GeNeViSTA

Spliceosome Variants: Insights into Disease Pathology, Current Detection Techniques, and Clinical Implications in Genetic Diseases

Prince Jacob[1#], Keerthana Kumari S[1], Rachanashree HN[1], Akila Prashant[1,2], Siddaramappa J Patil[3#]

[1]*Department of Medical Genetics, JSS Medical College, and JSS Hospital, JSS Academy of Higher Education and Research, Mysore, Karnataka, India*

[2]*Department of Biochemistry, JSS Medical College, and JSS Hospital, JSS Academy of Higher Education and Research, Mysore, Karnataka, India*

[3]*Division of Medical Genetics, Mazumdar Shaw Medical Center, Narayana Hrudayalaya Hospitals, Bangalore, India*

Correspondence to: Dr Siddaramappa J Patil *Email:* drsjpatil@gmail.com

& Dr Prince Jacob *Email:* princejacob@jssuni.edu.in

Abstract

The mechanism involved in pre-mRNA splicing represents a vital stage in eukaryotic gene expression, ensuring the precise excision of introns and the joining of exons to yield mature mRNA transcripts. Deviations in this process, often instigated by spliceosome variants, can result in irregular splicing patterns which impact gene function, and thus contribute to the development of various human diseases. This review examines the diverse forms of spliceosome variants and their implications in disease pathology, encompassing variants at donor and acceptor splice sites, deep intronic variants, exonic variants affecting splicing, and alterations in branch points. The array of methodologies, including bioinformatics tools, experimental procedures, and functional assays, utilized for the detection of spliceosome variants and the elucidation of their functional impacts, is discussed. A few variants identified at our centre in patients with different genetic disorders with a confirmed molecular diagnosis are enumerated in this study. Furthermore, the clinical significance of spliceosome variant detection in disease diagnosis, prognosis, and treatment is underscored in this study, emphasizing the potential of personalized medicine strategies. Finally, future avenues of research in spliceosome variant investigations are outlined, which underscores the necessity of interdisciplinary approaches and collaborative endeavors in advancing precision medicine and healthcare.

Keywords: Splice site variants, Spliceosome, pre-mRNA splicing, Disease pathology, Detection methodologies

Introduction

Pre-mRNA splicing, orchestrated by the spliceosome, is a fundamental process in eukaryotic gene expression. This highly regulated process involves the removal of intronic sequences and the ligation of exons to generate mature mRNA transcripts (Ares et al, 1999). The spliceosome, a dynamic macromolecular complex comprising both RNA and protein components, governs the fidelity and precision of splicing events. The splicing process, which occurs in the nucleus, relies on the interaction of cis and trans elements. Cis elements are DNA sequences crucial for splicing regulation, including donor and acceptor splice sites, branch points, polypyrimidine tracts, as well as splicing enhancers and silencers. These sequences collectively form consensus splice site sequences. The spliceosome, a complex composed of five small nuclear ribonucleoproteins (snRNPs) and

numerous proteins, catalyzes splicing. Through complementary RNA-RNA interactions facilitated by small nuclear RNAs (snRNAs) within snRNPs, the spliceosome accurately identifies specific splicing sites (Anna et al, 2018).

