Taylor & Francis
Taylor & Francis Group

REVIEW

Mutations in spliceosomal proteins and retina degeneration

Šárka Růžičková and David Staněk

Department of RNA Biology, Institute of Molecular Genetics AS CR, Prague, Czech Republic

ABSTRACT

A majority of human genes contain non-coding intervening sequences – introns that must be precisely excised from the pre-mRNA molecule. This event requires the coordinated action of five major small nuclear ribonucleoprotein particles (snRNPs) along with additional non-snRNP splicing proteins. Introns must be removed with nucleotidal precision, since even a single nucleotide mistake would result in a reading frame shift and production of a non-functional protein. Numerous human inherited diseases are caused by mutations that affect splicing, including mutations in proteins which are directly involved in splicing catalysis. One of the most common hereditary diseases associated with mutations in core splicing proteins is retinitis pigmentosa (RP). So far, mutations in more than 70 genes have been connected to RP. While the majority of mutated genes are expressed specifically in the retina, eight target genes encode for ubiquitous core snRNP proteins (Prpf3, Prpf4, Prpf6, Prpf8, Prpf31, and SNRNP200/Brr2) and splicing factors (RP9 and DHX38). Why mutations in spliceosomal proteins, which are essential in nearly every cell in the body, causes a disease that displays such a tissue-specific phenotype is currently a mystery. In this review, we recapitulate snRNP functions, summarize the missense mutations which are found in spliceosomal proteins as well as their impact on protein functions and discuss specific models which may explain why the retina is sensitive to these mutations.

Introduction

A vast majority of human genes contain non-coding intervening sequences – introns - that are removed from mRNA molecules during splicing. Intron excision and exon joining requires two transesterification reactions which are catalyzed by a large ribonucleoprotein complex called the spliceosome. The spliceosome dynamically changes its composition during splicing with the overall number of involved proteins reaching 170. In addition, the spliceosome contains five small nuclear RNAs (snRNAs), which together with ~65 proteins, enter splicing as preassembled small nuclear ribonucleoprotein particles (snRNPs) (reviewed in[1,2]).

Various human inherited diseases are caused by mutations that directly affect the accuracy and efficiency of pre-mRNA splicing.[3,4] One of the most interesting genetic mysteries is represented by inheritable retinitis pigmentosa (RP), which is caused by the mutation of more than 70 genes (sph.uth.edu/retnet). A majority of target genes are expressed specifically in the retina and are involved in different aspects of photoreceptor function (light detection, signal transduction, membrane components or structural elements of retina cells).[5] Surprisingly, mutations found in six small nuclear ribonucleoprotein particle (snRNP)-specific proteins (Table 1) and two non-snRNP splicing factors have been linked to RP as well. These ubiquitously expressed proteins are essential for almost every cell in the body and it remains enigmatic why mutations in these genes are associated with such a tissue specific phenotype.

RP is the most common type of inherited retinal degeneration characterized by the progressive loss of photoreceptor cells. It occurs in 1 in 3,000–5,000 individuals, without any apparent link to ethnic or racial background.[5] Pathological changes include atrophy of the retinal pigment epithelium (RPE), retina vessel attenuation, constricted visual fields, waxy pallor of the optic disc and the degeneration of photoreceptor cone and rod cells. Symptoms first appear as night blindness progressing to loss of peripheral vision, eventually leading to total blindness. However, the age of onset and the rate of progression varies among individuals. These two characteristics are likely dependent on genetic background.[6] RP can be transmitted in all three forms of mendelian inheritance: autosomal dominant (adRP), autosomal recessive (arRP) and X-linked (xlRP). 30%–40% cases account for adRP of which a quarter of these are caused by mutations in the rhodopsin gene. arRP represents about half of RP cases and ~10% of RP cases are X-linked.[6]

Small nuclear ribonucleoprotein particles

Spliceosomal snRNPs are complex particles consisting of small nuclear RNA (snRNA), a heptameric ring of Sm or Like-Sm (LSm) proteins and 1-13 proteins that are specific for each snRNP. Five major and four minor snRNPs have been described and named according to their snRNA composition. The major snRNPs are U1, U2, U4, U5 and U6 and the minor snRNPs are U11, U12, U4atac and U6atac. The U5 snRNP is common to both major and minor splicing pathways.

The most accepted view of spliceosome formation is a stepwise assembly model, which has been supported by numerous

Table 1. The list of snRNP proteins and their relation to RP.

snRNP/name of the protein	Function	Retinitis pigmentosa
U4 snRNP		
7 Sm proteins	Stability of the particle	−
SNU13 (15.5K/NHPX)	Binding of U4 snRNA, U4 snRNP formation	−
PRPF31 (hPrp31/61K)	Interaction with PRPF6, formation of the tri-snRNP	+
U6 snRNP		
LSm proteins 2-8	U6 snRNA stability, U4/U6 annealing	−
SART3	U4/U6 snRNA annealing	−
U4/U6 snRNP		
PRPF3 (hPrp3/90K)	Binding of the U4/U6 duplex, tri-snRNP stability	+
PRPF4 (hPrp4/60K)	Tri-snRNP stability	+
PPIH (USA-Cyp/CypH)	Pre-mRNA splicing?	−
U5 snRNP		
7 Sm proteins	Stability of the particle	−
TXNL4A (hDib1/15K)	The thioredoxin fold superfamily	−
SNRNP40 (WDR57/40K)	Protein-protein interaction?	−
CD2BP2 (52K/Snu40/Lin1)	Interacts directly with Prpf6 and TXNL4A, not part of the U4/U6•U5 tri-snRNP	−
DDX23 (hPrp28/100K)	DEAD-box RNA helicase motif but the ATPase activity not confirmed	−
PRPF6 (hPrp6/102K)	Interaction with PRPF31, formation of the tri-snRNP	+
EFTUD2 (hSnu114/116K)	Regulation of SNRNP200 activity	−
SNRNP200 (hBrr2/200K)	Unwinding of U4/U6 snRNA duplex during splicing, activation of the spliceosome	+
PRPF8 (hPrp8/220K)	Formation of U5 snRNP, regulation of SNRNP200 activity, pre-mRNA splicing	+
U4/U6•U5 tri-snRNP		
SNRNP27 (27K)	?	−
USP39 (hSad1/65K)	Ubiquitin specific peptidase, recruitment of the tri-snRNP to the spliceosome	−
SART1 (hSnu66/110K)	Recruitment of the tri-snRNP to the spliceosome	−

