Beyond Val30Met transthyretin (TTR): variants associated with age-at-onset in hereditary ATTRv amyloidosis

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ABSTRACT

Objectives: Val30Met in transthyretin (*TTR*) gene is causative for familial amyloid polyneuropathy (FAP). FAP shows a wide variation in age-at-onset (AO) between clusters, families, and among generations. We aim at identifying genetic modifiers of disease onset that may contribute to this variability in Portuguese patients by identifying other variants in *TTR locus*, beyond the TTR-FAP causing variant that could play a regulatory role in its expression level.

Methods: We analysed DNA samples of 330 Val30Met carriers (299 patients, 31 aged-asymptomatic carriers aged >40 years) from 120 families currently under follow-up. A generalized estimating equation analysis (GEE) was used to take into account non-independency of AO between relatives. An intensive *in silico* analysis was performed in order to understand a possible regulation of gene expression.

Results: We found 11 rare variants in the promoter, coding and intron/exon boundaries of the *TTR* gene associated with the onset of symptoms before and after age 40 years, namely 2 novel ones and a tandem CA-dinucleotide repeat. The seven Val30Met/Val30Met homozygous do not carry any of the variants identified in this study, including the common ones. *In silico* analysis disclosed significant alterations in the mechanism of splicing, transcription factors and miRNAs binding.

Conclusions: Variants within the promoter region may modify disease expressivity and variants in the 3'UTR can impact the efficacy of novel therapeutic interventions. Importantly, the putative mechanisms of regulation of gene expression within the *TTR* gene deserve to be better explored, in order to be used in the future as potential therapeutic targets.

Keywords: Age-at-onset, familial amyloid polyneuropathy, *TTR* gene, promoter, Hereditary transthyretin, genetic modifiers

INTRODUCTION

Transthyretin-related familial amyloid polyneuropathy (TTR-FAP) is an autosomal dominant neurodegenerative disease that presents as a progressive sensorimotor and autonomic polyneuropathy with involvement of the small myelinated and unmyelinated nerve fibers [1,2]. It is characterized by mutated protein deposition in the form of amyloid substance (ATTR_m). Without treatment, it leads to death within 10-20 years [1]. The Val30Met variant (p.Val30Met) in the transthyretin gene (*TTR*) is the commonest causative variant for TTR-FAP [3]. Significant phenotypic heterogeneity, namely in age-at-onset (AO), has been described in Val30Met patients. Variation between clusters has been widely documented, with Portuguese, Brazilian and Japanese patients showing mostly an early-onset (AO<40), whereas Swedish patients are known for their late-onset (≥50) [4-6]. However, within each cluster variation is equally large, and range in AO quite similar. In Portuguese patients clinical symptoms typically occur before age 40 years (early-onset), with late-onset patients being increasingly ascertained, leading to a wide variation in AO (19-82 years) [7]. The most intriguing feature is variation within the same family and the fact that late-onset patients often have very early-onset offspring, while the reverse was never observed [7].

In recent years, we explored the role of common variants in different candidate genes involved in different TTR pathways, taking into account the family structure and the non-independency of AO among members of the same family [8-10]. Although these results bring new insights on TTR pathways, AO variation is not fully explained. The observed intra-generational variability; the fact that late-onset patients have early-onset offspring and due to the main role of TTR in FAP, this led us to focus in another approach to identify modifiers closely linked to the *TTR* locus.

A small part of this variability may be explained by non-coding genetic variants in *cis*[11] and a *trans*-acting factor[12], but additional factors clearly remain to be discovered.

We speculated that variants within the regulatory regions of the *TTR* gene might account for the changed gene expression and ultimately culminate in phenotypic heterogeneity. The aim of this study was then to identify other variants and regulatory factors at *TTR* locus and understand how they may contribute as genetic modifiers of AO in TTR-FAP.

Subjects and Methods

Subjects and families

Unidade Corino de Andrade - Centro Hospitalar Universitário do Porto (UCA-CHUP, Porto) has the largest TTR-FAP Val30Met registry worldwide (more than 3000 patients), collected over 80 years and clinically well characterized. We selected 120 families currently under follow-up, with at least 2 generations affected and coming from all geographical areas of Portugal. Our study sample consists of 330 Val30Met carriers (299 patients, 5 homozygous; 31 asymptomatic carriers, 2 homozygous).