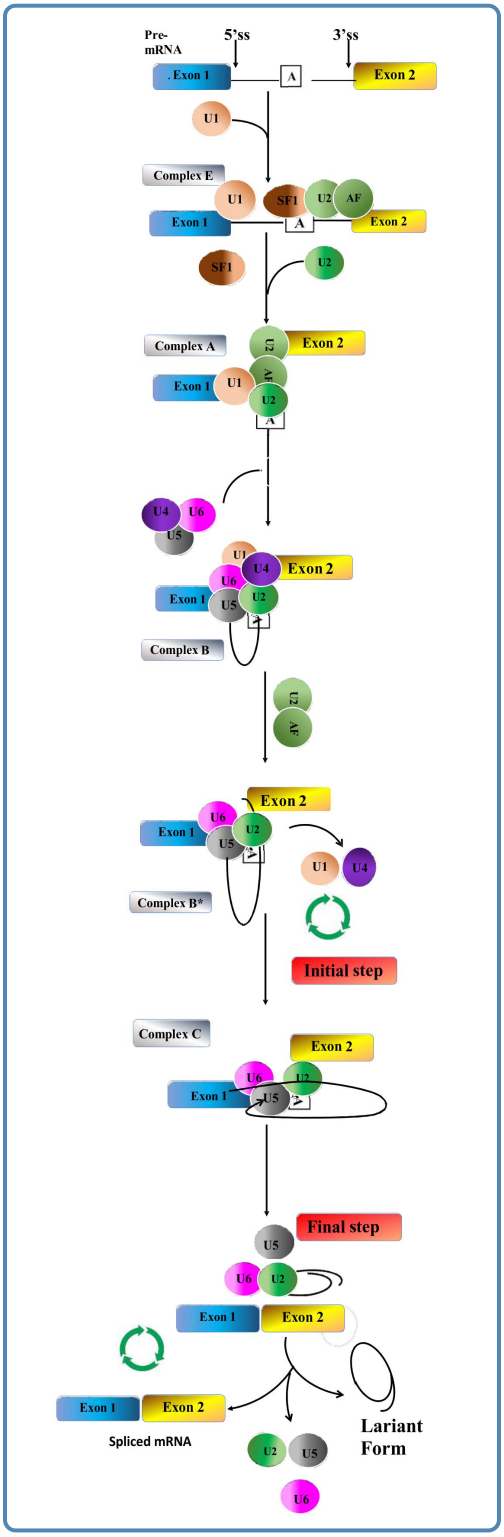

Figure 1 **Pictorial representation of the splicing process.** The process of splicing involves two main steps: recognizing the splicing sites at the intron/exon junctions and removing the introns while joining the exon ends. Initially, four complexes form between pre-mRNA and the spliceosome. The early (E) complex occurs when U1 snRNP binds to the AG-GU sequence at the 5' splice site, and SF1 binds to the branch point. This helps U2AF65 bind. U2 snRNP then displaces SF1 at the branch point, forming the ATP-dependent A complex. RNA helicases Prp5 and Sub2 stabilize these interactions, facilitating the recruitment of U4/5/6 tri-snRNP to form the B complex, or pre-catalytic spliceosome. Further RNA helicase activity rearranges the spliceosome, releasing U1 and U4 snRNPs and allowing U6 to interact with U2 snRNP, forming a pre-mRNA loop in the C complex. Within this complex, two transesterification reactions occur: the intron is removed, and the exon ends are joined.

The splicing process (**Figure 1**) involves two main steps: first, the recognition of splicing sites at intron/exon junctions, and second, the removal of introns and joining of exon ends. This process involves the formation of four complexes between the pre-mRNA and spliceosome. Initially, the early complex (E) forms are formed, where U1 snRNP binds to the donor splice site while SF1 and U2AF65 proteins bind to the branch point and polypyrimidine sequence, respectively. Subsequently, the ATP-dependent (A) complex is formed as SF1 is displaced by U2 snRNP. Stabilization of the branch point-U2snRNP interaction signals the recruitment of U4/5/6 tri-snRNP, leading to the formation of the B complex (pre-catalytic spliceosome). Further action by RNA helicases triggers spliceosome conformational changes, resulting in the release of U1 and U4 snRNPs and the formation of the C complex. Within this complex, two transesterification reactions occur, leading to intron removal and the joining of exon ends (Anna et al, 2018).

Disruption of spliceosome function, often

driven by variants, can lead to aberrant splicing patterns, resulting in the production of dysfunctional protein isoforms and contributing to the pathogenesis of various human diseases.

Canonical splice sites are characterized by the consensus sequences of GT (donor splice site) and AG (acceptor splice site) dinucleotide sequences (GT-AG) located at the 5' and 3' ends of introns. These sequences play a crucial role in accurately removing introns during mRNA maturation, with specific residues at positions +1 and +2 at the 5' donor splice site and positions -1 and -2 at the 3' acceptor splice site. Non-canonical splice site variants deviate from the usual GT-AG dinucleotide pairs found at the 5' and 3' ends of introns (donor and acceptor splice sites; GT-AG). These variants can disrupt normal splicing processes, affecting the maturation of mRNA. Research indicates that these non-canonical splice sites can produce abnormal transcripts, such as cryptic exons, with the extent of their occurrence influenced by the specific cellular environment. They are implicated in various genetic disorders such as congenital CD59 deficiency. Birt-Hogg-Dube syndrome, and ciliopathies. They are significant targets for identifying causative variants and enhancing understanding of disease mechanisms for better diagnosis and treatment (Anna et al, 2018; Chai et al., 2022). This article provides an overview of the current understanding regarding 'splice site variants', and techniques used for detecting these alterations in clinical diagnosis.

