Exonic splicing variant

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Clinical Details

Clinical features

- **Chronic axonal neuropathy**
  - Gross motor delay with progressive deterioration since 5 yo
  - Progressive motor weakness and loss of walking ability
- **Mild cognitive impairment**

Pathology testing

- Nerve/muscle biopsy:
  - **chronic axonal neuropathy**
  - **neurogenic muscle atrophy**

One frameshift variant in COX20 *in trans* with **NM_198076.6(*COX20*):c.41A>G p.(Lys14Arg)**

- Chr1: 244,835,755 (GRCh38)
- Rs ID : rs1057521790
- Located in exon 1 of 4

- Inheritance: Unknown

	Zygosity
Proband	Heterozygous
Mother	Homozygous ref

- GnomAD (v.2.1.1)= 1 in 41838
- GnomAD (v.3.1.2)= 7 in 148900

How should we classify this exonic COX20 variant?

Gene-disease association

- OMIM

  Mitochondrial complex IV deficiency, nuclear type 11

Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
1q44	Mitochondrial complex IV deficiency, nuclear type 11	619054	AR	3	COX20	614698

INHERITANCE
- Autosomal recessive

GROWTH

Height
- Short stature

Other
- Poor overall growth

HEAD & NECK

Neck
- Torticollis

MUSCLE, SOFT TISSUES
- Hypotonia

NEUROLOGIC

Central Nervous System
- Delayed walking (in some patients)
- Normal early development
- Speech delay (1 patient)
- Normal cognition
- Gait difficulties
- Loss of ambulation
- Cerebellar ataxia
- Dystonia
- Tremor
- Choreoathetosis
- Cerebellar atrophy on brain imaging

Peripheral Nervous System
- Sensory axonal neuropathy
- Foot drop

LABORATORY ABNORMALITIES
- Increased serum and CSF lactate
- Mitochondrial respiratory complex IV deficiency in patient tissues

MISCELLANEOUS
- Onset in childhood or adolescence
- Slowly progressive
- Variable severity
- Two unrelated families have been reported (last curated October 2020)

Patient's phenotypes

- Chronic axonal neuropathy (HP:0007267)
- Progressive muscle weakness (HP:0003323)