For each patient, AO was defined by the same team of neurologists specialized in Val30Met TTR-FAP, considering the presence of a whole set of symptoms characteristic of small fibers' neuropathy and not isolated and unspecific symptoms [10]. As for AO, it has long been accepted to classify patients in three groups: early (≤ 40), intermediate (40-49) and late (≥ 50) [13]. In this study, patients were divided in two groups only: early (≤ 40) and intermediate/late-onset (> 40 years). All asymptomatic carriers were aged 40 years or more and therefore were also included in the intermediate/late-onset group. The presence of Val30Met variant was confirmed in all subjects. DNA samples were collected at UCA-CHUP and stored at Centro de Genética Preditiva e Preventiva (CGPP-IBMC, Porto) biobank, authorized by CNPD (National Commission for Data Protection). The study was approved by the Ethics Committee of CHUP. Written informed consent was obtained from all patients.

Genotyping

Genomic DNA was extracted either from peripheral blood leucocytes or saliva, using the QIAamp® DNA Blood Mini Kit (QIAGEN™) and Oragene DNA Self Collection Kits (DNA Genotek, Ottawa, Canada) according to manufacturer's instructions, respectively.

The promoter, coding and all intron/exon boundaries of the *TTR* gene were amplified by polymerase chain reaction (PCR) using HotStarTaq DNA Polymerase® (Qiagen™) and primers forward and reverse for each amplicon (sequences available upon request).

Then the PCR products were examined by direct Sanger sequencing using dye terminator chemistry approach from Applied Biosystems™, with the same set of primers as for amplification, and samples loaded on an ABI-PRISM 3130 XL genetic analyzer (Applied Biosystems).

Statistical analysis

Association of genetic variants with AO (as a dichotomous variable), as in the case of the tandem repeat

and the two variants together, was explored using Chi-Square or Fisher's exact test. AO was also analyzed as a continuous variable: to account for non-independency of AO between members of the same family, we used generalized estimating equations (GEE); to correct for multiple testing, we applied a Bonferroni correction, dividing by the number of GEE tests performed. Therefore, statistical significance was set at $\alpha < 0.0125$. All statistical analyses were performed using IBM SPSS Statistics software (v.24).

In silico analysis

To predict the impact of sequence variants on TTR function, bioinformatics tools included in the Alamut Mutation Interpretation Software (Interactive Biosoftware, Rouen France) and Polyphen-2 were applied.

Also, we ran a comprehensive bioinformatics package, FuncPred[14], organized by NIH and one of the most reliable and popular function-prediction tools.

In order to predict potential consequences of variants on splicing events, we used two prediction programs (ESEfinder and Human Splicing Finder) in Alamut Software.

The JASPAR[15] database was utilized to explore the transcription factors binding (TFB) capacity. Transcription factors were filtered by expression in liver with an expression level greater than 1 Transcripts per Million (TPM) using Genotype-Tissue Expression (GTEx) project.

miRNA target sites in the 3'UTR *TTR*-wild type (WT) were predicted by miRWalk[16] and we used PITA algorithm, taking in account the conservation and the differences in the alignment scores [17].

Results

Study group demographic characteristics

This study included 330 Val30Met carriers (174 females, 156 males). Among the 299 patients, AO ranged from 21-74 years (mean 38.7; SD 12.9). The group with $AO \leq 40$ had 202 patients, whereas the group with $AO > 40$ consisted of 97 patients plus 31 asymptomatic carriers (aged 42 to 90 years) who might be considered similar to intermediate/late-onset patients.

Interestingly, of the 330 carriers, 7 were homozygous: 3 had onset > 50 , 2 were asymptomatic at age 50 or more and 2 had an onset < 50 .

We sequenced 2.1kb upstream *TTR* to include promoter region and the coding and flanking regions of the *TTR* gene. Location and frequency of the variants found are shown in Table 1 [near here].

Common variants

As shown in Table 1, we identified four common variants (MAF $> 10\%$), 3 in the promoter region and 1 in 3' near gene. TT genotypic of rs1791228 variant revealed on GEE analysis an association with AO ($p < 0.0125$) ($B = 23,6$; 95% CI, 20,2 to 27,0), significant after multiple-testing correction.