Different categories of splice site variants

Splice site variants can be categorized into various types depending on how they impact pre-mRNA splicing. These include:

Type 1: Variants at canonical splice sites and adjacent consensus sequences causing exon skipping

The first and most common category of splicing variants occur at the canonical splice sites, and lead to complete or partial exon skipping (**Figure 2A**). The most common variants typically impact the residues positioned one or two bases ahead of the 5' donor splice site and one or two bases before the 3' acceptor splice site. An analysis of splicing variants revealed a higher occurrence of variants at the donor splice site compared to those at the acceptor splice site when considering individual genes. Specifically, within the *NF1* gene, it was observed that variants affecting the 5' splice site were more frequent, accounting for 65% of cases, while variants affecting the 3' splice site occurred in 35% of cases (Anna et al., 2018). The impact of the variant at the canonical splice site may vary based on factors such as the strength of the splicing site, the presence of cryptic splice sites, the density of exonic splicing enhancers (ESE) and exonic splicing silencers (ESS), and the secondary structures formed by the pre-mRNA. The splicing complex primarily recognizes robust splice sites, and if the canonical splice site undergoes mutation, there is a higher likelihood of activating cryptic splice sites. In instances of weak splice sites, the likelihood of complete exon skipping is higher than the utilization of alternative splicing motifs (Anna et al., 2018).

Type 2: Intronic variants deep within the gene leading to the inclusion of a pseudo exon

The second category includes deep intronic variations that result in the inclusion of an intron fragment, the so-called cryptic exon or pseudo exon, into the mature transcript (**Figure 2B**). Functionally, such variants create novel acceptor /donor sites that are identified by the splicing and are used in combination with the existing intronic cryptic splice sites. One of the most common and well-known deep intronic change is a c.3718-2477C>T variant being one of the most frequent variants in *CFTR* gene responsible for cystic fibrosis (CF) (Anna et al., 2018).

Individuals with the c.3718-2477C>T variant in CF patients frequently exhibit a relatively mild phenotype, demonstrating variable disease expression. It has been observed that for the patients with CF the disease severity shows an inverse correlation with the abundance of accurately spliced transcripts, indicating that splicing regulation could serve as a significant modifier of the clinical course of cystic fibrosis in the presence of intronic variants (Anna et al., 2018).

Type 3: Variants in coding regions resulting in the loss of an exonic segment

Single nucleotide variants within exons can create new splice sites and can lead to the exclusion of an exon fragment (**Figure 2C**). These variants can establish a novel 5' or 3' splice site or activate a cryptic one that proves more robust than the original, thereby altering pre-mRNA processing and the loss of an exon fragment, referred to as type III splicing variant (Anna et al., 2018). It is important to note that variants in exons that lead to splicing changes are prone to