***COX20*-associated Mitochondrial complex IV deficiency, nuclear type 11 matches with patient's phenotypes**

Multiple *in silico* prediction tools predict the variant to be benign

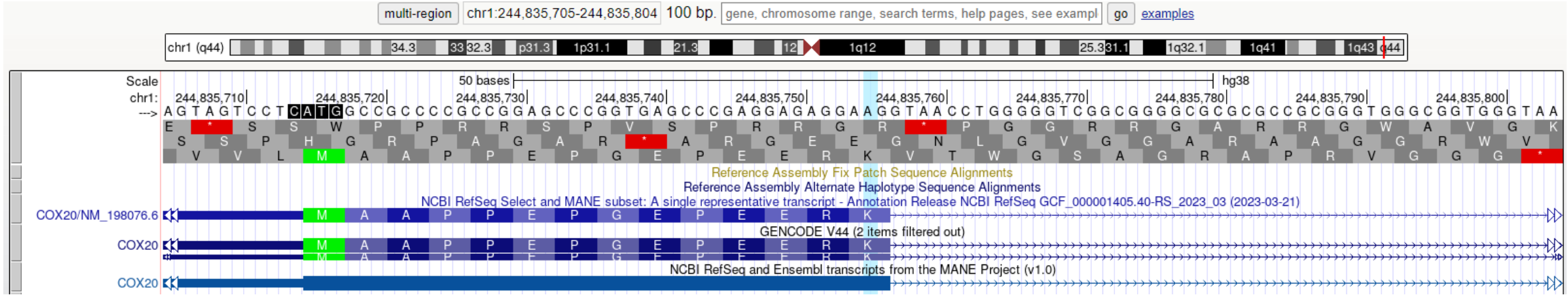
In-Silico Predictors

Meta scores 14

Show raw data | Alphabetically

Engine	Calibrated Prediction	Score	Version
REVEL	Benign Strong	0.078, 0.078	*dbNSFP version 4.3*
BayesDel addAF	Benign Moderate	addAF score -0.1523	*dbNSFP version 4.3*
BayesDel noAF	Benign Moderate	noAF score -0.4566	*dbNSFP version 4.3*
MetaLR	Benign Moderate	0.1052	*dbNSFP version 4.3*
MetaRNN	Benign Moderate	0.1281, 0.1334	*dbNSFP version 4.3*
MetaSVM	Benign Moderate	-1.0144	*dbNSFP version 4.3*

Further analysis reveals the variant locates near exon junction



MaxEntScan Uncertain Δ 3.8367

Δ type	Δ score ⓘ	pre-mRNA position ⓘ
Acceptor Loss	0.00	
Donor Loss	0.86	1 bp
Acceptor Gain	0.00	405 bp
Donor Gain	0.22	-19 bp

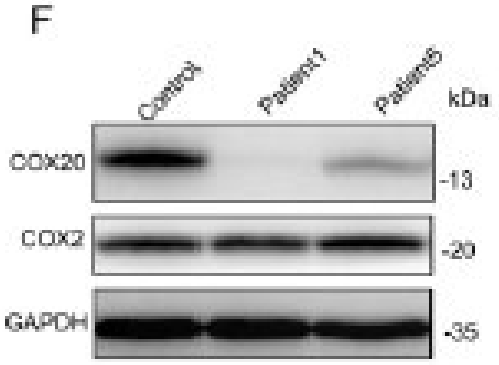
- **Prediction tools (MaxEntScan and SpliceAI) both predicts the variant to cause loss of splice donor**

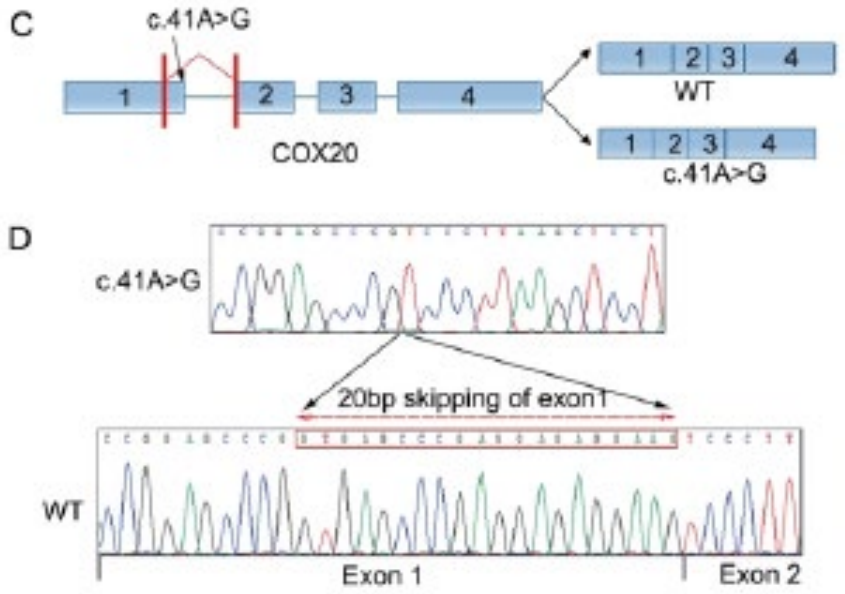
→ Search literature for functional evidence

c.41A>G causes 20bp skipping of exon 1 and result in frameshifting of *COX20* in multiple unrelated patients

Bi-allelic loss of function variants in *COX20* gene cause autosomal recessive sensory neuronopathy

