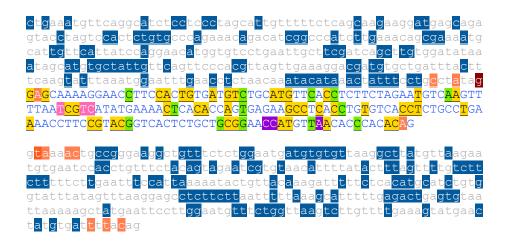**Ejemplo comparación de resultados predictores in sillico**

Cambio de estudio CTCFL c.755-1G>T (chr20:57519378 G/T, NM_001269040.2:c.755-1G>T)

Exón 3 e intrones adyacentes:

```
ctgaaatgttcaggcatctcctccctagcattgtttttctcagcaagaaggatgagcaga
gtacctagtccactctgtgcccagaaacagacatcggcccatcttgaaacagcgaaaatg
cattgttcattatccaggaacatggtgtcctgaattgcttcgatcagcttgtggatataa
atagcatgtgctattgttcagttcccacgttagttgaaaggacgatgtgctgatttactt
tcaagtatttaaatggaatttgaacctctaacaaatacataaactatttcctgcctatag
GAGCAAAAGGAACCTTCCACTGTGATGTCTGCATGTTCACCTCTTCTAGAATGTCAAGTT
TTAATCGTCATATGAAAACTCACACCAGTGAGAAGCCTCACCTGTGTCACCTCTGCCTGA
AAACCTTCCGTACGGTCACTCTGCTGCGGAACCATGTTAACACCCACACAG

gtaaaactgccgggaaggctgtttctctggaatgatgtgtgttaaggcttatgttaagaa
tgtgaatccacctgtttctacagtagaatcgtgtaacatttatactttagttttgtcttt
cttttcttgaatttccattaaaaatactgttacaaagatttttctcacatgcatctgtg
gtatttatagtttaaggagcctcttcttaatttttaaagcattttgagactgagtgtaa
ttaaaaagctatgaattcctggaatgtttctggttaagtcttgttttgaaagtatgaac
tatgtgattttacag
```

El cambio se encuentra en posición justo antes del exón 3 (la **g** en color granate).

Se va a obtener los resultados que produce analizar esta variable con los diferente predictores y ver cuál de ellos es más preciso.

NetGene2

```
Donor splice sites, direct strand
---------------------------------
          pos 5'->3'  phase strand  confidence  5'       exon intron     3'
            472          1     +        0.82        ACCCACACAG^GTAAAACTGC

Donor splice sites, complement strand
-------------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'       exon intron     3'
    401          386         0     -        0.87        GGTGACACAG^GTGAGGCTTC

Acceptor splice sites, direct strand
------------------------------------
          pos 5'->3'  phase strand  confidence  5'      intron exon       3'
            43           2     +        0.00        TTTTTCTCAG^CAAGAAGGAT
            112          2     +        0.07        CTTGAAACAG^CGAAAATGCA
            300          1     +        0.17        CTGCCTATAG^GAGCAAAAGG
            349          2     +        0.15        CCTCTTCTAG^AATGTCAAGT

Acceptor splice sites, complement strand
----------------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      intron exon       3'
    295          492         1     -        0.16        GCTCCTATAG^GCAGGAAATA
    191          596         1     -        0.07        TGAACAATAG^CACATGCTAT
    168          619         0     -        0.17        TATCCACAAG^CTGATCGAAG
```

```
Donor splice sites, direct strand
---------------------------------
          pos 5'->3'  phase strand  confidence  5'       exon intron     3'
            472          1     +        0.82        ACCCACACAG^GTAAAACTGC

Donor splice sites, complement strand
-------------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'       exon intron     3'
    401          386         0     -        0.87        GGTGACACAG^GTGAGGCTTC

Acceptor splice sites, direct strand
------------------------------------
          pos 5'->3'  phase strand  confidence  5'      intron exon       3'
            43           2     +        0.00        TTTTTCTCAG^CAAGAAGGAT
            112          2     +        0.07        CTTGAAACAG^CGAAAATGCA

Acceptor splice sites, complement strand
----------------------------------------
  pos 3'->5'  pos 5'->3'  phase strand  confidence  5'      intron exon       3'
    191          596         1     -        0.07        TGAACAATAG^CACATGCTAT
    168          619         0     -        0.17        TATCCACAAG^CTGATCGAAG
```

Se pierden dos sitios *acceptor* en la hebra directa y uno en la reversa (en rojo) en la secuencia mutante. El primero (en 300) coincide con el sitio *acceptor* del exón, mientras que el siguiente está en el interior del exón. Por lo tanto, es probable que esté afectando al *splicing*. Dados los niveles de *confidence* de los otros *acceptor*, es probable que se use el *acceptor* del siguiente exón, produciéndose *exon skipping.*