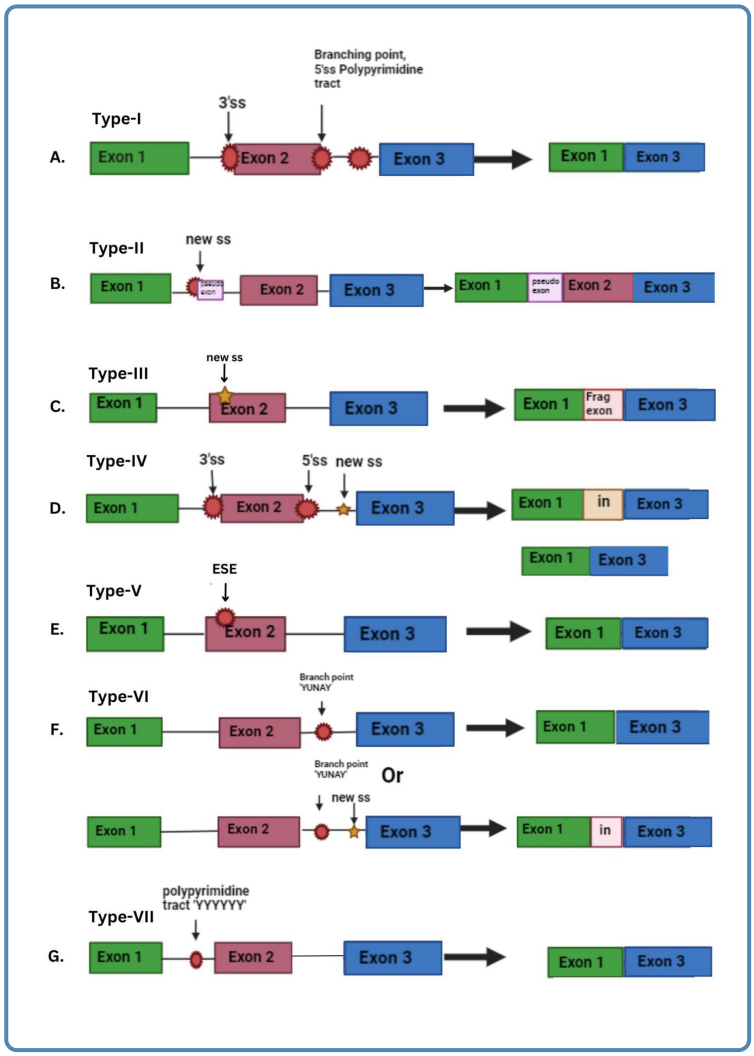

Figure 2 **Seven different types of splice site variants.** (A) Variants at canonical splice sites causing exon skipping. (B) Intronic variants deep within the gene leading to the inclusion of a pseudo exon. (C) Variants in coding regions results in the loss of an exonic segment. (D) Variants at canonical splice sites cause intron exclusion and skipping of exons. (E) Variant that disrupts ESE causes exon skipping or intron retention. (F) Variant at the branch point (YUNAY sequence) results in exon skipping. (G) Variant in Polypyrimidine Tract sequence [(Y)12–17] may lead to splicing alterations.

misclassification as synonymous, missense, or nonsense variants. Typically, the existence of these variants gives rise to two distinct transcripts from a mutated allele: one maintains the correct length but possesses a modified nucleotide, while the other is shorter and lacks an entire exon or a portion of it due to the nonspecific activity of the splicing complex (Ares et al.,1999). A good example of this is an exonic missense variant, c.887G>A in *MED12* resulting simultaneously in substitution of Arginine to Glutamine at codon position 296 and an aberrant splicing process leading to in-frame deletion of 42 bps in exon 7, r.847_888del (**Table 1**). Thus, through loss of the exonic segment and substitution, this missense variant leads to X-linked Ohdo syndrome (Togi et al., 2024).

Type 4: Variants at canonical splice sites cause intron exclusion and skipping of exons

Variants occurring in the canonical splice sites can alternatively (in contrast to type 1) lead to the activation of cryptic exonic or intronic splice sites. This activation results in the inclusion of an intron

fragment or the skipping of an exon fragment (**Figure 2D**). On analysis of the c.1525-1G>A variant in intron 9 of the *CFTR* gene, three distinct mRNA isoforms employing alternative splice sites within intron 10 and exon 10, positions c.1610–1611 and c.1678–1679 were identified. These isoforms were also found to lack the entire exon 10 or part of the fragment (Anna et al., 2018).

Type 5: Variants that disrupt ESE and lead to skipping of exon

Variants occurring within the exon can also (in contrast to type 3) cause the disruption of exonic splicing enhancers (ESE), which in turn leads to complete exon skipping (**Figure 2E**). Hence, the presence of exonic changes that result in the interference with exonic splicing enhancers is known as a type V splicing mutation. The utilization of RNA/cDNA sequencing in the diagnosis of genetic diseases is instrumental in identifying type V splicing variants (Ares et al., 1999; Anna et al., 2018). On analysis of *NF1* variants in the Leiden Open Variation Database (LOVD), it was found that 69% of the exonic variants were predicted to disrupt ESEs (Anna et al., 2018).

Type 6: Variants at the branch point (YUNAY sequence) results in exon skipping or intron retention

The branch point motif plays a crucial role in the early formation of the spliceosome complex. Changes in the branch point sequence can impact splicing accuracy. The branch point motif, positioned between −9 and − 400 base pairs downstream from the acceptor site, holds significance in humans for early spliceosome complex formation. It carries the consensus sequence YUNAY. Because branch point sequences are inherently variable, variants in this region may result in exon skipping. This occurs due to the improper binding of snRNP splicing proteins, leading to the disruption of the natural acceptor splicing site. Additionally, variants in the branch point sequence can induce intron retention if they generate a new 3' splice site (**Figure 2F**). Variants affecting the branch point sequence have been identified in the neurofibromatosis type 1 (*NF1*) gene. For instance, the variant 2410-18C>G in *NF1* leads to the partial retention (17 base pairs) of intron 15. This variant disrupts the original branch point sequence while creating a potential exonic splicing enhancer (ESE). Other splicing variants near this position include 2410-16A>G, 2410-15A>G, and 2410-12T>G. These findings underscore the critical role of this intronic fragment in facilitating the proper splicing of exons 15 and 16 (Anna et al., 2018).