Gray color indicates factors involved in RP development

in vitro and *in vivo* studies (e.g.[7,8]; reviewed in[2]). First, U1 snRNP interacts with the 5′ splice site via RNA-RNA base pairing of the U1 snRNA with the sequence at the 5′ splice site. This interaction is strengthened by the U1-C protein.[9] The sequence around the branch point is initially recognized by proteins SF1 and U2AF35/65,[10] which are subsequently replaced by U2 snRNP. Subsequently, the U2 snRNA forms a duplex with the intron sequence surrounding the adenine at the branch point.[11]

After U1 and U2 snRNPs define intron boundaries, U4/U6•U5 tri-snRNP joins the spliceosome. The U4-U6 helix is disrupted by the DExD/H box helicase SNRNP200 (also referred to as hBrr2).[12-14] While U4 snRNA together with U4/U6-specific proteins (including proteins mutated in RP Prpf3, Prpf4 and Prpf31 which are described in detail below) leave the spliceosome, U6 snRNA replaces U1 snRNA at the 5′ splice site resulting in a new interaction with U2 snRNA. U1 snRNP is released and a

catalytically competent spliceosome is formed. Several studies have suggested that the active splice site is formed by a dynamic snRNA-pre-mRNA network.[15-18] The crucial role of snRNAs, namely the U2, U5 and U6 snRNAs, in the splicing reaction was recently confirmed in *Saccharomyces pombe* where the spliceosome structure was elucidated.[19,20] snRNP proteins, mainly Prpf8, hold pre-mRNA in the correct orientation and provide structural support for snRNAs in the active site. During and after splicing, the spliceosome undergoes many structural rearrangements that are mediated by several RNA helicases including SNRNP200 and DHX38 (hPrp16) ([21], reviewed in[11]). Post-splicing snRNPs bound to the intron lariat are removed by the Prp43/Ntr1/Ntr2 complex. The lariat is then rapidly degraded and snRNPs are recycled for the next splicing reaction.[22]

In addition to splicing function, snRNPs, namely U1 snRNP, have been implicated in other nuclear processes, namely RNA polymerase II transcription and U1 snRNP associates with RNA polymerase II (Pol II).[23] The protein FUS, which interacts with both U1 snRNP and Pol II, was suggested to mediate Pol II-U1 association.[24] FUS mutations have been linked to the neurodegenerative disease, amyotrophic lateral sclerosis (ALS).[25] Interestingly, U1 snRNP-specific proteins also interact with SMN protein, which is linked to another motor neuron degenerative disease with early onset - spinal muscular atrophy.[26,27] It has been observed that depletion of U1 snRNP proteins leads to reduced growth of motor neurons.[26] This suggests that U1 snRNP might be a common link between diseases that affect motor neurons.

Mutations in splicing factors linked with retinitis pigmentosa

In this chapter, we summarize the current information regarding exonic missense mutations in six snRNP-specific and two non-snRNP proteins that are associated with RP. We have mostly omitted discussion related to deletions or mutations that affect splicing, which often lead to a reading frame shift, resulting in the introduction of a premature stop codon and subsequent destabilization of mRNA or protein. So far, RP linked mutations were identified only in U4/U6•U5 tri-snRNP-specific proteins. No mutations associated with RP have been detected in U1 or U2 snRNP-specific proteins (Table 1 and 2).

Prpf3

Prpf3 is a 90 kD protein, which forms a stable trimeric complex with Prpf4 and PPIH. The complex binds the U4/U6 snRNA duplex primarily via the interaction of Prpf3 with U6 and U4/U6 RNA.[28,29] Prpf3 further stabilizes the U4/U6•U5 tri-snRNP by interacting with Prpf6 and Sart1.[29,30] The C-terminus of Prpf3 is required for binding to RP9 (PAP-1), a protein also implicated in adRP.[31]

Two different missense mutations were discovered in two adjacent codons in exon 11 of the gene *PRPF3*, causing amino acid substitutions Thr494Met and Pro493Ser.[32] Finally, a third mutation in the same exon, which was identified in a Spanish family, was found to be responsible for the substitution at Ala489Asp.[33] Thr494Met is the most frequently observed substitution in Prpf3 while Ala489Asp occurs rather sporadically.

All three mutations are clustered in the C-terminal conserved region, which is important for protein-protein interactions. So far, only the Thr494Met mutation has been extensively studied. Mutation Thr494Met causes aggregation of the mutated protein when expressed in 661 W cells, which are used as *in vitro* model of photoreceptor cells. Nuclear aggregates were not observed when the Prpf3[Thr494Met] protein was expressed in HeLa.[34] Thr494 overlaps with a potential recognition motif for phosphorylation by casein kinase II (CKII). Gonzales-Santos and colleagues showed that Thr494Met substitution reduced Prpf3 phosphorylation and impaired the association with Prpf4 and U4/U6 snRNP.[35] Consistently, patients who express mutated Prpf3 exhibited altered levels of snRNPs, delayed spliceosome assembly and inefficient splicing.[36] The function of Prpf3 has also yielded interesting results in mice models. It was shown that Prpf3^{+/-} heterozygotic mice developed normally exhibiting compensatory expression from the Prpf3 wild-type allele.[37] However, pathological changes in the retinal pigment epithelium (RPE) of Prpf3[WT/Thr494Met] knock-in mice were detected in two year old mice.[38] Further research has revealed additional defects in RPE phagocytic function in Prpf3[Thr494Met/Thr494Met] mice,[39] which points to RPE as a primary target tissue and photoreceptor death as a consequence of RPE dysfunction. However, two year mice are at the end of their life and thus this results should be considered with caution.

Prpf4

Prpf4 is a 60 kD protein that complexes with Prpf3 and PPIH and is part of both U4/U6 and U4/U6•U5 snRNPs. In 2011, Linder and his colleagues suggested that Prpf4 might be involved in RP because Prp4 reduction caused the same eye phenotype as reduced expression of Prp31, a protein linked to RP (see below).[40] Indeed, two amino acid substitutions (Arg192His and Pro315Leu) in the *PRPF4* gene have been recently connected with RP.[41,42] In addition, a third mutation that causes the substitution of Pro187Ala was found in a patient with adRP, but the connection of this substitution to the disease phenotype was not fully established.[43] Cells expressing the mutated Prpf4[Pro315Leu] exhibited altered levels of splicing factors as well as changes in the nuclear morphology of the splicing factor compartment.[41] Detailed molecular analysis showed that a Arg192His substitution disrupts the association of Prpf4[Arg192His] with Prpf3 and its incorporation into snRNPs.[42] The reduced expression of Prpf4 protein in zebrafish led to morphological changes and degeneration of photoreceptor cells.[42] Additionally, the expression of Prpf4[Pro315Leu] and Prpf4[Arg192His] proteins in zebrafish were shown to be linked to embryonic and retinal defects.[41,42]

Prpf6

Prpf6 is a 102 kD protein U5 snRNP-associated protein that is essential for the interaction between U5 and U4/U6 particles and the formation of U4/U6•U5 tri-snRNP.[30,44,45] Only one mutation in *PRPF6* gene has been identified thus far, c.2185C>T transition in the TPR domain resulting in Arg729Trp substitution.[46] The mutated Prpf6[Arg729Trp] has been shown to accumulate in Cajal bodies. Because defective snRNPs accumulate in these nuclear structures,[47] this finding suggests that Arg729Trp substitution inhibits snRNP assembly. The Arg729 is in a close proximity to Prpf4 suggesting that it is involved in formation and stabilization of the tri-snRNP.[19] In addition, inefficiently spliced pre-mRNAs were detected in patient cells expressing Prpf6[Arg729Trp].[46] Interestingly, a missense mutation, c.1430A>G, found within the *PRPF6* gene co-segregated with progressive neurodegenerative genetic Kufs disease. It was observed that patients who carried this missense mutation suffered visual impairment.[48] However, additional studies are needed to confirm the role of this mutation in either Kufs disease or the visual defect.