Interestingly one variant is a tandem repeat composed by a CA-dinucleotide (rs71383038) in the promoter region (Table 1). While the most common allele in the Portuguese population (including our sample) was CA₁₀, followed by the minor allele CA₉, we found an unreported allele with seven CA-repeats (CA₇). When we compared CA₉ carriers with CA₁₀, the only two CA₉ homozygotes for the tandem repeat rs71383038 showed onset after 60 years; 56.1% of the patients presented the common genotype (CA₁₀/CA₁₀) and 42.7% the CA₉/CA₁₀ genotype. Worth mentioning, the only patient with a deletion of 3 CA-repeats (CA₇) showed a very early-onset (30 years).

None of the other common variants presented significant differences in frequencies when comparing early- and intermediate/late-onset patients or when analyzing AO as a continuous variable. The variants rs3764479 and rs3794885 were the most common alterations in the promoter region (43.5% and 42.8%, respectively).

Noteworthy, in the $AO \leq 40$ group, more patients carried simultaneously the minor allele of rs3764479 and of rs3764478 ($p = 0.03$), with a 2.22-fold risk for symptoms to start before age 40 (OR=2.22, 95% CI=1.05 to 4.70).

Rare Variants

In the promoter region, 12 rare variants were found, including one novel (c. -1993 G>T). Seven rare variants in the coding and flanking regions of the *TTR* gene were identified, including two novel ones: c.105A>G, in exon 3 (p.Lys35Lys), a synonymous variant and c.200+107 T>C, in intron 2. Variants already reported included 2 intronic, two at the 3' UTR and 2 exonic ones. Importantly, rare variants: c. -1993 G>T; rs13381522; rs546878244; rs3764477; rs540872876; rs58616646; 146750662 and rs996949357 in the promoter region and c.200+107 T>C; rs28933981; and rs1053907197 in the coding and flanking regions of *TTR* gene showed an association with AO ($p < 0.0125$), significant after multiple-testing correction (Table 2 near here).

Regarding the coding variant, TTR Ser6 (rs1800458, p.Gly26Ser), ten patients showed an early-onset and eight patients a late-onset, ($p > 0.05$), showing that TTR Ser6 is not associated with early or late-onset. All compound heterozygotes for TTR Val30Met/Thr119Met (p.Val50Met/Thr139Met) showed a later-onset, as previously described [18].

Homozygous individuals

Val30Met/Val30Met homozygous do not carry any of the variants identified in this study: they are also homozygous for the common allele of each variant.

Haplotype Analysis

We performed a haplotype analysis (using the Haploview software) with the variants identified in this study and we did not find any significant results (Table 3 near here).

Putative effects of the rare genetic variants

Using JASPAR database and GTEx project, we predicted that the combination of rs3764479/rs3764478 variants (as they were found to be present mostly together) create 69 new potential transcription-factor binding sites and may disrupt another 18, all expressed in the liver, where TTR is synthesized (Table 4 near here).

We also assess how likely a splicing change would occur as a result of the presence of DNA variants. Table 5 [near here] shows the variants that may alter splicing significantly (consensus value (CV) higher than 70). We found a new acceptor site, 2 nucleotides downstream rs1800458, probably an active splice site. A branch point, probably inactive in the wild-type sequence, is predicted to become a strongly active splice site in the presence of rs1791228. Also, presence of rs36204272 leads to loss of a donor site

probably inactive in wild-type TTR. Disruption of four exonic splicing enhancers was observed in rs28933981, while creation of three new ones was seen in rs1800458.

Using miRNA-target prediction programs, we detected two miRNAs: mir-200a and mir-141, which are predicted target-sites for the TTR 3'UTR. Presence of rs62093482 or rs1053907197 (located in TTR 3'UTR) does not interfere with miRNA binding. rs62093482 creates a putative target site to mir-622. The rare allele of rs1053907197 is a putative target site to mir-138 and mir-622.

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Discussion

TTR-FAP phenotypic variability is evident not only between different disease-associated variants but also for patients with the same disease-causing variant that display different clinical manifestations. The main focus of this study was on the phenotypic variability of the TTR-FAP Val30Met individuals regarding AO.

Phenotypic effect of variants

Using a parametric test (GEE), which is a quite powerful statistical method, we found interesting results regarding the association of some of these variants with AO, which showed that rare variants in fact seem to modulate AO variability. The effect size of the variants found could be small, which can be a limitation in order to achieve a significant association with AO, due to statistical power. Likewise, how to point out rare variants as a causative effect on AO remains challenging, as they may not be shared by all affected individuals. Although the role of environmental factors cannot be excluded, even as, the contribution of other genetic factors (common or rare) and a global role of them in modulation of specific phenotypes in the presence of the same disease causing variant.

