et al., 2019 & Liu et al., 2001 & Gundorova et al., 2021 & Gu et al., 2014 & Han et al., 2015
5	c.281A>T (p.Asp94Val)	PM2, PM3_Su, PP2, PP3, PP4	LP	Khatami et al., 2017 & Khani et al., 2021
6	c.286G>A (p.Asp96Asn)	PM3_VS, PS3_Su, PM2, PP2, PP4	P	Khani et al., 2021 & Liu et al., 2001 & Han et al., 2015 & Cao et al., 2014 & Imamura et al., 1999
7	c.293C>T (p.Pro98Leu)	PM3_Su, PM2, PP2, PP3, PP4	LP	Khani et al., 2021 & Chiu et al., 2012
8	c.297C>A (p.Tyr99Ter)	PVS1, PM2, PM3, PP4	P	Khatami et al., 2017
9	c.317C>T (p.Thr106Met)	PM3_VS, PM2, PP2, PP3, PP4	P	Khatami et al., 2017 & Khani et al., 2021 & Leuzzi et al., 2010 & Hong et al., 2015 & Gundorova et al., 2021 & Han et al., 2015 & Manti et al., 2020
10	c.331G>A (p.Ala111Thr)	PM2, PM3_Su, PP2, PP3, PP4	LP	Khatami et al., 2017 & Khani et al., 2021 & Chiu et al., 2012 & Li et al., 2018
11	c.351C>A (p.Asn117Lys)	PM2, PM3_Su, PP2, PP4	VUS	Khatami et al., 2017 & Khani et al., 2021
12	c.367C>T (p.Pro123Ser)	PM2, PM3_Su, PP2, PP3, PP4	LP	Khani et al., 2021 & Almannai et al., 2019
13	c.373G>A (p.Gly125Arg)	PM2, PM3, PP2, PP3, PP4	LP	Khani et al., 2021
14	c.400G>A (p.Glu134Lys)	PM3_S, PM2, PP2, PP3, PP4	LP	Khatami et al., 2017 & Gundorova et al., 2021 & Almannai et al., 2019
15	c.407A>T (p.Asp136Val)	PM3_VS, PM2, PS3_Su, PP2, PP4	P	Khani et al., 2021 & Dudešek et al., 2001 & Żekanowski et al., 1998 & Oppliger et al., 1997
16	c.412A>C (p.Asn138His)	PM2, PM3_Su, PP2, PP3, PP4	LP	Khani et al., 2021
17	c.84-3C>G	PM3_VS, PS3_Su, PM2, PP4	P	Khatami et al., 2017 & Khani et al., 2021 & Romstad et al., 1999 & Gundorova et al., 2021 & Manti et al., 2020, Oppliger et al., 1997
18	c.163+2T>C	PVS1, PM2, PP4	P	Khatami et al., 2017
19	c.164-1G>C	PVS1, PM2, PP4	P	Manti et al., 2020
20	c.315-1G>A	PVS1, PM2, PM3, PP4	P	Gundorova et al., 2021
21–54	The ACMG-AMP classification of other variants were as follows: c.83+84A>T, c.84-97_84-96del, c.84-7dup, c.84-7del, c.163+14T>C, c.164-97T>C, c.187-81A>G, and c.243+94T>A (all with BA1, BS2, BP4 criteria and benign classification) **&** c.186+184dup, c.187-52G>A, c.243+154C>T (all with BS1, BS2, BP4 criteria and benign classification) **&** c.163+92C>T, c.163+136G>T, c.164-92C>G, c.164-36A>G, c.186+122T>C, c.186+132G>T, c.243+119G>A, c.243+136A>G, and c.315-94C>T (all with PM2, BP4 criteria and VUS classification) **&** c.84-6G>T, c.84-5G>T, c.84-3C>T, c.186+166A>G, c.186+169G>C, c.243+11G>A, and c.243+97C>T (all with BP4 criterion and VUS classification) **&** c.312G>A (p.Val104=), c.318G>A (p.Thr106=), and c.420G>T (p.Val140=) (all with BP7 criterion and VUS classification) **&** c.314+20G>A (with BS2, BP4 criteria and likely benign classification) **&** c.405T>C (p.Thr135=) (with BA1, BS2, BP7 criteria and benign classification) **&** c.-19C>T and c.*16T>C (both without any criteria and with VUS classification)				

VUS: variant of uncertain significance, P: pathogenic, LP: likely pathogenic

Variant	Splice site	Sequence (in NM_000317.3)	Var SEAK	MaxEnt Scan	NN Splice	Net Gene2	ASSP	CRYP-SKIP	GEN SCAN	Outcome
c.84-3C>G	Authentic ASS	R gtcagTAAATTTCTAAGTGA	+38.77 %	+5.75	0.70	0.18	8.01	0.7	5.29	Exon skipping
		V gtgagTAAATTTCTAAGTGA	-22.38 %	-5.29	loss	loss	loss	loss	loss	
	Cryptic ASS activated	V gtgagtaaatttctaagTGA	-	-	-	-	-	-	-	p.Lys29_Ser32del (Oppliger et al., 1997 functional study)

Fig. 2 In silico analysis of *PTS*:c.84-3C>G variant using splicing predictive tools. Although exon skipping was predicted by these tools, according to Oppliger et al. (1997) functional study, the use of a cryptic splicing site would result in a deletion of 12 nucleotides at the beginning of exon 2 and finally, a deletion of four amino acids (p.Lys29_Ser32del)

(Table 1). Both of c.164-1G>C and c.315-1G>A are rare splicing variants [26, 31]. Both of these variants cause loss of function of ASS (Table 3), however, because of assigning of PVS1 criterion, PP3 was not applied [9]. The other intronic or synonymous variants had no effect on splicing process (Tables 1 and 3) and were classified as B, LB, or VUS (Table 4). The splicing effect analysis of some of these variants are shown in Table 3.

In conclusion, the collection of all reported *PTS* gene variants in the Iranian population, their analysis using in silico predictive tools, and their classification according to ACMG-AMP criteria were the three main areas in the present study. The ACMG-AMP criteria need to be updated depending on the type of disease. In addition, to the best of our knowledge, no template has been described for classifying the variants identified in PTPS deficiency. Therefore, this study can be a good reference for future studies in this area.

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Author contributions
KM had the idea of the topic. Literature search and data collection was performed by KM, SK and MK. Data analysis was performed by KM. The first draft of the manuscript was written by KM and critically revision of the manuscript was performed by all authors. All authors read and approved the final manuscript.

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Declarations

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Kermanshah University of Medical Sciences, Kermanshah, Iran (Ethics code: IR.KUMS.REC.1400.635, project number: 4000706).

Consent for publication
Not applicable.

Competing interests
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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