Prpf8

At 220kD, Prpf8 is the largest protein found in the spliceosome. Prpf8 is a highly conserved protein that lies in the center of the spliceosome where interacts with the U5 snRNA as well as the 5′ and 3′splice sites of pre-mRNA.[49] Eleven missense mutations causing amino acid substitutions were found in the *PRPF8* gene and have been linked to adRP (Table 2). Currently, all mutations identified cluster in the C-terminal Jab1/MPN domain, which interacts with SNRNP200 and regulates its helicase activity.[50-54] The Jab1/MPN domain has apparently two opposing functions in SNRNP200 regulation. While the entire Jab1/MPN domain stimulates SNRNP200 helicase activity,[51,54] the C-terminal tail is inserted into the active site of the SNRNP200 helicase and sterically inhibits its activity.[52,53] Consistently, RP mutations that shorten the C-terminus increase the stimulatory effect, likely by removing the inhibitory tail. It has been also described that the mutation reducing Prpf8-SNRNP200 interaction has a negative effect on helicase stimulation.[51,53,54] Coincidently, SNRNP200 is also linked to RP and mutations within SNRNP200 are known to reduce its helicase activity (see below).

The effect of *PRPF8* mutations in cell metabolism was studied mostly in yeast providing similar, but not fully consistent, results exhibiting different growth defects which are likely dependent on the genetic background of the studied yeast strain.[52,54,55] However, experiments in yeast have pointed to an additional defect caused by RP mutations - inhibition of U5 snRNP assembly. Several labs have reported that yeast Prp8p mutated variants mimicking human Prpf8 RP mutations interacted less efficiently with U5 proteins yBrr2p and ySnu114p, which resulted in splicing defects.[50,54,55] Similarly, inefficient spliceosome assembly and reduced splicing were detected in cells derived from RP patients carrying *PRPF8* mutations.[36]

Currently, it is unclear how mutated Prpf8s induce the RP phenotype. One possibility is via misregulation of SNRNP200 resulting in changes in splicing. Alternatively, Prpf8 proteins containing exonic substitutions may inhibit the formation of snRNPs and thus reduce the effective concentration of splicing competent snRNPs. Recently, two studies observed gene specific splicing defects after Prpf8 knockdown suggesting that the reduction of Prpf8 concentration can target only a subset of susceptible genes.[56,57] Interestingly, several somatic *PRPF8* missense mutations were identified in patients with myeloid syndromes such as acute myeloid leukemia.[58] Although, the presence of *PRPF8* mutations in patients with acute myeloid leukemia was associated with a poor disease prognosis, their

Table 2. List of missense mutations and amino acid substitutions of spliceosomal factors linked to RP.

Gene/Exon No.	Nucleotide mutation	Amino acid substitution	Reference
Prpf3			
11	c.1466C>A	Ala489Asp	[33]
11	c.1478C>T	Pro493Ser	[32]
11	c.1482C>T	Thr494Met	[32,98]
Prpf4			
5	c.556C>G	Pro187Ala*	[43]
6	c.575G>A	Arg192His	[42]
10	c.944C>T	Pro315Leu	[41]
Prpf6			
16	c.2185C>T	Arg729Trp	[46]
Prpf8			
38	c.6353C>T	Ser2118Phe	[99]
42	c.6901C>A	Pro2301Thr	[100]
42	c.6901C>T	Pro2301Ser	[101]
42	c.6912C>G	Phe2304Leu	[100]
42	c.6926A>C	His2309Pro	[100]
42	c.6926A>G	His2309Arg	[100]
42	c.6926G>A	Arg2310Lys	[100]
42	c.6928A>G	Arg2310Gly	[100]
42	c.6930G>C	Arg2310Ser	[99]
42	c.6942C>A	Phe2314Leu	[100]
42	c.7000T>A	Tyr2334Asn	[102]
42	c.7006T>C	Term2336Arg	[98]
Prpf31			
3	C319C>G	Leu107Val	[66]
4	c.413C>A	Thr138Lys	[103]
6	c.581C>A	Ala194Glu	[60]
6	c.646G>C	Ala216Pro	[60]
6	C669G>A	Gly224Arg	[104]
8	c.871G>C	Ala291Pro	[86]
8	c.895T>C	Cys299Arg	[86]
SNRNP200/Brr2			
16	c.2041C>T	Arg681Cys	[81]
16	c.2042G>A	Arg681His	[81,105]
16	c.25047G>T	Val683Leu	[81]
16	c.2066A>G	Tyr689Cys	[81]
20	c.2653C>G	Gln885Glu	[80]
25	c.3260C>T	Ser1087Leu	[78,81]
25	c.3269G>T	Arg1090Leu	[106]
DHX38/Prpf16			
7	c.995G>A	Gly332Asp	[91]
RP9/PAP1			
6	c.509A>G	Asp170Gly	[83]

Notes.
*Link to RP phenotype is not fully established
**Extension of the protein by 41aa
***This mutation causes partial skipping of exon 4 resulting in a frameshift

impact on the final function of the protein as well as on the malignant phenotype is currently unknown.