Splice Site Prediction by Neural Network (NNSplice)

Donor site predictions for 85.53.15.54.25403.0 :

Start	End	Score	Exon	Intron
465	479	0.95	cacacag**gt**aaaact	

Acceptor site predictions for 85.53.15.54.25403.0 :
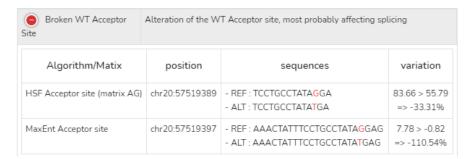
Start	End	Score	Intron	Exon
23	63	0.95	ccctagcattgtttttctc**ag**caagaaggatgagcagagta	
117	157	0.46	aatgcattgttcattatcc**ag**gaacatggtgtcctgaattg	
181	221	0.46	atagcatgtgctattgttc**ag**ttcccacgttagttgaaagg	
280	320	0.82	taaactatttcctgcctat**ag**gagcaaaaggaaccttccac	
329	369	0.77	ctgcatgttcacctcttct**ag**aatgtcaagtttaatcgtc	

Donor site predictions for 85.53.15.54.25733.0 :

Start	End	Score	Exon	Intron
465	479	0.95	cacacag**gt**aaaact	

Acceptor site predictions for 85.53.15.54.25733.0 :

Start	End	Score	Intron	Exon
23	63	0.95	ccctagcattgtttttctc**ag**caagaaggatgagcagagta	
117	157	0.46	aatgcattgttcattatcc**ag**gaacatggtgtcctgaattg	
181	221	0.46	atagcatgtgctattgttc**ag**ttcccacgttagttgaaagg	
329	369	0.77	ctgcatgttcacctcttct**ag**aatgtcaagtttaatcgtc	

Se pierde uno de los sitios *acceptor* (en rojo) en la secuencia mutante. Este sitio coincide con el sitio *acceptor* del exón, por lo que es probable que esté afectando al *splicing*.

Spliceman

Point mutation	Wildtype (wt)	Mutation (mt)	L1 distance	Ranking (L1)	
ctata(g/t)gagca	tatagg	tatatg	27975	61%	

Human Splicing Finder

	Broken WT Acceptor Site	Alteration of the WT Acceptor site, most probably affecting splicing

Algorithm/Matix	position	sequences	variation
HSF Acceptor site (matrix AG)	chr20:57519389	- REF : TCCTGCCTATAGGA - ALT : TCCTGCCTATATGA	83.66 > 55.79 => -33.31%
MaxEnt Acceptor site	chr20:57519397	- REF : AAACTATTTCCTGCCTATAGGAG - ALT : AAACTATTTCCTGCCTATATGAG	7.78 > -0.82 => -110.54%

SVM-BPfinder (no hay cambios)