We found significant results for some rare variants: despite the fact that the heterozygous genotype appeared only once, this is in accordance with the allelic frequencies described for European (Non-Finnish) in Genome Aggregation Database (GnomAD). Moreover, we could not preclude the possibility that high effect risk alleles were present. Thus, we consider that these rare variants results are equally important and should be reported, because their identification may have implications for genetic screening and personalized treatment.

The protective role of rs1800458 (p.Gly26Ser) has already been hypothesized[11,19]; however, results do not allow us to state that this coding variant was protective in our Val30Met patients, since it did not significantly associate with late-onset.

Both Thr119Met carriers found had a very late AO (61 and 63 years), though they belonged to an early-onset family. This supports the theory that stability of the TTR molecule may be an important factor to prevent amyloidogenesis, offering a protection from the Val30Met effects [18,20].

As described in other clusters [21,22], the homozygous status does not appear to be related to the onset of symptoms, as we found late-onset carriers of Val30Met/Val30Met. Interestingly, the 7 homozygous analyzed in this study do not carry any of variants identified. This leads us to hypothesize that the absence of these variants in the TTR vicinity may compensate the double genetic dose of TTR

Val3oMet.

Noteworthy, it is very promising that the combination of the minor alleles of rs3764479 and rs3764478 (which appear frequently together) showed a high risk for an early-AO. This approach deserves our future attention, as clusters of patient-specific variant combinations have already been found [23].

Putative effect on splicing activity, TFBSs and miRNA target sites

Analysis of non-coding regions suggests the presence of four non-coding variants, possibly *cis*-regulatory elements of the *TTR* gene that modulate the effect of Val3oMet on the disease phenotype.

To the best of our knowledge, no other studies about genetic alterations in splicing activity on *TTR* gene have been performed, except for a recent association study for variants in the *TTR* gene in Han Chinese patients with Alzheimer disease [24]. It is important to emphasize that some of the differences between WT and variant sequences could alter splicing either by re-directing the spliceosome or by altering the binding of auxiliary factors, such as SR proteins, exonic and intronic splicing enhancers and silencers [25]. For instance, results showed that variant rs1791228 was significantly associated with AO, and the association survived Bonferroni correction. According to the splicing events' prediction programs, this variant may influence the constitutive splicing of *TTR* pre-mRNA.

The *TTR* gene is regulated by the promoter at the transcriptional level in liver cells [26]. In this study, we identified two variants in the promoter region that together reduce significantly AO. *In silico*, these variants not only disrupt potential binding sites once occupied by transcription factors, but also create new ones. Human transcription factors were predicted with a relative profile score threshold >80% and filtered by expression in liver.

miRNA mir-200a and mir-141 inhibit *TTR* expression, by directly binding to the 3'UTR of *TTR*, which is reversed by variants in the miRNA binding site [27]. rs62093482 and rs1053907197 variants do not affect the 3'UTR miRNA binding sites, but they create new ones. Two novel therapeutic drugs recently approved for treatment of FAP modulate *TTR* expression at the RNA level: patisiran, the first small interfering RNA-based drug approved by the FDA [28]; and inotersen, an antisense oligonucleotide [29]. Since both drugs prevent the production of *TTR* by targeting its 3' UTR, we hypothesized that variants in this region could modify their efficacy.

As we are aware, this is the largest screening study of *TTR* variants in Val3oMet *TTR*-FAP. Our data suggest possible mechanisms that may account for expression of *TTR* as a potential modulator of the course of FAP. This reinforces the idea that molecular diagnosis should go beyond simply screening for the disease-causing variant.

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The other authors have no conflicts of interest.

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Table 1: Variants found in the *TTR* locus: dbSNP ID, location, frequency in AO ≤40 and >40 onset patients, and minor allele frequency in Val30Met Portuguese patients and in the Genome Aggregation Database (GnomAD).