Prpf31

The human *PRPF31* gene is comprised of 14 exons and encodes a highly conserved 61 kDa protein of 499 amino acids.[45] Prpf31 is an essential protein that is involved in the assembly and stability of the U4/U6•U5 tri-snRNP. Prpf31 is part of the U4 snRNP, which binds the U5-specific protein Prpf6, creating a bond which is essential for the formation of the splicing competent U4/U6•U5 tri-snRNP.[30,45,59]

Mutations in *PRPF31* were first described 15 year ago by Vithana et al..[60] To date, ~60 mutations have been reported according to http://www.hgmd.cf.ac.uk[60-65] Most mutations in *PRPF31* are deletions, frameshifts or mutations that inhibit splicing and thus include introns that contain many premature stop codons. All of these types of mutations destabilize Prpf31 mRNA, which results in reduced concentration of functional Prpf31, which has been suggested to be a major cause of RP in patients carrying *PRPF31* mutations.[61,66] The correlation between reduced Prpf31 expression and eye malfunction was further confirmed in mouse, zebrafish and fruit fly model organisms.[39,40,67] However, several individuals carrying mutated *PRPF31* did not present RP symptoms. It was shown that Prpf31 insufficiency can be compensated by higher expression from a wild type allele. Several genomic loci that enhance Prpf31 expression and prevent disease development have been identified.[61,66,68,69]

In addition to mutations reducing the level of Prpf31 mRNA, several missense mutations, which segregate with RP, have been described. Two of these substitutions, Ala194Glu and Ala216Pro, found in the NOP domain, which has been identified in several RNP proteins. Both mutated variants exhibit reduced import to the cell nucleus.[70,71] It was further determined that Prpf31[Ala216Pro] was not properly incorporated into U4/U6 snRNP but surprisingly, Prpf31[Ala216Pro] interacted with U5-specific Prpf6 both *in vitro* and *in vivo*.[72,73] These results suggest a cytotoxic effect of the Ala216Pro substitution via the blocking of Prpf6 and thus preventing U4/U6 snRNP interaction with U5 snRNP. Interestingly, this negative effect of Prpf31[Ala216Pro] can be reverted by higher Prpf6 expression .[72] Consistently, the Prpf31 mutation reduces the concentration of active snRNPs as well as the splicing efficiency of reporter and endogenous genes.[36,72,73]

SNRNP200/Brr2

SNRNP200 is a large nuclear protein of 200 kDa, which interacts extensively with U5-specific proteins EFTUD2 (hSnu114) and Prpf8. SNRNP200 is a DExD/H box RNA helicase composed of two helicase modules, one active and the other possessing a regulatory function.[74,75] SNRNP200 unwinds U4/U6 snRNAs during spliceosome activation and the helicase activity is regulated by the C-terminus of Prpf8 and GTPase activity of EFTUD2 proteins.[52,53,76,77] To date, six mutations have been identified in SNRNP200 (Table 2). Two mutations (Ser1087Leu and Arg1090Leu) are in the Sec63-like domain of the enzymatically active N-terminal helicase module. Both of these mutations reduce unwinding activity and promote usage of cryptic splice sites, which indicate an influence on splicing fidelity.[78,79] Interestingly, in contrast to mutations in other snRNP proteins, these two mutations do not affect snRNP formation and are normally incorporated into snRNPs.[79] Five mutations (Arg681Cys, Arg681His, Val683Leu, Tyr689Cys and Gln885Glu) were recently found in the Ski2-like helicase domain of the N- terminal helicase module.[80,81] These data together with mutations found in the Prpf8 domain, which regulates SNRNP200 function, suggests that malfunction or ill-timing of SNRNP200 has a deleterious effect on splicing resulting in cell death.
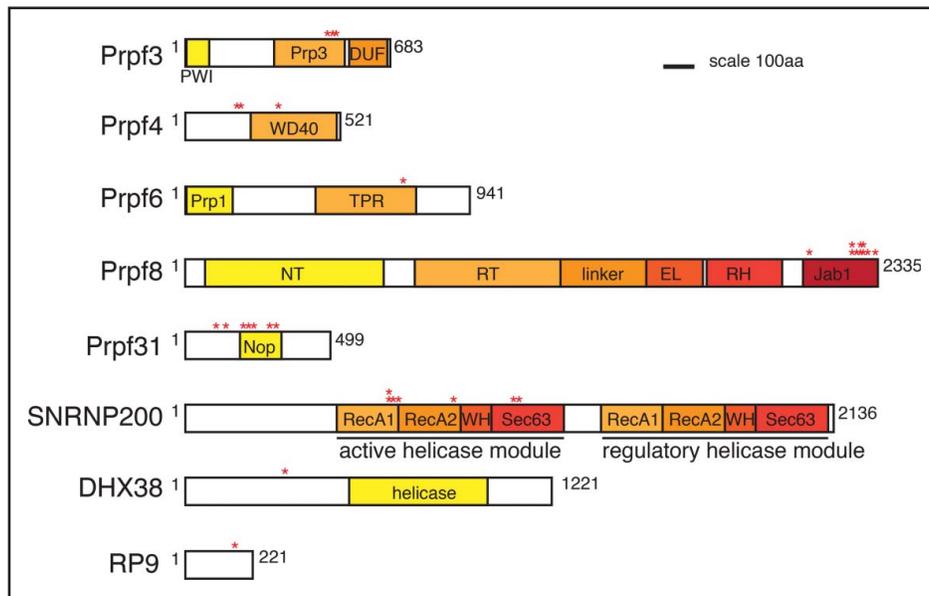
Figure 1. Domain structure and RP mutations of splicing factors. The length of proteins and position of mutations (marked by red asterisks) are approximately in scale. Abbreviations: DUF - DUF1115 domain involved in RNA binding; NT- N-terminal domain; RT- reverse transcriptase -like domain; EL - endonuclease-like domain; RH - RNase H-like domain.

RP9/PAP-1

RP9 (also called PAP-1) is a 221 amino acid protein originally identified as a target and an interacting partner of Pim-1 kinase.[82] Later, two amino acid substitutions within RP9 (His137Leu and Asp170Gly) were associated with adRP.[83] RP9 protein localizes to the nuclear splicing factor compartment, which contains many splicing factors. In addition, RP9 interacts with Prpf3, a U4/U6 snRNP specific protein.[31] The authors who described the RP9 interaction with Prpf3 also observed an association of RP9 with tri-snRNPs, however, other studies analyzing the composition of snRNPs did not detected RP9 protein in di- or tri-snRNPs.[84] RP9 exhibits splicing activity both *in vitro* and *in vivo* but the exact role of RP9 in splicing remains to be clarified. It should be mentioned that only the RP9[Asp170Gly] mutated variant exhibited splicing defects.[85] A pseudogene of the *RP9* gene was found at the same chromosomal locus. Two different studies reported that the *RP9* pseudogene carries mutations that cause either His137Leu[86] or Asp170Gly substitutions.[87] These findings together with the unclear function of RP9 during splicing, presents the question of whether mutations in the *RP9* gene are causative in adRP.

DHX38

DHX38 is a 140 kDa DEAH-box helicase which acts during splicing. Its function was extensively studied in yeast where the DHX38 homolog, Prp16p, interacts with the Nineteen complex and Brr2p (the yeast homolog of SNRNP200).[88,89] Recent data suggest that Prp16p is important for Nineteen complex mediated remodeling of U2-U6 snRNA helix during splicing.[90] In 2014, Ajmal et al. found that a homozygotic single nucleotide mutation, which causes Gly332Asp substitution, correlated with early onset RP.[91] Thus, this is the first example of a recessive mutation in a splicing factor linked to RP.