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
wt	14	482	tgttcagtt	-0.936103376215	0.545073375262	34	9	20	-1.7545342
wt	14	470	acgttagtt	-4.16710626799	0.541935483871	22	9	20	-2.261058
wt	14	465	agttgaaag	-1.23803372578	0.54347826087	17	9	20	-0.79719788
wt	14	449	tgctgattt	1.36450697501	0.551801801802	1	9	20	1.2372812
wt	14	445	gatttactt	-1.78346790692	0.55	47	15	25	-2.8610252
wt	14	439	ctttcaagt	-1.83772196331	0.546082949309	41	15	25	-2.5037434
wt	14	431	tatttaaat	-3.73657653647	0.546948356808	33	15	25	-2.7405686
wt	14	430	atttaaatg	-1.42149293897	0.548235294118	32	15	25	-1.77039
wt	14	419	atttgaacc	-0.857433885774	0.553140096618	21	15	25	-0.85166862
wt	14	411	ctctaacaa	2.16290643036	0.551724137931	13	15	25	0.83686663
wt	14	400	acataaact	-1.3386580836	0.556962025316	2	15	25	0.16381184
wt	14	357	ctgtgatgt	1.01399465301	0.5625	9	14	27	0.66231865
wt	14	343	tgttcacct	1.498804313	0.559171597633	57	23	33	-2.1313548
wt	14	326	atgtcaagt	-2.67314196672	0.554517133956	40	23	33	-2.6903007
wt	14	319	gttttaatc	-3.85299618365	0.554140127389	33	23	33	-2.7093029
wt	14	318	ttttaatcg	0.161437794563	0.555910543131	32	23	33	-1.0735922
wt	14	312	tcgtcatat	-1.77637254255	0.553745928339	26	23	33	-1.4532457
wt	14	307	atatgaaaa	-2.65644077255	0.556291390728	21	23	33	-1.4805203
wt	14	300	aactcacac	1.45800836703	0.559322033898	14	23	33	0.57454809
wt	14	291	cagtgagaa	-1.46322150475	0.562937062937	5	23	33	0.0016009269
wt	14	282	gcctcacct	2.4775368871	0.56678700361	1	18	26	1.7338199
wt	14	273	gtgtcacct	0.35371237824	0.563432835821	1	9	17	0.81731631
wt	14	262	gcctgaaaa	0.0969378054342	0.556420233463	16	10	16	-0.2442786
wt	14	244	cggtcactc	0.975936811446	0.556485355649	52	10	22	-2.1229324
wt	14	223	atgttaaca	-3.38532897759	0.55504587156	31	10	22	-2.5017743
wt	14	222	tgttaacac	1.89294176597	0.557603686636	30	10	22	-0.37095739
wt	14	207	aggtaaaac	-0.453647565016	0.564356435644	15	10	22	-0.33810223
wt	14	176	gaatgatgt	0.733410065584	0.584795321637	1	8	9	0.8983586
wt	14	167	gtgttaagg	-3.52948988474	0.586419753086	23	15	24	-2.023069
wt	14	166	tgttaaggc	-0.713850267615	0.590062111801	22	15	24	-0.85613826
wt	14	160	ggcttatgt	-0.528493855973	0.593548387097	16	15	24	-0.4026466
wt	14	155	atgttaaga	-4.44269790822	0.593333333333	11	15	24	-1.6188204
wt	14	154	tgttaagaa	-1.52449890476	0.597315436242	10	15	24	-0.41162308
wt	14	146	atgtgaatc	-0.786235863209	0.617021276596	2	15	24	0.39019278
wt	14	115	gtgtaacat	1.23064209505	0.636363636364	1	33	64	1.6220749
wt	14	108	attttatac	-3.89107634378	0.631067961165	1	26	54	-0.47818868
wt	14	101	actttagtt	-4.94576733473	0.625	2	18	46	-1.0309349
wt	14	80	tcttgaatt	-1.31875938321	0.573333333333	2	8	17	0.1023638
wt	14	70	ccattaaaa	-3.16084715997	0.553846153846	19	10	22	-1.6546866
wt	14	69	cattaaaaa	-0.940681067348	0.5625	18	10	22	-0.7192934
wt	14	58	ctgttacaa	-1.93776069672	0.584905660377	7	10	22	-0.4061793
wt	14	43	ttctcacat	1.3220729687	0.578947368421	14	7	11	0.32271276
wt	14	24	tatttatag	-3.24663928808	0.578947368421	19	0	0	-1.8851199
wt	14	17	agttttaagg	-4.37973679438	0.583333333333	12	0	0	-1.8842764
wt	14	16	gtttaagga	-2.60300243808	0.636363636364	11	0	0	-1.1081754