<i>TTR</i> Region	dbSNP ID	Location NM_000371.3 (HGVS)	N	Variant frequency		Allele Frequency	
				AO ≤40	AO >40	Val30Met	European (Non-Finish)
PROMOTER	rs3764479	c.-2041A>G	111	64	47	0.222	0.328
		c. -1993 G>T	1	1	0	0.002	-
	rs13381522	c.-1933C>T	7	3	4	0.014	0.046
	rs546878244	c.-1700G>A	1	1	0	0.002	0.000
	rs3764478	c.-1383G>T	39	27	12	0.078	0.111
	rs71383038	c.-1232_-1231delCA	112	64	48	0.220	-
	rs72922940	c.-1168A>G	35	18	17	0.069	0.116
	rs3764477	c.-1157G>A	9	4	5	0.018	0.045
	rs540872876	c.-1156G>A	1	0	1	0.002	0.000
	rs58616646	c.-1136C>T	8	4	4	0.016	0.046
	rs146750662	c.-950C>T	2	2	0	0.004	0.004
	rs116409170	c.-833C>T	6	3	3	0.012	0.025
	rs3794885	c.-743A>T	109	63	46	0.218	0.296
	rs79748512	c.-682G>A	6	2	4	0.012	0.039
	rs996949357	c.-543T>C	1	0	1	0.002	-
Coding and flanking regions	In 2	c.200+107 T>C	1	0	1	0.002	-
	Ex 2	rs1800458 c.76G>A, p.Gly26Ser	18	10	8	0.027	0.074
	In 3	rs36204272 c.337-18G>C	7	3	4	0.011	0.037
	Ex 3	c.105A>G, p.Lys35Lys	1	0	1	0.002	-
	Ex 4	rs28933981 c.416C>T, p.Thr139Met	2	0	2	0.003	0.003
	3'UTR	rs62093482 c.*261C>T	6	3	3	0.009	0.028
	3'UTR	rs1053907197 c.*75A>C	1	1	0	0.002	-
	3' near gene	rs1791228 c.*402C>T	127	87	40	0.194	0.463

AO, age-at-onset. Novel variants are highlighted in bold

. Table 2: Statistically significant results for rare variants associated with AO variation, using GEE analysis, after Bonferroni correction.

<i>TTR Region</i>	Rare variant	Genotypes	B	95% CI	P- value
<i>Promoter</i>	Intercept	-	40.1	[36.7; 43.6]	<0.001
	c. -1993 G>T	GG (Ref)	-	-	-
		GT	-6.1	[-9.6; -2.7]	<0.001
	rs13381522	CC (Ref)	-	-	-
		CT	21.4	[8.5; 34.3]	0.001
	rs546878244	GG (Ref)	-	-	-
		GA	-8.1	[-11.6; -4.7]	<0.001
	rs3764477	GG (Ref)	-	-	-
		GA	-15.5	[-18.2; -12.9]	<0.001
	rs540872876	GG (Ref)	-	-	-
		GA	18.4	[14.8; 21.9]	<0.001
	rs58616646	CC (Ref)	-	-	-
		CT	-18.4	[-21.9; -14.8]	<0.001
	rs146750662	CC (Ref)	-	-	-
		CT	-8.1	[-11.6; -4.7]	<0.001
<i>Coding and flanking regions</i>	rs996949357	TT (Ref)	-	-	-
		TC	11.9	[8.4; 15.3]	<0.001
	Intercept	-	42.0	[39.5; 44.5]	<0.001
	c.200+107 T>C	TT (Ref)	-	-	-
		TC	8.19	[5.3; 11.1]	<0.001
	rs28933981	CC (Ref)	-	-	-
		CT	21.59	[17.3; 25.9]	<0.001
	rs1053907197	AA (Ref)	-	-	-
		AC	-7.81	[-10.7; -4.9]	<0.001

Ref, Reference genotype; B, unstandardized coefficient (estimated quantitative effect of each genotype on mean AO variation according to the intercept, compared with the reference genotype); CI, confidence interval; significance level set to 0.0125

Table 3: Haplotype association analysis of all variants identified in *TTR* locus on Val30Met TTR carriers.