Conlusions

The list of RP mutations in splicing proteins and their diverse effects on protein functions does not provide a clear clue of the molecular mechanisms underlying the RP phenotype. One mutation depicts only a mild splicing phenotype while another one significantly limits protein function. Nevertheless, splicing defects are most often mentioned when explaining adRP which is caused by mutations in genes that code for splicing proteins. Retina cells express unusually high amounts of spliceosome components, which indicates a high demand for splicing.[36,92] Moreover, inefficient splicing and alterations in alternative splicing have been reported in cell culture experiments, animal models and blood cells derived from RP patients. However, it should be mentioned that general splicing defects do not always lead to retina degeneration because mutation of splicing factors FUS and SMN cause motor neuron degeneration while the photoreceptors seem to be not affected. The explanation of these differences could lie in recent studies that analyzed splicing after depletion of various splicing factors. First, Pleiss et al. showed in yeast that the depletion of core splicing proteins resulted in transcript specific splicing defects.[93] In human cell culture, the knockdown of different core splicing factors exhibited a gene-specific alternative splicing phenotype. In addition, different tri-snRNP-specific proteins affected the same set of genes.[57] Finally, the reduction of Prpf8 resulted in gene-specific splicing changes and affected mainly genes with weak 5′ splice site.[56] This differential sensitivity of various genes to a reduced concentration of core splicing factors could explain the tissue specific phenotype of mutations in generic splicing factors. Mutations in tri-snRNP proteins affect all splicing events but some retina specific transcripts are overly sensitive to reduced levels of functional tri-snRNP. Potential targets could include genes, which are involved in retinal metabolism or those genes that protect against light induced oxidative damage, to which

retina cells are quite susceptible. However, retina-specific mis-spliced variants have not yet been identified and further research is necessary to compare splicing variants in wild-type retina and retina expressing mutated proteins. It would be also interesting to see whether splicing defects are in major U2 introns or in U12 introns as was suggested for SMA.[94]

Another interesting question is why many patients carrying splicing mutations develop RP in the second or third decade of their life. A similar later onset has been documented for patients with ALS as well. Two potential splicing factors FUS and TDP-43 were found mutated in several ALS patients and in both cases mutations stimulated protein aggregation.[25,95] Recently, it was shown that FUS protein aggregation increases over time and is enhanced by ALS mutations.[96] Many RP mutations in splicing factors abrogate their incorporation into mature snRNPs, likely by inhibiting proper protein folding. Is it thus possible that protein aging, as suggested in several neurodegenerative disorders and laminopathies, is responsible for RP phenotypes? Similarly to prions, a mutation in a splicing factor induces protein-folding defects that slowly propagate in a healthy protein population and inactivates it. Indeed, large aggregates and amorphous deposits have been observed in photoreceptors and RPE expressing mutated proteins.[34,38] It should be also noted that photoreceptor cells do not regenerate and thus any protein defect or stress will accumulate over time possibly resulting in an increased probability of cell death. An alternative hypothesis is based on the unfolded protein response, which detects mis-folded proteins and alter cellular metabolism. Constant production of mis-folded snRNP proteins creates long-lasting stress that together with photooxidative damage, retina cells are prone to, trigger apoptosis. Consistently, it was shown recently that mutation in the *RHO* gene promotes retinal degeneration in rats likely via persistent activation of unfolded protein response and induced changes in calcium homeostasis.[97]

Finally, findings that mice expressing RP mutants exhibit primary defects in the phagocytic function of RPE[38] as opposed to photoreceptors indicate that we are still far from a complete understanding of molecular principles underlying RP. Hence, further research is necessary to elucidate the molecular cause of the disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

Acknowledgment

Funding

References

1. Matera AG, Wang Z. A day in the life of the spliceosome. Nat Rev Mol Cell Biol 2014; 15:108-21; PMID:24452469; http://dx.doi.org/10.1038/nrm3742
2. Wahl MC, Will CL, Luhrmann R. The spliceosome: design principles of a dynamic RNP machine. Cell 2009; 136:701-18; PMID:19239890; http://dx.doi.org/10.1016/j.cell.2009.02.009
3. Chabot B, Shkreta L. Defective control of pre-messenger RNA splicing in human disease. J Cell Biol 2016; 212:13-27; PMID:26728853; http://dx.doi.org/10.1083/jcb.201510032
4. Linder B, Fischer U, Gehring NH. mRNA metabolism and neuronal disease. FEBS Lett 2015; 589:1598-606; PMID:25957814; http://dx.doi.org/10.1016/j.febslet.2015.04.052
5. Daiger SP, Sullivan LS, Bowne SJ. Genes and mutations causing retinitis pigmentosa. Clin Genetics 2013; 84:132-41; PMID:23701314; http://dx.doi.org/10.1111/cge.12203
6. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet 2006; 368:1795-809; PMID:17113430; http://dx.doi.org/10.1016/S0140-6736(06)69740-7
7. Huranova M, Ivani I, Benda A, Poser I, Brody Y, Hof M, Shav-Tal Y, Neugebauer KM, Stanek D. The differential interaction of snRNPs with pre-mRNA reveals splicing kinetics in living cells. J Cell Biol 2010; 191:75-86; PMID:20921136; http://dx.doi.org/10.1083/jcb.201004030
8. Gornemann J, Kotovic KM, Hujer K, Neugebauer KM. Cotranscriptional spliceosome assembly occurs in a stepwise fashion and requires the cap binding complex. Mol Cell 2005; 19:53-63; PMID:15989964; http://dx.doi.org/10.1016/j.molcel.2005.05.007
9. Kondo Y, Oubridge C, van Roon AM, Nagai K. Crystal structure of human U1 snRNP, a small nuclear ribonucleoprotein particle, reveals the mechanism of 5′ splice site recognition. Elife 2015; 4: e04986
10. Sickmier EA, Frato KE, Shen H, Paranawithana SR, Green MR, Kielkopf CL. Structural basis for polypyrimidine tract recognition by the essential pre-mRNA splicing factor U2AF65. Mol Cell 2006; 23:49-59; PMID:16818232; http://dx.doi.org/10.1016/j.molcel.2006.05.025
11. Papasaikas P, Valcarcel J. The Spliceosome: The Ultimate RNA Chaperone and Sculptor. Trends Biochem Sci 2016; 41:33-45; PMID:26682498; http://dx.doi.org/10.1016/j.tibs.2015.11.003
12. Laggerbauer B, Achsel T, Luhrmann R. The human U5-200 kD DEXH-box protein unwinds U4/U6 RNA duplices in vitro. Proc Natl Acad Sci U S A 1998; 95:4188-92; PMID:9539711; http://dx.doi.org/10.1073/pnas.95.8.4188
13. Kim DH, Rossi JJ. The first ATPase domain of the yeast 246-kDa protein is required for in vivo unwinding of the U4/U6 duplex. RNA 1999; 5:959-71; PMID:10411139; http://dx.doi.org/10.1017/S135583829999012X
14. Berglund JA, Chua K, Abovich N, Reed R, Rosbash M. The splicing factor BBP interacts specifically with the pre-mRNA branchpoint sequence UACUAAC. Cell 1997; 89:781-7; PMID:9182766; http://dx.doi.org/10.1016/S0092-8674(00)80261-5
15. Keating KS, Toor N, Perlman PS, Pyle AM. A structural analysis of the group II intron active site and implications for the spliceosome. RNA 2010; 16:1-9; PMID:19948765; http://dx.doi.org/10.1261/rna.1791310
16. Anokhina M, Bessonov S, Miao Z, Westhof E, Hartmuth K, Luhrmann R. RNA structure analysis of human spliceosomes reveals a compact 3D arrangement of snRNAs at the catalytic core. EMBO J 2013; 32:2804-18; PMID:24002212; http://dx.doi.org/10.1038/emboj.2013.198
17. Jaladat Y, Zhang B, Mohammadi A, Valadkhan S. Splicing of an intervening sequence by protein-free human snRNAs. RNA Biol 2011; 8:372-7; PMID:21445000; http://dx.doi.org/10.4161/rna.8.3.15386
18. Valadkhan S. Role of the snRNAs in spliceosomal active site. RNA Biol 2010; 7:345-53; PMID:20458185; http://dx.doi.org/10.4161/rna.7.3.12089
19. Yan C, Hang J, Wan R, Huang M, Wong CC, Shi Y. Structure of a yeast spliceosome at 3.6-Å resolution. Science 2015; 349:1182-91; PMID:26292707; http://dx.doi.org/10.1126/science.aac7629