Hai-Lin Dong,[1] Yin Ma,[1] Hao Yu,[1] Qiao Wei,[1] Jia-Qi Li,[1] Gong-Lu Liu,[1] Hong-Fu Li,[1] Lei Chen,[1,2] Dian-Fu Chen,[1,2] Ge Bai[1,2,3] and Zhi-Ying Wu[1,2,3,4]





- results in p.(Gly8ValfsTer2) and is predicted to undergo NMD
- reduced COX20 protein
- PVS1 (RNA)

Patient1: homozygous c.41A>G
Patient5: heterozygous c.41A>G + 1 p.W74C

Overall classification of c.41A>G :

Pathogenic

PVS1 (RNA), PM3, PP4, PM2_Supporting

Bi-allelic loss of function variants in COX20 gene cause autosomal recessive sensory neuronopathy. Brain. 2021. PMID: 33751098

In class exercise

- NM_001101426.4(*CRPPA*):c.1251G>A
  - Patient with on set of muscular dystrophy since childhood
  - Answer: Pathogenic, PM3_VeryStrong, PVS1_Moderate (RNA), PM2_Supporting

- NM_004006.3(*DMD*):c.3603G>T
  - Patient clinically diagnosed with Duchenne Muscular Dystrophy with negative DMD IHC staining in muscle biopsy
  - Answer: VUS, PP3, PM2_Supporting, PP4
  - Potential upgrade:
    - Sequencing parental samples to test for de novo occurance
    - Muscle biopsy for RNA-seq to confirm splicing effect

Supplementary slides for exercise cases

NM_001101426.4(*CRPPA*):c.1251G>A p.(Gln417=)

- Genomic coordinate (GRCh38): chr7-16216066-C-T
- Located at exon 9 of 10
- Inheritance – paternal:

	Zygosity
Proband	Heterozygous
Mother	Homozygous for WT
Father	Heterozygous

- GnomAD (v.3.1.2): 0
- GnomAD (v.2.1.1): 0

- ACMG classification: pathogenic (PM3_VeryStrong, PP3, PM2_Supporting)

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Reported variant effect: Exon 9 skipping

PMID: 31909476

Received: 30 October 2019 | Revised: 10 December 2019 | Accepted: 11 December 2019
DOI: 10.1111/cge.13695