Type 7: Variants in the polypyrimidine tract sequence [(Y)12–17] may lead to splicing alterations

Variants occurring in the polypyrimidine tract (situated upstream of the 3' splice site) or the pyrimidine-rich region (situated downstream of the 5' splice site) can lead to splicing alterations. These types of variants are rare. This sequence is crucial for binding the U2AF65 spliceosome subunit and the polypyrimidine tract-binding protein, both of which play a role in the regulation of alternative splicing (**Figure 2G**). Variants within the polypyrimidine tract have been observed in Hemophilia B, such as the variant c.253-19_253-16del in *F9*. This variant leads to the reduction of the polypyrimidine tract length from 24 nucleotides to 20. Consequently, this alteration causes inefficient splicing, resulting in the skipping of exon 3 (Anna et al., 2018).

Point variants at the branch point and polypyrimidine tract are infrequent and challenging to detect when analyzing genomic DNA, especially in coding sequences. Identifying their precise location is difficult, making it challenging to draw conclusions about the potential impact of a specific variant in these regions solely through genomic DNA analysis. To address this, RNA/cDNA sequencing is often employed, or their effects are evaluated through functional studies, such as minigene assays (Anna et al., 2018).

Synonymous variations in splice sites can cause diseases

Synonymous variants change the DNA sequence of a gene without affecting the amino acid sequence of the encoded protein. Though these types of variants are considered non-pathogenic, some synonymous variants can affect RNA splicing, translational efficiency, and mRNA stability. Synonymous variants can occur in a gene that has been directly associated with disease pathogenesis, as has been shown in the case of Treacher–Collins syndrome 17, Xlinked infantile spinal muscular atrophy 19, Seckel syndrome 20 and cystic fibrosis (Sauna et al., 2011). One investigation revealed that a synonymous variant within the *IL2RG* gene resulted in an abnormal splice pattern, leading to decreased expression of the common gamma chain (γc) and the onset of late onset combined

Table 1 Analysis of different splice site variants from our centre using *in-silico* prediction tools.

Gene	Variant details	Zygosity	Splice site tool predictions		OMIM Disease [MIM#]
			Splice AI (Δ score)	Human splice finder	
BTD	NM_001370658.1: c.400-3T>G	Homozygous	Moderate (0.2)	Alteration of the WT acceptor site, most probably affecting splicing	Biotinidase deficiency [253260]
CREEBP	NM_004380.3: c.3779+5G>C	Heterozygous	Strong (0.53)	Alteration of the WT donor site, most probably affecting splicing	Rubinstein-Taybi syndrome 1 [180849]
SMPD1	NM_000543.5: c.1341-10_1363dup	Compound heterozygous with another variant in *cis*	Strong acceptor gain (0.84)	No significant impact on splicing signals	Niemann-Pick disease, type A [257200]
VPS33B	NM_018668.5: c.96G>A p.Gln32=	Homozygous	Donor loss (0.37)	Alteration of the WT donor site, most probably affecting splicing	Keratoderma-ichthyosis-deafness syndrome, autosomal recessive [620009]
MED12	NM_005120.3:c.887G>A p.Arg296Gln; r.847_888del	Hemizygous	Acceptor gain (0.38)	Alteration of auxiliary sequences: Significant alteration of ESE / ESS motifs ratio (-9)	Ohdo syndrome, X-linked [300895]

immunodeficiency. Another study examined both disease-causing and neutral exonic point variants, concluding that synonymous variants primarily induce disease phenotypes by disrupting splicing. Furthermore, computational predictors were utilized to pinpoint splice-disruptive variants, encompassing missense or synonymous variants. Notably, deep learning-based predictors trained on gene model annotations exhibited the most effective performance in distinguishing disruptive from neutral variants. These findings underscore the significance of considering synonymous variations at splice sites in the investigation of disease genetics (Ares et al.,1999). We identified a synonymous variant, c.96G>A (p. Gln32=) in *VPS33B* leading to autosomal recessive keratoderma-ichthyosis-deafness (KID) syndrome. The prediction tools were consistent in predicting that this variant could potentially disrupt the WT donor site, and thus be causative (**Table 1**). It is worth noting here that according to the revised American College of Genetics and Genomics/ Association for Molecular Pathology (ACMG- AMP) recommendations (Walker et al., 2023), synonymous variants which are present in the first or last three bases of the exon and are predicted to impact splicing can be considered as disease-causing, and BP7 criteria should not be applied for the same. This variant in *VPS33B* mentioned in Table 1 is present in the last base of exon 1, and was thus considered for diagnosis, as the patient had a concordant phenotype.

