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

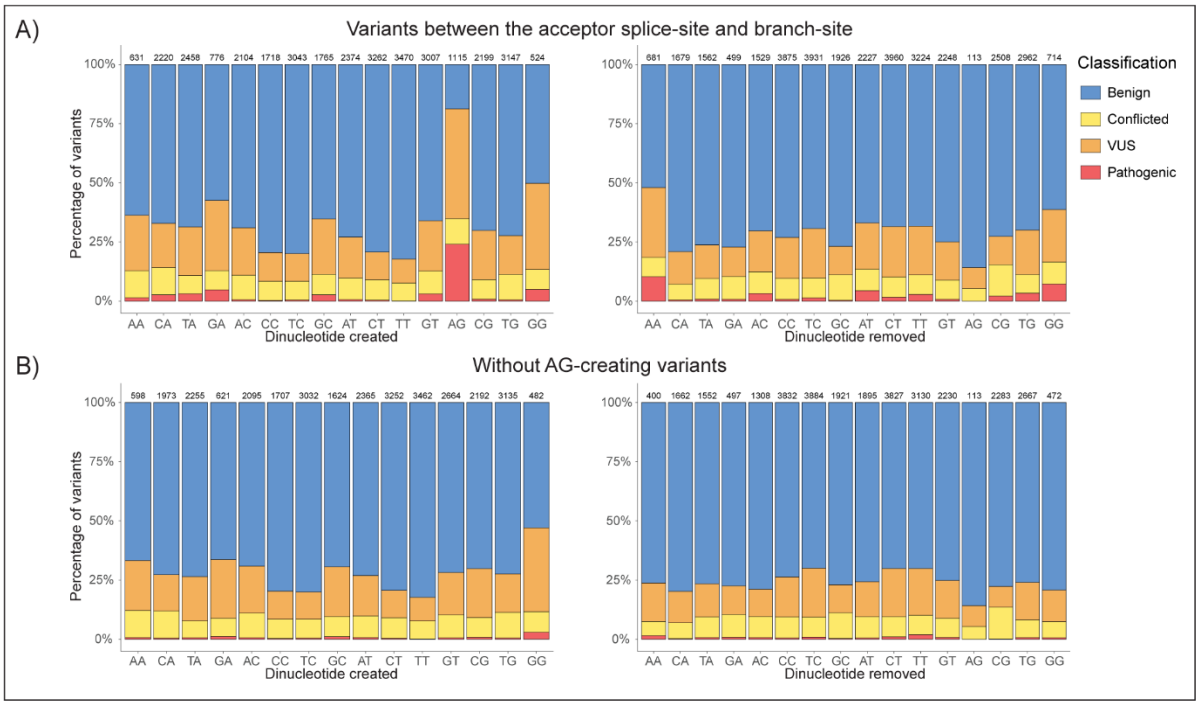

Figure S1. All dinucleotide combinations created and removed by variants in ClinVar and LOVD between the acceptor splice-site and branch-site. A) Each variant from the groups AG-creating, loss of 1Y, loss of ≥2Y and other (described in Figure 1A) were determined to create and remove dinucleotide combinations. 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 ≤ 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	CACTGGTGTCGTGGAGTTTGTACGG 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 CATTCAGCCTAGTGCAGAGC	347 bp	64/90/x35	*DMD* PCR 2
EGFP_F *DMD* Ex27_R	ATCACTCTCGGCATGGACGA CATTCAGCCTAGTGCAGAGC	541 bp	64/120/x35	*DMD* PCR 3
Neo_F Neo_R	GATGGATTGCACGCAGGTTC TCAGAGCAGCCGATTGTCTG	86 bp	60/50/x25,x35	NEO

Supplemental Materials and Methods

$DMD_{ex25\text{-}27}$ construct sequences

$DMD_{ex25\text{-}27}$ 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 7A*ii*). GRCh37 co-ordinates of partial *DMD* sequences included: chrX:32481711-32481265; chrX:32473240-32471457; chrX:32467155-32466573.

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

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CACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACAT

GAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCA

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> *DMD*$_{ex25\text{-}27}$ (variant, V)

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

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GCCTAATAAAAAACATTTATTTTCATTGCAA**ACGCGT**

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

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TTCCCTAAGTCCAACTACTAAACTGGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTC

TGCCTAATAAAAAACATTTATTTTCATTGCAA**ACGCGT**

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

GCGATCGCGGTACCTGCAGGACCGGTCGCCACCATGGCGGATTACAAGGATGACGACG

ATAAGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTG

GACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCAC

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CACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACAT

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GGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGC

AGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG

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TGTACAAGGGCGGCCGCAGC**GCT**GGAGGACTTTTAGTCAGTGATATTCAGACAATTCAG

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GGATGACACAAGCTGAAGAAGAGTATCTTGAGAGAGATTTTGAATATAAAACTCCAGAT

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TTCCCTAAGTCCAACTACTAAACTGGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTC

TGCCTAATAAAAAACATTTATTTTCATTGCAA**ACGCGT**

> *DMD*$_{ex25\text{-}27}$ (alternative AG No.2, AG2)

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GGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGC

AGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTG

CAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCC

CGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCG

ATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGC

TGTACAAGGGCGGCCGCAGC**GCT**GGAGGACTTTTAGTCAGTGATATTCAGACAATTCAG

CCCAGTCTAAACAGTGTCAATGAAGGTGGGCAGAAGATAAAGAATGAAGCAGAGCCAG

AGTTTGCTTCGAGACTTGAGACAGAACTCAAAGAACTTAAC**A**CTCAGTGGGATCACATGT

GCCAACAG**gt**atagacaatctctttcactgtggcttgcctcaacgtacttaactaagatttcctaatgtctcccttcaccgttacttttggttaaggc

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AAGGGAGGTTTGGAGAAAACTGTAAGCCTCCAGAAAGATCTATCAGA**GATGCACGAAT**

GGATGACACAAGCTGAAGAAGAGTATCTTGAGAGAGATTTTGAATATAAAACTCCAGAT

GAATTACAGAAAGCAGTTGAAGAGATGAAG**gt**aaaaaaaaaaaaaagaaaaactaagtaaaacaaaggaaataaatg

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TCCCTAAGTCCAACTACTAAACTGGGGGATATTATGAAGGGCCTTGAGCATCTGGATTCT

GCCTAATAAAAAACATTTATTTTCATTGCAA**A<u>CGCGT</u>**