

HGGA, Volume 3

Supplemental information

**Prevalence, parameters, and pathogenic mechanisms
for splice-altering acceptor variants
that disrupt the AG exclusion zone**

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Supplemental Information

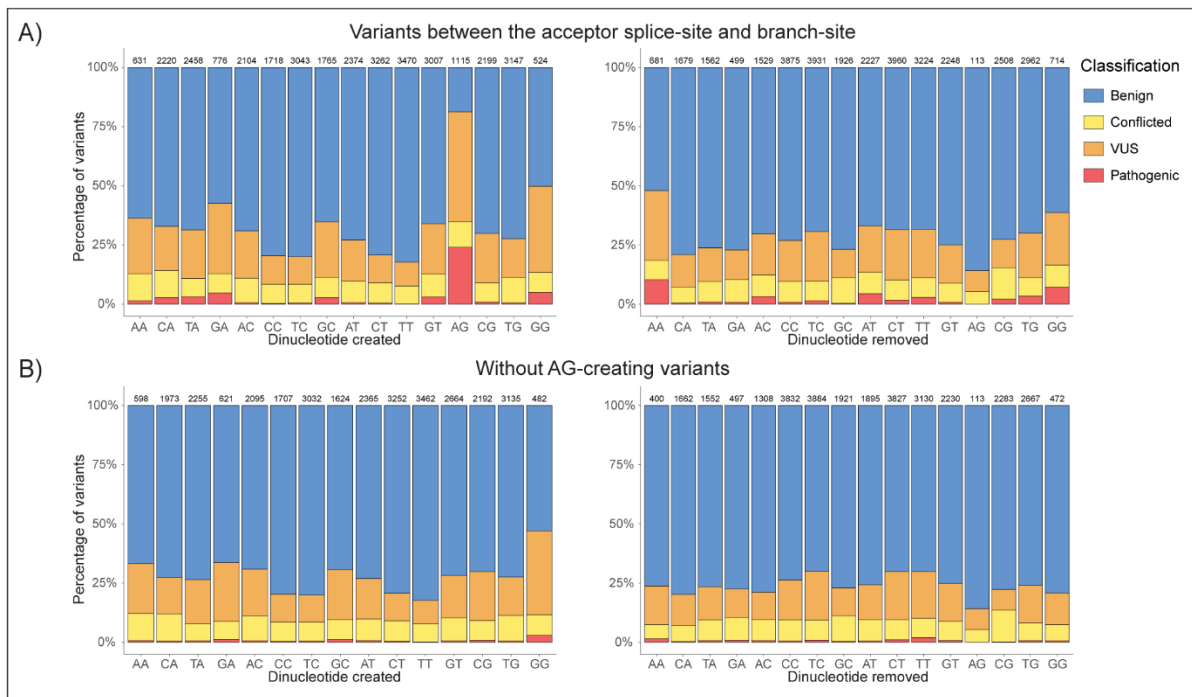


Figure S1. All dinucleotide combinations created and removed by variants in ClinVar and LOVD between the acceptor splice-site and branch-site. A) Each variant from the groups AG-creating, loss of 1Y, loss of $\geq 2Y$ and other (described in Figure 1A) were determined to create and remove dinucleotide combinations. The percentage of variants with each clinical classification are shown for each combination, revealing 24.0% of created AG dinucleotides are pathogenic. All other dinucleotide combinations are 0.1-5.0% pathogenic. As multiple dinucleotide combinations were created or removed by each variant, these groups are not mutually exclusive, eg. an AG-creating variant may simultaneously create a GA dinucleotide and remove an AA dinucleotide. Thus, **B)** this analysis was repeated excluding all AG-creating variants, revealing that 2.9% of created GG dinucleotides are pathogenic and all other groups of created or removed dinucleotides were $\leq 1.1\%$ pathogenic. The total number of variants creating or removing each combination are shown above the graphs.