Techniques/ Technologies for Detecting Spliceosome Variants

Bioinformatic approaches

Current methods for detecting and interpreting splice site variants include *in-silico* tools utilizing machine learning algorithms. Bioinformatics tools such as SpliceFinder integrate functional annotation tools and splice site prediction programs to analyze next-generation sequencing (NGS) data. Position Weight Matrix-based tools are effective in predicting the consequences of variants on mRNA splicing. Recent studies have shown that machine learning classifiers, particularly Random Forest (RF), outperform Support Vector Machine (SVM) in splice site prediction. Additionally, Convolutional Neural Network (CNN) architectures have been developed to predict splice sites and evaluate the impact of genomic variants on splicing. These technologies offer precise and reliable approaches for identifying splice site variants, aiding in the recognition of disease-causing variants and their influence on mRNA splicing (Anna et al., 2018)

These tools were initially created for research purposes but can potentially be integrated into routine diagnostics. They vary in their algorithms, focusing on consensus splicing sites and requiring sequence input within specific positions. Additionally, there are tools designed to assess the impact of distant variants on splicing, predict exon skipping, cryptic site activation, or the generation of aberrant transcripts, as well as algorithms specifically tailored to predict the influence of single nucleotide variants on branch site sequences or polypyrimidine tracts, such as the Branch Site Analyzer and SVM-BP finder (Anna et al., 2018)

When dealing with exonic variants, it is crucial to evaluate their potential effects on exon splicing enhancers (ESEs) or silencers (ESSs). Various algorithms are available for this assessment, such as ESE Finder, and ESRsearch employing a unanimous enrichment approach with hexameric sequence frequencies. Some models like FAS-ESS are based on functional analyses of random sequences through minigene assays, while others like SpliceAid2 rely on the direct interaction between splicing factors and RNA target motifs. Additionally, bioinformatic programs such as mFold or pFold can be employed to predict whether a variant might impact mRNA secondary structure (Chai et al., 2022)

To enhance user convenience, various programs employing different algorithms have been developed and are accessible via websites. Prominent examples include Human Splicing Finder (HSF), Splice AI and SROOGLE, which predict the presence of *cis*-splicing elements in provided sequences or offer predictions for specific variants in particular genes. Additionally, MutPredSplice is an online tool capable of analyzing individual variants or sets of variants uploaded in a VCF file format. Advanced tools used for annotating variants, particularly those derived from next-generation sequencing data, often integrate splicing prediction algorithms. For instance, the Variant Effect Predictor tool, accessible online, incorporates specialized plugins for splicing analysis utilizing the MaxEntScan model and the dbscSNV matrix from the dbNSFP database (Chai et al., 2022). We have enlisted few examples of splice site variants (**Table 1**) identified in patients from our centre with the scores of analysis from two commonly used and efficient *in-silico* prediction tools.

Experimental Techniques

Experimental approaches, including polymerase chain reaction (PCR)-based methods, RNA sequencing (RNA-seq), and mass spectrometry, provide complementary strategies for detecting spliceosome variants at the transcriptomic and proteomic levels. PCR-based assays, such as allele-specific PCR, enable the targeted amplification and quantification of splicing isoforms harboring specific variants. These assays offer high sensitivity and specificity for detecting spliceosome variants in patient samples, facilitating the identification of disease-associated variants and their correlation with clinical phenotypes (Togi et al., 2024)

RNA-seq, on the other hand, offers a genome-wide perspective on alternative splicing events and allows for the identification of novel spliceosome variants in disease-relevant tissues. By profiling the transcriptome of patient samples, researchers can identify dysregulated splicing events and prioritize candidate genes for further functional characterization. Moreover, RNA-seq enables the detection of fusion transcripts resulting from gene fusions and alternative splicing events, providing insights into the molecular mechanisms driving disease pathogenesis (Togi et al., 2024)

Mass spectrometry-based proteomics facilitates the characterization of spliceosome protein complexes and enables the detection

of post-translational modifications associated with spliceosome dysfunction. By profiling the proteome of spliceosome complexes, researchers can identify disease-associated variants and elucidate their functional consequences on spliceosome assembly and activity. Furthermore, mass spectrometry enables the quantification of protein expression levels and the identification of dysregulated splicing factors in disease states, providing insights into the molecular mechanisms underlying spliceosome-mediated splicing regulation (Sauna et al., 2011)