20. Hang J, Wan R, Yan C, Shi Y. Structural basis of pre-mRNA splicing. Science 2015; 349:1191-8; PMID:26292705; http://dx.doi.org/10.1126/science.aac8159

21. Konig SL, Liyanage PS, Sigel RK, Rueda D. Helicase-mediated changes in RNA structure at the single-molecule level. RNA Biol 2013; 10:133-48; PMID:23353571; http://dx.doi.org/10.4161/rna.23507

22. Fourmann JB, Schmitzova J, Christian H, Urlaub H, Ficner R, Boon KL, Fabrizio P, Lührmann R. Dissection of the factor requirements for spliceosome disassembly and the elucidation of its dissociation products using a purified splicing system. Genes Dev 2013; 27:413-28; PMID:23431055; http://dx.doi.org/10.1101/gad.207779.112

23. Spiluttini B, Gu B, Belagal P, Smirnova AS, Nguyen VT, Hebert C, Schmidt U, Bertrand E, Darzacq X, Bensaude O. Splicing-independent recruitment of U1 snRNP to a transcription unit in living cells. J Cell Sci 2010; 123:2085-93; PMID:20519584; http://dx.doi.org/10.1242/jcs.061358

24. Yu Y, Reed R. FUS functions in coupling transcription to splicing by mediating an interaction between RNAP II and U1 snRNP. Proc Natl Acad Sci U S A 2015; 112:8608-13; PMID:26124092; http://dx.doi.org/10.1073/pnas.1506282112

25. Kwiatkowski TJ, Jr, Bosco DA, Leclerc AL, Tamrazian E, Vanderburg CR, Russ C, Davis A, Gilchrist J, Kasarskis EJ, Munsat T, et al. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 2009; 323:1205-8; PMID:19251627; http://dx.doi.org/10.1126/science

26. Yu Y, Chi B, Xia W, Gangopadhyay J, Yamazaki T, Winkelbauer-Hurt ME, Yin S, Eliasse Y, Adams E, Shaw CE, et al. U1 snRNP is mislocalized in ALS patient fibroblasts bearing NLS mutations in FUS and is required for motor neuron outgrowth in zebrafish. Nucleic Acids Res 2015; 43:3208-18; PMID:25735748; http://dx.doi.org/10.1093/nar/gkv157

27. Stejskalova E, Stanek D. The splicing factor U1-70K interacts with the SMN complex and is required for nuclear gem integrity. J Cell Sci 2014; 127:3909-15; PMID:25052091; http://dx.doi.org/10.1242/jcs.15538

28. Nottrott S, Urlaub H, Luhrmann R. Hierarchical, clustered protein interactions with U4/U6 snRNA: a biochemical role for U4/U6 proteins. Embo J 2002; 21:5527-38; PMID:12374753; http://dx.doi.org/10.1093/emboj/cdf544

29. Liu S, Mozaffari-Jovin S, Wollenhaupt J, Santos KF, Theuser M, Dunin-Horkawicz S, Fabrizio P, Bujnicki JM, Lührmann R, Wahl MC. A composite double-/single-stranded RNA-binding region in protein Prp3 supports tri-snRNP stability and splicing. ELife 2015; 4: e07320; PMID:26161500; http://dx.doi.org/10.7554/eLife.07320

30. Liu S, Rauhut R, Vornlocher HP, Luhrmann R. The network of protein-protein interactions within the human U4/U6.U5 tri-snRNP. RNA 2006; 12:1418-30; PMID:16723661; http;//dx.doi.org/10.1261/rna.55406

31. Maita H, Kitaura H, Ariga H, Iguchi-Ariga SM. Association of PAP-1 and Prp3p, the products of causative genes of dominant retinitis pigmentosa, in the tri-snRNP complex. Exp Cell Res 2005; 302:61-8; PMID:15541726; http://dx.doi.org/10.1016/j.yexcr.2004.08.022

32. Chakarova CF, Hims MM, Bolz H, Abu-Safieh L, Patel RJ, Papaioannou MG, Inglehearn CF, Keen TJ, Willis C, Moore AT, et al. Mutations in HPRP3, a third member of pre-mRNA splicing factor genes, implicated in autosomal dominant retinitis pigmentosa. Hum Mol Genet 2002; 11:87-92; PMID:11773002; http://dx.doi.org/10.1093/hmg/11.1.87

33. Gamundi MJ, Hernan I, Muntanyola M, Maseras M, Lopez-Romero P, Alvarez R, Dopazo A, Borrego S, Carballo M. Transcriptional expression of cis-acting and trans-acting splicing mutations cause autosomal dominant retinitis pigmentosa. Human mutation 2008; 29:869-78; PMID:18412284; http://dx.doi.org/10.1002/humu.20747