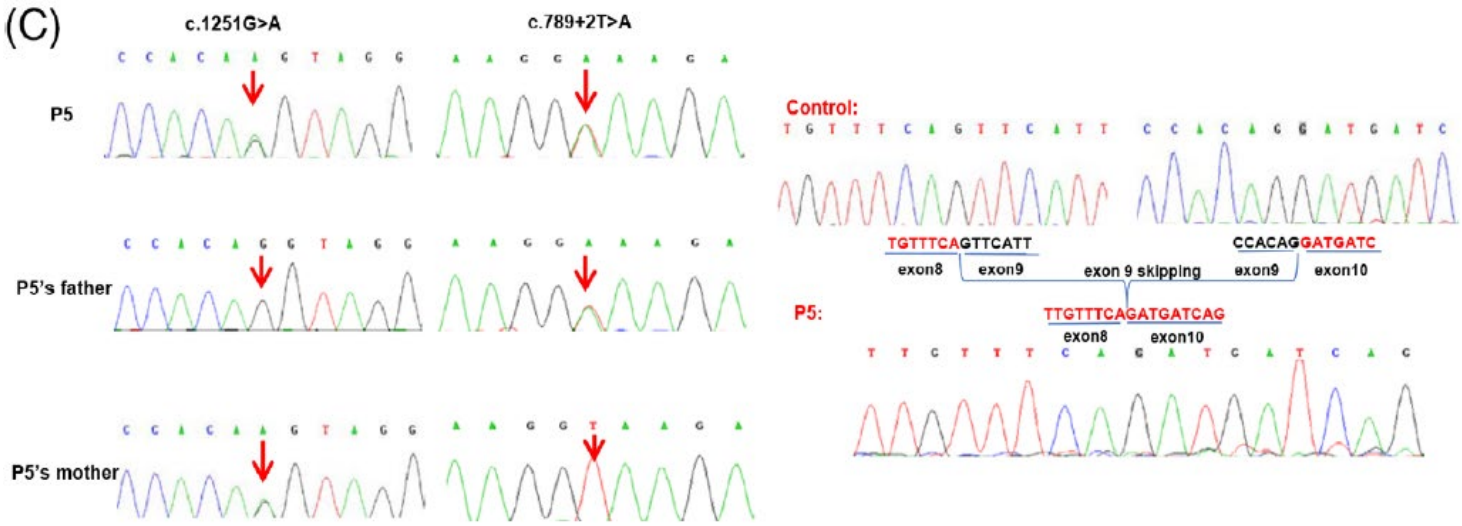
CLINICAL GENETICS WILEY

LETTER TO THE EDITOR

A splice site mutation c.1251G>A of *ISPD* gene is a common cause of congenital muscular dystrophy in Chinese patients

Five unrelated cases were detected to have the same unreported synonymous mutation (Hg19, g.chr7:16255691, c.1251G>A). The G to A transition located on the last nucleotide of exon 9 of *ISPD* was predicted to affect exon splicing. We performed reverse transcription-polymerase chain reaction to analyze the mRNA and confirmed our hypothesis. The full-length cDNA was amplified with two designed primers, one located in junction of exons 7 and 8 of *ISPD* (primer F 5′-TTGTTTGTGTGAATGTTACAACCTC-3′) and the other in exon 10 (primer R 5′-GCACAATTAAGATACGCAAATAGAT-3′). Direct sequencing of products revealed exon 9 skipping (Figure 1) caused by

The human *ISPD* protein has two domains: an N-terminal cytidyltransferase domain (exon1-5) and a C-terminal domain (exon6-10).[5] Although c.1251G>A causing exon 9 skipping influenced the structure of the non-catalytic domain, the patients with c.1251G>A and another truncating mutation have obvious mental retardation and brain involvement. Therefore, we predict that exon 9 also plays an important role in *ISPD*. The non-catalytic domain and catalytic domain are both functionally important.



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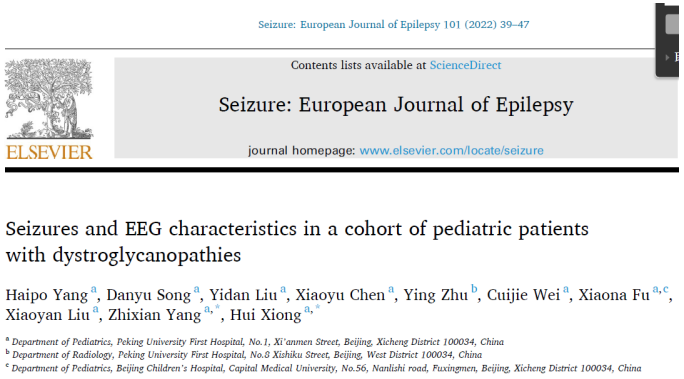
Evidence of *in trans* occurrence

PMID: 31909476

Patient	Exon(s)	Nucleotide change	Predicted amino acid change	Variation type	Parental derivation	Reported/ novel	Polyphen2 (score)	Mutation taster	SIFT (score)	Phenotype
4	3	c.659A>T	p.Asp220Val	missense	mother	Novel	Probably damaging (0.996)	Disease causing	Damaging (0.02)	CMD
	9	c.1251G>A	p.Val374_Gln417del	splicing	father	Novel	/	/	/	
5	4	c.789+2T>G	/	splicing	father	Reported	/	/	/	MEB
	9	c.1251G>A	p.Val374_Gln417del	splicing	mother	Novel	/	/	/	
6	7	c.990delC	p.Ile331Serfs*2	frameshift	mother	Novel	/	/	/	MEB
	9	c.1251G>A	p.Val374_Gln417del	splicing	father	Novel	/	/	/	
7	6-9	exon6-9 del	/	CNV	mother	Novel	/	/	/	CMD with MR
	9	c.1251G>A	p.Val374_Gln417del	splicing	father	Novel	/	/	/	
8	7	c.1026+1G>A	/	splicing	father	Novel	/	/	/	CMD/LGMD
	8	c.1114_1116 delGTT	p.Val372del	deletion	mother	Reported	/	/	/	
9	7	c.1026+1G>A	/	splicing	father	Novel	/	/	/	LGMD
	9	c.1124A>G	/	predicted splicing	mother	Novel				
10	8	c.1186G>T	p.Glu396*	nonsense	father	Reported	/	/	/	CMD with MR
	9	c.1251G>A	p.Val374_Gln417del	splicing	mother	Novel	/	/	/	