Functional assays

Bioinformatic algorithms serve as valuable tools for evaluating potential effects of identified changes. However, it is important to emphasize that these tests provide predictive outcomes, and the precise impact of the variant must be confirmed through functional studies. Another approach to validate the pathogenic effect of a specific splicing variant is to analyze its segregation with the disease in affected and unaffected family members at the DNA level. Nonetheless, laboratory testing is still necessary to ascertain the exact splicing effect (Walker et al., 2023)

The most straightforward and efficient functional assay to ascertain if the chosen variant impacts splicing involves analyzing RNA extracted from pertinent patient tissue or cell lines derived from patient cells. Sequencing RNA/cDNA following reverse transcription PCR (RT-PCR) enables confirmation of whether the identified variant affects the mRNA sequence. However, a significant challenge with this method is the potential occurrence of nonsense-mediated decay (NMD), which could obscure the effect of the presumed splicing mutation. To mitigate this limitation, patient cells can be treated with NMD inhibitors like puromycin, which halt RNA degradation (Walker et al., 2023)

If suitable material for functional RNA sequencing is not accessible, an alternative option is a minigene assay, a laboratory technique that acts as an in vitro hybrid system enabling ”exon trapping.” This method proves particularly beneficial for analyzing genes with low expression levels in leukocytes or fibroblasts. In the minigene assay, a fragment of the gene under scrutiny, such as a specific exon along with adjacent intronic sequences with and without variants, is amplified and then inserted into a specialized expression plasmid, facilitating the examination of pre-mRNA splicing. This approach serves to validate whether the potential splicing variant impacts splicing efficiency or triggers the activation of alternative cryptic splicing sites. Additionally, it allows for the investigation of the role of cis-acting elements in splicing regulation (Thanapattheerakul et al., 2020)

Lastly, CRISPR-based genome editing technologies enable the generation of isogenic cell lines carrying precise spliceosome variants, allowing for the elucidation of genotype-phenotype correlations and the identification of therapeutic targets. By introducing specific variants into the endogenous genome, researchers can assess the functional consequences of spliceosome variants on pre-mRNA splicing and gene expression regulation. Moreover, CRISPR-based genome editing enables the development of cellular models for studying disease pathogenesis and evaluating therapeutic interventions, paving the way for personalized medicine approaches (Jian et al., 2014) A comprehensive list of all the approaches is enlisted in **Table 2**.

Clinical Implications and Future Directions

The detection of spliceosome variants holds significant clinical implications for disease diagnosis, prognosis and therapeutic intervention. Characterization of spliceosome variant profiles in patient populations can inform personalized treatment strategies and guide the selection of targeted therapies tailored to individual molecular profiles. Furthermore, the integration of spliceosome variant detection into routine clinical practice has the potential to revolutionize precision medicine approaches and improve patient outcomes (Hu et al., 2013)

For example, small molecule modulators of spliceosome function, such as splice-switching oligonucleotides and small molecule splicing modulators, hold promise as therapeutic interventions for diseases characterized by aberrant splicing patterns. By targeting specific spliceosome components or splicing regulatory elements, these compounds can modulate splicing efficiency and restore normal gene expression patterns. Clinical trials evaluating the efficacy of spliceosome modulators in various disease settings are currently underway, with promising results reported in preclinical studies (Hu et al., 2013).

Moreover, the identification of spliceosome variants as prognostic biomarkers in cancer and other diseases has important implications for

Table 2 Different approaches for analysis of splice site mutations

Approaches	Tools/ techniques	Website links
1.Bioinformatic approaches (In-silico prediction tools)	Human Splice Finder	www.umd.be/HSF/
	Splice AI	https://spliceailookup.broadinstitute.org/
	Splice site prediction program	www.fruitfly.org/seq_tools/splice.html
	SPANER	http://tools.genes.toronto.edu/
	SpliceAid2	http://193.206.120.249/splicing_tissue.html
	NetGene2	http://www.cbs.dtu.dk/services/NetGene2/
	MutPredSplice	http://www.mutdb.org/mutpredsplice/submit.htm
	ESE finder	http://exon.cshl.org/ESE
2.Experimental Approaches	Polymerase chain reaction-based method	https://doi/10.1002/wrna.1364
	RNA sequencing (RNA-seq)	https://doi.org/10.1038/s41598-021-89938-2
	Mass spectrometry	https://doi.org/10.1371/journal.pone.0265766
3.Functional assays	Minigene splicing assay	https://doi.org/10.1002/humu.22624
	Reverse transcription PCR analysis	https://doi.org/10.1186/s12867-016-0060-1
	Protein truncation test (PTT)	https://10.1007/978-1-59745-388-2_8
	CRISPR-based genome editing technologies	https://doi.org/10.1002/gcc.22784

disease management and treatment planning. Patients harboring specific spliceosome variants may benefit from targeted therapies aimed at correcting splicing defects and restoring normal gene expression patterns. Furthermore, the development of companion diagnostic tests for detecting spliceosome variants could enable the stratification of patient populations and facilitate the selection of appropriate therapeutic interventions (Zhai et al., 2013).