34. Comitato A, Spampanato C, Chakarova C, Sanges D, Bhattacharya SS, Marigo V. Mutations in splicing factor PRPF3, causing retinal degeneration, form detrimental aggregates in photoreceptor cells. Hum Mol Genet 2007; 16:1699-707; PMID:17517693; http://dx.doi.org/10.1093/hmg/ddm118

35. Gonzalez-Santos JM, Cao H, Duan RC, Hu J. Mutation in the splicing factor Hprp3p linked to retinitis pigmentosa impairs interactions within the U4/U6 snRNP complex. Hum Mol Genet 2008; 17:225-39; PMID:17932117; http://dx.doi.org/10.1093/hmg/ddm300

36. Tanackovic G, Ransijn A, Thibault P, Abou Elela S, Klinck R, Berson EL, Chabot B, Rivolta C. PRPF mutations are associated with generalized defects in spliceosome formation and pre-mRNA splicing in patients with retinitis pigmentosa. Hum Mol Genet 2011; 20:2116-30; PMID:21378395; http://dx.doi.org/10.1093/hmg/ddr094

37. Graziotto JJ, Inglehearn CF, Pack MA, Pierce EA. Decreased levels of the RNA splicing factor Prpf3 in mice and zebrafish do not cause photoreceptor degeneration. Invest Ophthalmol Visual Sci 2008; 49:3830-8; PMID:18552388; http://dx.doi.org/10.1167/iovs.07-1483

38. Graziotto JJ, Farkas MH, Bujakowska K, Deramaudt BM, Zhang Q, Nandrot EF, Inglehearn CF, Bhattacharya SS, Pierce EA. Three gene-targeted mouse models of RNA splicing factor RP show late-onset RPE and retinal degeneration. Invest Ophthalmol Visual Sci 2011; 52:190-8; PMID:20811066; http://dx.doi.org/10.1167/iovs.10-5194

39. Farkas MH, Lew DS, Sousa ME, Bujakowska K, Chatagnon J, Bhattacharya SS, Pierce EA, Nandrot EF. Mutations in pre-mRNA processing factors 3, 8, and 31 cause dysfunction of the retinal pigment epithelium. Am J Pathol 2014; 184:2641-52; PMID:25111227; http://dx.doi.org/10.1016/j.ajpath.2014.06.026

40. Linder B, Dill H, Hirmer A, Brocher J, Lee GP, Mathavan S, Bolz HJ, Winkler C, Laggerbauer B, Fischer U. Systemic splicing factor deficiency causes tissue-specific defects: a zebrafish model for retinitis pigmentosa. Hum Mol Genet 2011; 20:368-77; PMID:21051334; http://dx.doi.org/10.1093/hmg/ddq473

41. Chen X, Liu Y, Sheng X, Tam PO, Zhao K, Chen X, Rong W, Liu Y, Liu X, Pan X, et al. PRPF4 mutations cause autosomal dominant retinitis pigmentosa. Hum Mol Genet 2014; 23(11):2926-39; PMID:24419317; http://dx.doi.org/10.1093/hmg/ddu005

42. Linder B, Hirmer A, Gal A, Ruther K, Bolz HJ, Winkler C, Laggerbauer B, Fischer U. Identification of a PRPF4 loss-of-function variant that abrogates U4/U6.U5 tri-snRNP integration and is associated with retinitis pigmentosa. PLoS One 2014; 9:e111754; PMID:25383878; http://dx.doi.org/10.1371/journal.pone.0111754

43. Benaglio P, San Jose PF, Avila-Fernandez A, Ascari G, Harper S, Manes G, Ayuso C, Hamel C, Berson EL, Rivolta C. Mutational screening of splicing factor genes in cases with autosomal dominant retinitis pigmentosa. Mol Vision 2014; 20:843-51; PMID:24959063

44. Makarov EM, Makarova OV, Achsel T, Luhrmann R. The human homologue of the yeast splicing factor prp6p contains multiple TPR elements and is stably associated with the U5 snRNP via protein-protein interactions. J Mol Biol 2000; 298:567-75; PMID:10788320; http://dx.doi.org/10.1006/jmbi.2000.3685

45. Makarova OV, Makarov EM, Liu S, Vornlocher HP, Luhrmann R. Protein 61K, encoded by a gene (PRPF31) linked to autosomal dominant retinitis pigmentosa, is required for U4/U6center dotU5 tri-snRNP formation and pre-mRNA splicing. Embo J 2002; 21:1148-57; PMID:11867543; http://dx.doi.org/10.1093/emboj/21.5.1148

46. Tanackovic G, Ransijn A, Ayuso C, Harper S, Berson EL, Rivolta C. A missense mutation in PRPF6 causes impairment of pre-mRNA splicing and autosomal-dominant retinitis pigmentosa. Am J Hum Genet 2011; 88:643-9; PMID:21549338; http://dx.doi.org/10.1016/j.ajhg.2011.04.008

47. Novotny I, Malinova A, Stejskalova E, Mateju D, Klimesova K, Roithova A, Švéda M, Knejzlík Z, Staněk D. SART3-Dependent accumulation of incomplete spliceosomal snRNPs in cajal bodies. Cell Reports 2015; 10:429-40; PMID:25600876; http://dx.doi.org/10.1016/j.celrep.2014.12.030

48. Velinov M, Dolzhanskaya N, Gonzalez M, Powell E, Konidari I, Hulme W, Staropoli JF, Xin W, Wen GY, Barone R, et al. Mutations in the gene DNAJC5 cause autosomal dominant Kufs disease in a proportion of cases: study of the Parry family and 8 other families. PLoS One 2012; 7:e29729; PMID:22235333; http://dx.doi.org/10.1371/journal.pone.0029729

49. Galej WP, Nguyen TH, Newman AJ, Nagai K. Structural studies of the spliceosome: zooming into the heart of the machine. Curr Opin Struct Biol 2014; 25:57-66; PMID:24480332; http://dx.doi.org/10.1016/j.sbi.2013.12.002

50. Pena V, Liu S, Bujnicki JM, Luhrmann R, Wahl MC. Structure of a multipartite protein-protein interaction domain in splicing factor prp8 and its link to retinitis pigmentosa. Mol Cell 2007; 25:615-24; PMID:17317632; http://dx.doi.org/10.1016/j.molcel.2007.01.023

51. Nguyen TH, Li J, Galej WP, Oshikane H, Newman AJ, Nagai K. Structural basis of Brr2-Prp8 interactions and implications for U5 snRNP biogenesis and the spliceosome active site. Structure 2013; 21:910-19; PMID:23727230; http://dx.doi.org/10.1016/j.str.2013.04.017