Table S1. Primer sequences for RT-PCR

Primer name	Sequence (5' to 3')	Product size (normal splicing)	Annealing temperature (°C)/ extension time (s)/No. of cycles	PCR reaction
COL6A2_ Ex26F1 COL6A2_ Ex28R	TCCACGAGAAGCACGAGAG GCCTTGTGGAAGTTCTGCTC	268 bp	58/60/x35	COL6A2 PCR 1
COL6A2_ Ex26F1 COL6A2_ Ex27R	TCCACGAGAAGCACGAGAG CTGTTTGGCAGGGAAGGTC	174 bp	58/60/x35	COL6A2 PCR 2
COL6A2_ Ex26F2 COL6A2_ In27R	CATCGATGACATGGAGGAC TGCTAGTCTGGGGTGGTG	110 bp	58/60/x35	COL6A2 PCR 3
COL6A2_ In26F COL6A2_ Ex28R	AGCCGCTGTCTAGCGTGAG GCCTTGTGGAAGTTCTGCTC	367 bp	58/60/x35	COL6A2 PCR 4
GAPDH_ Ex3F GAPDH_ Ex6R	TCACCAGGGCTGCTTTTAAC GGCAGAGATGATGACCCTTT	317 bp	58/60/x25,x30	GAPDH
SF2_ Ex3F SF2_ Ex4R	CACTGGTGTCTCGTGGAGTTTGTACGG GGGCAGGAATCCACTCCTATG	N/A	58/60/x35	SF2
EGFP_ F DMD_ Ex26_ R	ATCACTCTCGGCATGGACGA GTGTCATCCATTCGTGCATC	298 bp	64/90/x35	DMD PCR 1
DMD_ Ex25/26J_ F DMD_ Ex27_ R	CATGTGCCAACAGGTCTATG CATTACGCCTAGTGCAGAGC	347 bp	64/90/x35	DMD PCR 2
EGFP_ F DMD_ Ex27_ R	ATCACTCTCGGCATGGACGA CATTACGCCTAGTGCAGAGC	541 bp	64/120/x35	DMD PCR 3
Neo_ F Neo_ R	GATGGATTGCACGCAGGTTTC TCAGAGCAGCCGATTGTCTG	86 bp	60/50/x25,x35	NEO

Supplemental Materials and Methods*DMD_{ex25-27} construct sequences*

DMD_{ex25-27} sequences cloned between AsiSI (SgfI)/MluI sites (underlined) of pCMV6-Entry EGFP-DYS. Canonical *DMD* exons 25, 26 and 27 highlighted in blue, EGFP highlighted in green and pseudo-exon highlighted in purple. *DMD* introns 25 and 26 were shortened by 8024 bp and 4301 bp, respectively, relative to the reference (indicated by a blue triangle in figure 7Aii). GRCh37 co-ordinates of partial *DMD* sequences included: chrX:32481711-32481265; chrX:32473240-32471457; chrX:32467155-32466573.

> *DMD_{ex25-27}* (wild-type, W)

**GCGATCGCGGTACCTGCAGGACCGGTCGCCACCATGCGGGATTACAAGGATGACGACG
ATAAGGTGAGCAAGGGCGAGGAGCTGTTACCGGGGTGGTGCCCATCCTGGTTCGAGCTG**

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CTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCC
CACCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACAT
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GGGGCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCGACAAGC
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> *DMD*_{ex25-27} (variant, V)

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> *DMD*_{ex25-27} (wild-type + extra T, +T)

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> *DMD*_{ex25-27} (reverse silencer, GA)

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> *DMD*_{ex25-27} (alternative AG No.1, AG1)

GCGATCGCGGTACCTGCAGGACCGGTCGCCACC ATGGCGGATTACAAGGATGACGACG
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> *DMD*_{ex25-27} (alternative AG No.2, AG2)

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GCCTAATAAAAAACATTTATTTTCATTGCAA**ACGCGT**