Classification of Splice Variants

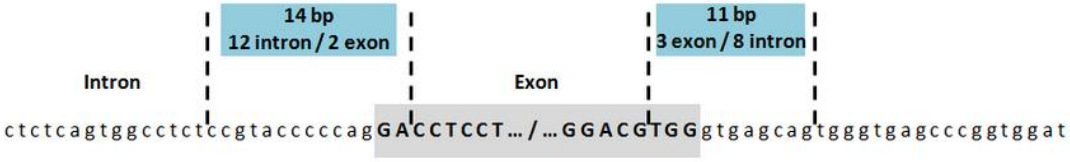
All variants are classified according to the guidelines of the American College for Medical Genetics and Genomics (ACMG).[4] Variants not included in this scheme (e.g., intronic variants with a potential effect on correct splicing) are assessed with a classification system based on the guidelines published by Houdayer et al.[2] in order to prioritize variants for further (e.g., cDNA) analysis.

1. Variants of conserved splice sites (AG/gt or ag/GT)

Variants at the position +/- 1 and 2 are to be classified according to **ACMG Guidelines**[4] (criterium PVS1; see ACMG Classification of Sequence Variants). These variants normally lead to a functional disruption of the gene product. There are, however, exceptions:

1. Truncating variants ("loss of function" and/or "null" variants) must be described as a pathomechanism of disease in the corresponding gene (e.g., in the gene *MYH7*, only missense variants are associated with the disease).
2. A nearby cryptic splice site (AG/GT) is activated and the (predicted) new exon is spliced in-frame.
3. The (predicted) skipped exon (or exons) is alternatively spliced physiologically (see e.g. UCSC browser). For example, while *BRCA2* c.68-7T>A results in exon 3 skipping, alternative splicing leaves the remainder of the protein intact.
4. The (predicted) skipped exon (or exons) is sliced in-frame and contains no known functional domains (see e.g. www.nextprot.org/)
5. The (predicted) alternative splice leads to a small change in protein length (in-frame deletions or insertions of only a few amino acids) with no effect on protein function (e.g. located outside of fuctional domains).

2. Variants of 5´and 3´ splice site consensus according to Cartegni[2]



Cartegni consensus splice site variants (see image) which fulfill the following criteria but none of the ACMG criteria are classified as **Class 3** variants:

- **MaxEntScan (MES) analysis**: difference between wild type (WT) and mutation is **greater than 15%** (sensitivity >96%)
- **Splice Site Finder-like (SSF) analysis**: difference between wild type (WT) and mutation **greater than 5%** (specificity >87%)

Additional analyses (e.g., segregation or cDNA analysis, mini-gene assays) may be considered for these cases.

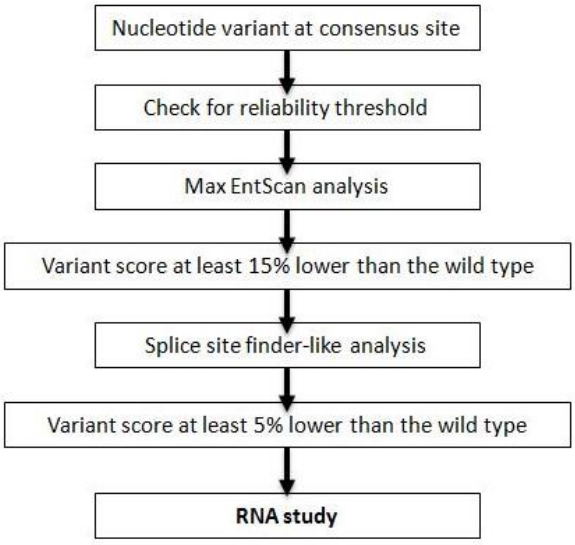
Please note:

- This applies only to physiological splice sites that are adequately recognized by prediction software. The thresholds for reliable classification of WT splice sites are **MES >3** and **SSF >60**.

- Cartegni consensus splice site variants in very large exons (e.g. *BRCA1/BRCA2* exon 11) and very small exons (e.g. *KCNQ3* exon 9) cannot be reliably classified.
- Significant indications of potential pathogenicity, such as definitive clinical symptoms, immunohistochemistry, two recessive inherited mutations, known pathogenic variants at the same site, high nucleotide conservation, etc., may be grounds for further analysis.

Variants that do <u>not</u> fulfill these criteria are to be classified as Class 2 variants.

Algorithm:



3. Variants excluded from the Cartegni splice site consensus ("deep" intronic or exonic variants) fulfilling <u>none</u> of the ACMG criteria.[4]

- **Deep intronic variants** are considered **Class 3** variants only if the splicing module above suggests a *de novo* splice site or the activation of a cryptic splice site. Variants which do not fulfil these criteria are considered **Class 2** variants.[2,3]
- **Deep exonic variants** are considered **Class 3** variants only if the splicing module above (in this case, all 5 Alamut splicing modules apply) suggest a *de novo* splice site or the activation of a cryptic splice site. Variants which do not fulfil these criteria are considered **Class 2 (in the case of silent variants)** or **Class 3 (in the case of missense variants)**.[2,3]

ESE and branch point prediction modules are **NOT** recommended for diagnostic use, since the sensitivity and specificity are too low.[2]

<u>References</u>

[1] Cartegni et al., 2002. *Nat Rev Genet 3*

[2] Houdayer et al., 2013. *Hum Mutat* 33

[3] Jian et al., 2014. *Nucleic Acids Research* 1

[4] Richards et al.; 2015. *Genetics in Medicine 3*