seq_id	agez	ss_dist	bp_seq	bp_scr	y_cont	ppt_off	ppt_len	ppt_scr	svm_scr
mut	14	482	tgttcagtt	-0.936103376215	0.547169811321	34	9	20	-1.7538571
mut	14	470	acgttagtt	-4.16710626799	0.544086021505	22	9	20	-2.2603635
mut	14	465	agttgaaag	-1.23803372578	0.545652173913	17	9	20	-0.79649578
mut	14	449	tgctgattt	1.36450697501	0.554054054054	1	9	20	1.2380086
mut	14	445	gatttactt	-1.78346790692	0.552272727273	47	15	25	-2.8602912
mut	14	439	ctttcaagt	-1.83772196331	0.548387096774	41	15	25	-2.5029992
mut	14	431	tatttaaat	-3.73657653647	0.549295774648	33	15	25	-2.7398105
mut	14	430	atttaaatg	-1.42149293897	0.550588235294	32	15	25	-1.7696301
mut	14	419	atttgaacc	-0.857433885774	0.555555555556	21	15	25	-0.85088849
mut	14	411	ctctaacaa	2.16290643036	0.554187192118	13	15	25	0.83766212
mut	14	400	acataaact	-1.3386580836	0.559493670886	2	15	25	0.16462949
mut	14	380	atatgagca	-1.84275007492	0.554666666667	11	9	17	-0.67851698
mut	14	357	ctgtgatgt	1.01399465301	0.5625	9	14	27	0.66231865
mut	14	343	tgttcacct	1.498804313	0.559171597633	57	23	33	-2.1313548
mut	14	326	atgtcaagt	-2.67314196672	0.554517133956	40	23	33	-2.6903007
mut	14	319	gttttaatc	-3.85299618365	0.554140127389	33	23	33	-2.7093029
mut	14	318	ttttaatcg	0.161437794563	0.555910543131	32	23	33	-1.0735922
mut	14	312	tcgtcatat	-1.77637254255	0.553745928339	26	23	33	-1.4532457
mut	14	307	atatgaaaa	-2.65644077255	0.556291390728	21	23	33	-1.4805203
mut	14	300	aactcacac	1.45800836703	0.559322033898	14	23	33	0.57454809
mut	14	291	cagtgagaa	-1.46322150475	0.562937062937	5	23	33	0.0016009269
mut	14	282	gcctcacct	2.4775368871	0.56678700361	1	18	26	1.7338199
mut	14	273	gtgtcacct	0.35371237824	0.563432835821	1	9	17	0.81731631
mut	14	262	gcctgaaaa	0.0969378054342	0.556420233463	16	10	16	-0.2442786
mut	14	244	cggtcactc	0.975936811446	0.556485355649	52	10	22	-2.1229324
mut	14	223	atgttaaca	-3.38532897759	0.55504587156	31	10	22	-2.5017743
mut	14	222	tgttaacac	1.89294176597	0.557603686636	30	10	22	-0.37095739
mut	14	207	aggtaaaac	-0.453647565016	0.564356435644	15	10	22	-0.33810223
mut	14	176	gaatgatgt	0.733410065584	0.584795321637	1	8	9	0.8983586
mut	14	167	gtgttaagg	-3.52948988474	0.586419753086	23	15	24	-2.023069
mut	14	166	tgttaaggc	-0.713850267615	0.590062111801	22	15	24	-0.85613826
mut	14	160	ggcttatgt	-0.528493855973	0.593548387097	16	15	24	-0.4026466
mut	14	155	atgttaaga	-4.44269790822	0.593333333333	11	15	24	-1.6188204
mut	14	154	tgttaagaa	-1.52449890476	0.597315436242	10	15	24	-0.41162308
mut	14	146	atgtgaatc	-0.786235863209	0.617021276596	2	15	24	0.39019278
mut	14	115	gtgtaacat	1.23064209505	0.636363636364	1	33	64	1.6220749
mut	14	108	attttatac	-3.89107634378	0.631067961165	1	26	54	-0.47818868
mut	14	101	actttagtt	-4.94576733473	0.625	2	18	46	-1.0309349
mut	14	80	tcttgaatt	-1.31875938321	0.573333333333	2	8	17	0.1023638
mut	14	70	ccattaaaa	-3.16084715997	0.553846153846	19	10	22	-1.6546866
mut	14	69	cattaaaaa	-0.940681067348	0.5625	18	10	22	-0.7192934
mut	14	58	ctgttacaa	-1.93776069672	0.584905660377	7	10	22	-0.4061793
mut	14	43	ttctcacat	1.3220729687	0.578947368421	14	7	11	0.32271276
mut	14	24	tatttatag	-3.24663928808	0.578947368421	19	0	0	-1.8851199
mut	14	17	agttttaagg	-4.37973679438	0.583333333333	12	0	0	-1.8842764
mut	14	16	gtttaagga	-2.60300243808	0.636363636364	11	0	0	-1.1081754

Variant Effect Predictor tool

ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000243914.7	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000371196.6	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, NMD_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000422109.6	nonsense_mediated_decay	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000422869.6	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000423479.7	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, NMD_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000426658.6	nonsense_mediated_decay	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000429804.7	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000432255.6	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000433949.7	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000481655.2	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000502686.6	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000539382.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, non_coding_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000607923.5	retained_intron	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, non_coding_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608108.1	retained_intron	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608158.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608263.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608425.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608440.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, NMD_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608720.5	nonsense_mediated_decay	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant, non_coding_transcript_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608858.5	processed_transcript	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000608903.5	protein_coding	-	-1	-
ENST00000432255.6:c.755-1G>T	20:57519378-57519378	A	splice_acceptor_variant	CTCFL	ENSG00000124092	Transcript	ENST00000609232.5	protein_coding	-	-1	-

Se trata de una mutación que afecta al *splicing*, concretamente al *acceptor* del exón.

ESEfinder

Solo se obtiene un resultado positivo con la posición de interés en las matrices 3'SS:

| 286 (-501) | atttcctgcctatagGAGCAAAAGGAACCT | -14.48960 | | 286 (-501) | atttcctgcctatagGAGCAAAAGGAACCT | 9.58400 | | 286 (-501) | atttcctgcctatagGAGCAAAAGGAACCT | -11.08790 | | 286 (-501) | atttcctgcctatagGAGCAAAAGGAACCT | 9.51010 |

Sin embargo, estas puntuaciones en la secuencia mutante son negativas:

| 286 (-501) | atttcctgcctatatGAGCAAAAGGAACCT | -18.45940 | | 286 (-501) | atttcctgcctatatGAGCAAAAGGAACCT | -2.50360 | | 286 (-501) | atttcctgcctatatGAGCAAAAGGAACCT | -15.01110 | | 286 (-501) | atttcctgcctatatGAGCAAAAGGAACCT | -3.44330 |

Por lo tanto, la mutación está generando la pérdida de un sitio *acceptor,* afectando al *splicing.*