<i>Haplotype</i>	Haplotype Frequency	Haplotype Frequency		P-value	P-permuted*
		Early-onset	Late-onset		
<i>ACGCAGGCCAGTGGCCC</i>	0.664	0.706	0.625	0.1829	0.6341
<i>GCGAAGGCCTGTGGCCT</i>	0.071	0.053	0.087	0.3089	0.9956
<i>GCTAAGGCCTGTGGCCT</i>	0.036	0.059	0.016	0.0721	0.2231
<i>GCTAAGGCCTGTGGCCC</i>	0.034	0.036	0.032	0.8428	10.000
<i>GCGAGGGCCTGTGGCCT</i>	0.033	0.035	0.032	0.8956	10.000
<i>GCGAAGGCCTGTGGCCC</i>	0.026	0.018	0.033	0.4819	10.000
<i>ACGCAGGCCAGTGGCCT</i>	0.019	0.021	0.017	0.8564	10.000
<i>GCGAGGGCCTGTGGCCC</i>	0.017	0.018	0.016	0.9188	10.000
<i>ATGCAAGTCAGTGCCCC</i>	0.017	0.000	0.032	0.055	0.1582
<i>ACGCAGGCCAGTGGCTC</i>	0.012	0.009	0.016	0.6208	10.000
<i>ACGCAGGCCAGTAGCCT</i>	0.012	0.017	0.008	0.5058	10.000

* Permutation for 10,000 times.

Table 4: Predicted transcription factors (TFs) which have lost (disrupted) or gain (new) affinity to bind in the presence of rs3764479/rs3764478 variants.

Disrupted TF	New TF		
NR1H4	JUNB	REL	RREB1
MAFG	JUND	NFATC2	CTCF
ATF4	EGR1	AR	HSF1
IRF1	RELA	TCF7L2	HLF
NFIL3	KLF9	MXI1	CEBPG
MLX	HMBOX1	ZBTB7A	RARA
ATF7	NRF1	MYC	HNH4G
NFYA	KLF4	ESRRA	TEAD2
MEF2B	HEY2	ZBTB7B	ZBTB33
TGIF2	NR4A2	JUN	GMEB2
GLIS3	THAP1	USF2	NR3C1
NKX3-1	ZNF263	BHLHE40	RORA
SOX9	ETV6	SREBF1	MAFF
POU6F1	RELB	NR2C2	TFCP2
MSC	TFDP1	ZIC1	GABPA
HSF2	NFKB2	TFAP4	GATA6
FOXP1	SP1	MNT	MTF1
ESR1	SP3	ELK3	HESX1
	TEAD1	TFEB	RUNX1
	ZNF740	CLOCK	
	TBX15	NFATC3	
	SPI1	RUNX3	
	RBPJ	CEBPD	
	ELK4	SREBF2	
	HEY1	HIF1A	

Table 5: Prediction of splice sites and splicing enhancers (ESE) alterations by HSF and the ESEfinder software.

Variant	Splice sites	Wild-type CV	Val30Met CV	CV variation	Distance from variant
rs1800458	acceptor site	0	77	77	2 nt downstream
rs36204272	donor site	68.6	0	-68.6	-
rs1791228	branch point	60.2	85	24.8	2 nt downstream
Variant	SR protein	Wild-type MS	Val30Met MS	MS variation	Distance from variant
rs1800458	SC35	0	2.73	2.73	6 nt upstream
	SC35	0	2.67	2.67	2 nt upstream
	SC35	3.02	2.57	-0.45	7 nt upstream
	SF2/ASF site	0	1.99	1.99	6 nt upstream
	SF2/ASF site	4.64	2.73	-1.91	4 nt upstream
	SF2/ASF (IgM-BRCA1) site	4.56	2.86	-1.70	4 nt upstream
rs28933981	SRp40	2.81	0	-2.81	6 nt upstream
	SRp55	3.89	0	-3.89	2 nt upstream
	SF2/ASF site	2.03	0	-2.03	-
	SF2/ASF (IgM-BRCA1) site	2.87	0	-2.87	-
rs62093482	SC35	3.73	3.5	-0.23	3 nt upstream
rs1791228	SRp40	3.05	3.71	0.66	3 nt upstream
	SF2/ASF (IgM-BRCA1) site	2.33	2.58	0.25	1 nt upstream

CV, Consensus value; MS, Matrix score; nt, nucleotides; SR protein: Serine and arginine-rich (SR) proteins; SC35, Serine/arginine-rich splicing factor; SF2/ASF, pre-mRNA-splicing factor SF2/alternative splicing factor; SRp40, Splicing factor, arginine/serine rich 40 kDa; SRp55, Splicing factor, arginine/serine rich 55 kDa.