Dystroglycanopathy causes a wide spectrum of clinical severities. The severe phenotypes include Walker-Warburg syndrome (**WWS**), muscle-eye-brain disease (**MEB**), and Fukuyama congenital muscular dystrophy (**FCMD**).
The clinical features of **WWS** include severe muscle weakness, death in infancy, absent psychomotor development, neuronal migration disorder, and ocular abnormalities.
The clinical features of **MEB** include severe muscle weakness, mental retardation, epilepsy, neuronal migration disorder, and ocular abnormalities.
The clinical features of **FCMD** include severe proximal and axial weakness, mental retardation, epilepsy, and neuronal migration disorder.


Seizure: European Journal of Epilepsy 101 (2022) 39–47

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Seizure: European Journal of Epilepsy

journal homepage: www.elsevier.com/locate/seizure

PMID: 35863218

Seizures and EEG characteristics in a cohort of pediatric patients with dystroglycanopathies

Haipo Yang [a], Danyu Song [a], Yidan Liu [a], Xiaoyu Chen [a], Ying Zhu [b], Cuijie Wei [a], Xiaona Fu [a,c], Xiaoyan Liu [a], Zhixian Yang [a,*], Hui Xiong [a,*]

[a] Department of Pediatrics, Peking University First Hospital, No.1, Xi'anmen Street, Beijing, Xicheng District 100034, China
[b] Department of Radiology, Peking University First Hospital, No.8 Xishiku Street, Beijing, West District 100034, China
[c] Department of Pediatrics, Beijing Children's Hospital, Capital Medical University, No.56, Nanlishi road, Fuxingmen, Beijing, Xicheng District 100034, China

13	*ISPD*	c.724C>T c.1251G>A	P. Q242X p.Val374_Gln417del	MEB	Unreported

Evidence of *in trans* occurrence

PMID	Variants in trans with c.1251G>A	Protein change	Clinical phenotype	Evidence	Classification	Point
31909476	c.659A>T	p.Asp220Val	CMD	PP3, PM2_supporting	VUS	0.25
31909476	c.789+2T>G	/	MEB	PVS1, PM2_supporting	LP	1
35863218	c.724C>T	p. Gln242*	MEB	PVS1, PM2_supporting	LP	1
31909476	c.990delC	p.Ile331Serfs*2	MEB	PVS1, PM2_supporting	LP	1
31909476	c.1186G>T	p.Glu396*	CMD	PVS1, PM2_supporting	LP	1

Table 1. Points awarded per in *trans* proband

Classification/Zygosity of other variant [1]	Points per Proband	
	Confirmed in *trans*	Phase unknown
Pathogenic or Likely pathogenic variant	1.0	0.5 (P) 0.25 (LP)
Homozygous occurrence *(max point 1.0)*	0.5	N/A
Uncertain significance variant *(max point 0.5)*	0.25	0.0

[1]All variants should be sufficiently rare (meet PM2 specification); P - Pathogenic; LP - Likely pathogenic

Table 2. Recommendation for determining the appropriate evidence strength level for PM3