Future research directions in spliceosome variant studies encompass a broad spectrum of interdisciplinary approaches, including the development of novel detection

methods, elucidation of disease-specific splicing signatures, and exploration of therapeutic modalities targeting spliceosome dysfunction. Integration of multi-omics data, including genomics, transcriptomics, and proteomics, will facilitate a comprehensive understanding of spliceosome-mediated splicing regulation and its implications for human health and disease. Moreover, collaborative efforts between academia, industry, and regulatory agencies are essential for translating basic research findings into clinical applications and improving patient outcomes (Zhai et al., 2013)

Conclusion

Splice site variants can cause diseases by disrupting the proper recognition of exons and altering mRNA splicing. These variants can result in exon skipping, the formation of new exon/intron boundaries, or the activation of cryptic exons. Synonymous variants can also affect splicing by disrupting consensus sequences. To detect splice site variants, various technologies can be used. Bioinformatic algorithms can be applied to predict the effect of identified changes, but functional studies are necessary to confirm the exact impact of specific variants. In vitro transcription and variant analysis via a hybrid minigene system are commonly used methods for functional studies. These approaches can help in the diagnosis of splice site variants and provide insights into the mechanisms underlying splicing-related diseases. By leveraging cutting-edge technologies and interdisciplinary approaches, researchers can elucidate the molecular mechanisms underlying spliceosome-associated diseases and pave the way for innovative diagnostic and therapeutic strategies. Continued investment in spliceosome variant research holds the promise of transformative advances in precision medicine and personalized healthcare.

References

1. Anna A, et al. Splicing mutations in human genetic disorders: examples, detection, and confirmation. J Appl Genet. 2018; 59(3): 253–268.
2. Ares M Jr, et al. A handful of intron-containing genes produces the lion's share of yeast mRNA. RNA. 1999; 5(9):1138-1139.
3. Baralle D. Splicing in action: assessing disease causing sequence changes. J Med Genet. 2005; 42(10): 737–748.
4. Bashari A, et al. Targeting splicing factors for cancer therapy. RNA. 2023; 29(4): 506–515.
5. Chai JN, et al. A splice site mutation associated with congenital CD59 deficiency. Hematol Rep. 2022; 14(2): 172–178.
6. Hauss O, et al. The protein truncation test in mutation detection and molecular diagnosis. In: In Vitro Transcription and Translation Protocols. Totowa, NJ: Humana Press; 2007. p. 151–164.
7. Hu Y, et al. DiffSplice: the genome-wide detection of differential splicing events with RNA-seq. Nucleic Acids Res. 2013; 41(2): e39–e39.
8. Jian X, et al. In silico tools for splicing defect prediction: a survey from the viewpoint of end users. Genet Med. 2014;16(7): 497–503.
9. Sauna ZE, et al. Understanding the contribution of synonymous mutations to human disease. Nat Rev Genet. 2011; 12(10): 683–691.
10. Taylor J, et al. Mutations in spliceosome genes and therapeutic opportunities in myeloid malignancies. Genes Chromosomes Cancer. 2019; 58(12): 889–902.
11. Thanapattheerakul T, et al. Predicting the effect of variants on splicing using convolutional neural networks. PeerJ. 2020; 8: e9470.
12. Togi S, et al. Qualitative and quantitative analysis of MED12 c.887G>A causing both missense and splicing variants in X-linked Ohdo syndrome. Am J Med Genet A. 2024: e63628.
13. Walker LC, et al. Using the ACMG/AMP framework to capture evidence related to predicted and observed impact on splicing: Recommendations from the ClinGen SVI Splicing Subgroup. Am J Hum Genet. 2023; 110(7): 1046-1067.
14. Zhai LH, et al. Proteomic characterization of post-translational modifications in drug discovery. Acta Pharmacol Sin. 2022; 43(12): 3112–3229.