PM3_Supporting	PM3	PM3_Strong	PM3_VeryStrong
0.5	1.0	2.0	4.0

The variant has been detected *in trans* with likely pathogenic variants in 5 unrelated patients (PM3_VeryStrong)

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ACMG Classification for NM_001101426.4(*CRPPA*):c.1251G>A p.(Gln417=)

1. The variant is found *in trans* in the proband and has been detected *in trans* with a likely pathogenic variant in 4 unrelated patients (PM3_VeryStrong)

2. The variant causes exon 9 skipping which is predicted to cause in-frame deletion of 44 a.a. (PVS1_Moderate (RNA))

3. This variant is absent from gnomAD population databases (PM2_Supporting)

According to ACMG guideline, the variant is classified as pathogenic.

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NM_004006.3(*DMD*):c.3603G>T p.(Lys1201Asn)

- Genomic coordinate (GRCh38):
  ChrX:32454662
- rs ID: rs1265370991
- Located in exon 26 of 79

- GnomAD (v.3.1.2) = Absent
- GnomAD (v.2.1.1) = Absent
- REVEL = 0.197

- ACMG classification: **VUS**

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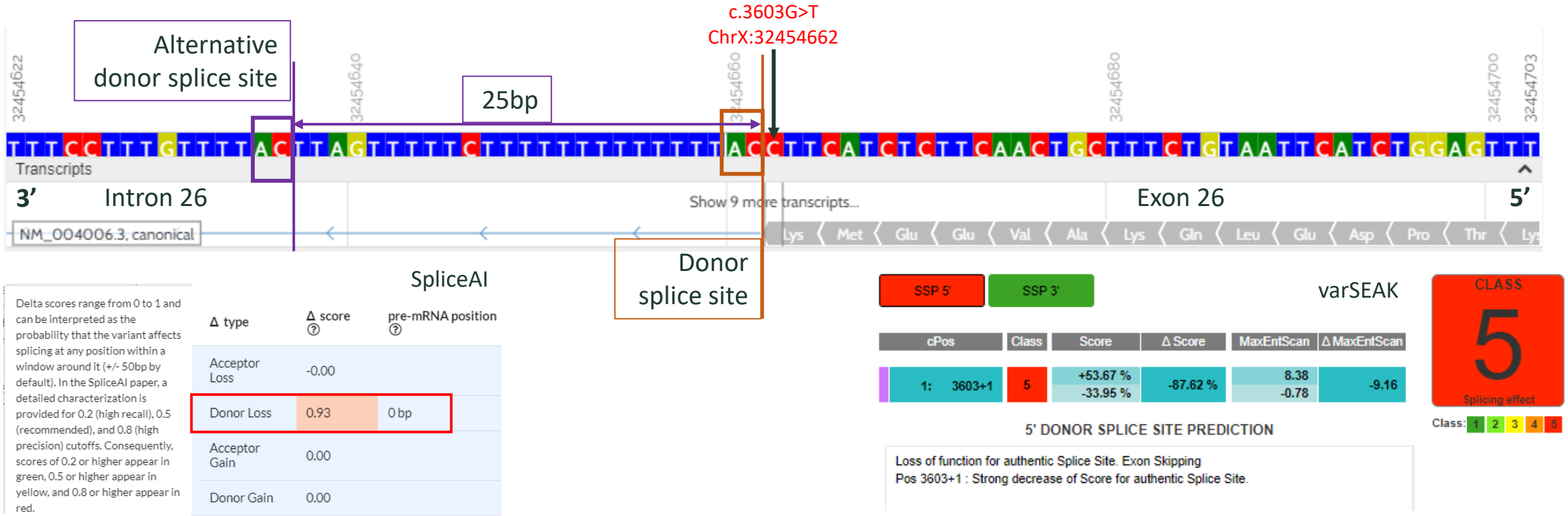
c.3603G>T is Predicted to Affect the Canonical Splice Site



- SpliceAI & varSEAK predicted a high chance to loss the donor splice site on intron 26 followed by the mutation on the last nucleotide of exon 26.

- By manual searching, an alternative splice site is found 25bp downstream from the variant.
  - As 25bp is not divisible by 3, it is predicted to undergo frameshift and nonsense mediated decay.

Silent Variant c.3603G>A is Reported in Literature

Our variant: c.3603G>**T** p.(Lys1201Asn)

> J Mol Diagn. 2010 Jan;12(1):65-73. doi: 10.2353/jmoldx.2010.090074. Epub 2009 Dec 3.

One hundred twenty-one dystrophin point mutations detected from stored DNA samples by combinatorial denaturing high-performance liquid chromatography

Annalaura Torella [1], Amelia Trimarco, Francesca Del Vecchio Blanco, Anna Cuomo, Stefania Aurino, Giulio Piluso, Carlo Minetti, Luisa Politano, Vincenzo Nigro

We screened 153 DNA samples from unrelated DMD or BMD patients. These samples were extracted and studied many years ago without obtaining a genetic diagnosis (Figure 1). We preliminarily excluded deletions or duplications by MLPA and Log-PCR.[3,4]

Table 6. Putative Splicing Defects

Sample	Position	DNA change	Splice site	New	Disease
TU22-TU77	Intron 2	c.94−1 G>A	Acceptor	No	BMD
TU34	Intron 5	c.358−2 A>G	Acceptor	No	DMD
TU296	Intron 5	c.358−2 A>T	Acceptor	No	DMD
TU219	Intron 6	c.530+1 G>A	Donor	Yes	DMD/BMD
TU309	Intron 11	c.1331+2 T>C	Donor	Yes	DMD/BMD
2082	Intron 11	c.1332−9 A>G	Acceptor	No	DMD
TU124	Intron 26	c.3433−1 G>A	Acceptor	No	DMD
TU164	Exon 26	c.3603 G>A	Donor	Yes	DMD
TU105	Intron 35	c.5026−6 A>G	Acceptor	No	DMD
TU332	Intron 48	c.7098+1 G>A	Donor	No	DMD
TU379	Exon 58	c.8668 G>A	Donor	No	DMD
TU114	Intron 58	c.8668+1 G>A	Donor	Yes	DMD

Reported with impact on donor splice site.

> Muscle Nerve. 2012 Dec;46(6):917-25. doi: 10.1002/mus.23481.

Upregulation of chemokines and their receptors in Duchenne muscular dystrophy: potential for attenuation of myofiber necrosis

Boel De Paepe [1], Kim K Creus, Jean-Jacques Martin, Jan L De Bleecker

Patient	Age (y)	Mutation	Muscle damage	Dystrophin	Utrophin
1	8	Exon 2 duplication	Severe	0 to ±	+++
2	19	Exon 26 3603G>A	Severe	ND	ND
3	2	Exon 37 5299–5302duplATTT	Definite	0 to ±	+++
4	8	Exon 63 9470delA	Severe	0 to ± (DYS1), 0 (DYS2,3)	+++
5	3	Exon 65 A9527G	Severe	0 to ± (DYS1), 0 (DYS2,3)	+ to ++
6	4	Exons 46 to 52 deletion	Severe		
7	9	Exon 2 duplication	Severe	0 t	
8	7	Exon 4 198delA	Severe		
9	2	Exons 2 to 15 deletion	Severe	0 to ± (DYS1), 0 (DYS2,3)	++

Did not mention exclusion of deletions/ duplication events by MLPA.

- At the same location, a silent variant c.3603G>**A** p.(Lys1201=) has been reported in **2** DMD affected individuals.
  - One of the case reported with impact on donor splice site without explanation.
- No additional patient information or mutation impact was explained in these studies.

c.3603G>T is Absent from Normal Population Database

Variant is absent from normal population on gnomAD v2.1.1 & v3.1.2 database.

The region is well covered by WGS reads on gnomAD v3.1.2.

PM2_Supporting