52. Mozaffari-Jovin S, Wandersleben T, Santos KF, Will CL, Luhrmann R, Wahl MC. Inhibition of RNA helicase Brr2 by the C-terminal tail of the spliceosomal protein Prp8. Science 2013; 341:80-4; PMID:23704370; hhtp://dx.doi.org/10.1126/science.1237515

53. Mozaffari-Jovin S, Wandersleben T, Santos KF, Will CL, Luhrmann R, Wahl MC. Novel regulatory principles of the spliceosomal Brr2 RNA helicase and links to retinal disease in humans. RNA Biol 2014; 11:298-312; PMID:24643059; http://dx.doi.org/10.4161/rna.28353

54. Maeder C, Kutach AK, Guthrie C. ATP-dependent unwinding of U4/U6 snRNAs by the Brr2 helicase requires the C terminus of Prp8. Nat Struct Mol Biol 2009; 16:42-8; PMID:19098916; http://dx.doi.org/10.1038/nsmb

55. Boon KL, Grainger RJ, Ehsani P, Barrass JD, Auchynnikava T, Inglehearn CF, Beggs JD. prp8 mutations that cause human retinitis pigmentosa lead to a U5 snRNP maturation defect in yeast. Nat Struct Mol Biol 2007; 14:1077-83; PMID:17934474; http://dx.doi.org/10.1038/nsmb1303

56. Wickramasinghe VO, Gonzalez-Porta M, Perera D, Bartolozzi AR, Sibley CR, Hallegger M, Ule J, Marioni JC, Venkitaraman AR. Regulation of constitutive and alternative mRNA splicing across the human transcriptome by PRPF8 is determined by 5′ splice site strength. Genome Biol 2015; 16:201; PMID:26392272; http://dx.doi.org/10.1186/s13059-015-0749-3

57. Papasaikas P, Tejedor JR, Vigevani L, Valcarcel J. Functional splicing network reveals extensive regulatory potential of the core spliceosomal machinery. Mol Cell 2015; 57:7-22; PMID:25482510; http://dx.doi.org/10.1016/j.molcel.2014.10.030

58. Kurtovic-Kozaric A, Przychodzen B, Singh J, Konarska MM, Clemente MJ, Otrock ZK, Nakashima M, Hsi ED, Yoshida K, Shiraishi Y, et al. PRPF8 defects cause missplicing in myeloid malignancies. Leukemia 2015; 29:126-36; PMID:24781015; http://dx.doi.org/10.1038/leu.2014.144

59. Schaffert N, Hossbach M, Heintzmann R, Achsel T, Luhrmann R. RNAi knockdown of hPrp31 leads to an accumulation of U4/U6 di-snRNPs in Cajal bodies. Embo J 2004; 23:3000-9; PMID:15257298; http://dx.doi.org/10.1038/sj.emboj.76600296

60. Vithana EN, Abu-Safieh L, Allen MJ, Carey A, Papaioannou M, Chakarova C, Al-Maghtheh M, Ebenezer ND, Willis C, Moore AT, et al. A human homolog of yeast pre-mRNA splicing gene, PRP31, underlies autosomal dominant retinitis pigmentosa on chromosome 19q13.4 (RP11). Mol Cell 2001; 8:375-81; PMID:11545739; http://dx.doi.org/10.1016/S1097-2765(01)00305-7

61. Rivolta C, McGee TL, Rio Frio T, Jensen RV, Berson EL, Dryja TP. Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations. Hum Mutation 2006; 27:644-53; PMID:16708387; http://dx.doi.org/10.1002/humu. 20325

62. Sato H, Wada Y, Itabashi T, Nakamura M, Kawamura M, Tamai M. Mutations in the pre-mRNA splicing gene, PRPF31, in Japanese families with autosomal dominant retinitis pigmentosa. Am J Ophthalmol 2005; 140:537-40; PMID:16139010; http://dx.doi.org/10.1016/j.ajo.2005.02.050

63. Wang L, Ribaudo M, Zhao K, Yu N, Chen Q, Sun Q, Wang L, Wang Q. Novel deletion in the pre-mRNA splicing gene PRPF31 causes autosomal dominant retinitis pigmentosa in a large Chinese family. Am J Med Genetics Part A 2003; 121A:235-9; PMID:12923864; http://dx.doi.org/10.1002/ajmg.a.20224

64. Sullivan LS, Bowne SJ, Seaman CR, Blanton SH, Lewis RA, Heckenlively JR, Birch DG, Hughbanks-Wheaton D, Daiger SP. Genomic rearrangements of the PRPF31 gene account for 2.5% of autosomal dominant retinitis pigmentosa. Invest Ophthalmol Visual Sci 2006; 47:4579-88; PMID:17003455; http://dx.doi.org/10.1167/iovs.06-0440

65. Xia K, Zheng D, Pan Q, Liu Z, Xi X, Hu Z, Deng H, Liu X, Jiang D, Deng H, et al. A novel PRPF31 splice-site mutation in a Chinese family with autosomal dominant retinitis pigmentosa. Mol Vision 2004; 10:361-5; PMID:15162096

66. Rio Frio T, Wade NM, Ransijn A, Berson EL, Beckmann JS, Rivolta C. Premature termination codons in PRPF31 cause retinitis pigmentosa via haploinsufficiency due to nonsense-mediated mRNA decay. J Clin Invest 2008; 118:1519-31; PMID:18317597; http://dx.doi.org/10.1172/JCI34211

67. Ray P, Luo X, Rao EJ, Basha A, Woodruff EA, 3rd, Wu JY. The splicing factor Prp31 is essential for photoreceptor development in Drosophila. Protein Cell 2010; 1:267-74; PMID:21203973; 10.100/s13238-010-0035-9

68. Rose AM, Shah AZ, Venturini G, Krishna A, Chakravarti A, Rivolta C, Bhattacharya SS. Transcriptional regulation of PRPF31 gene expression by MSR1 repeat elements causes incomplete penetrance in retinitis pigmentosa. Scientific Reports 2016; 6:19450; PMID:26781568; http://dx.doi.org/10.1038/srep19450

69. Vithana EN, Abu-Safieh L, Pelosini L, Winchester E, Hornan D, Bird AC, Hunt DM, Bustin SA, Bhattacharya SS. Expression of PRPF31 mRNA in patients with autosomal dominant retinitis pigmentosa: a molecular clue for incomplete penetrance? Invest Ophthalmol Visual Sci 2003; 44:4204-9; PMID:14507862

70. Deery EC, Vithana EN, Newbold RJ, Gallon VA, Bhattacharya SS, Warren MJ, Hunt DM, Wilkie SE. Disease mechanism for retinitis pigmentosa (RP11) caused by mutations in the splicing factor gene PRPF31. Hum Mol Genet 2002; 11:3209-19; PMID:12444105; http://dx.doi.org/ 10.1093/hmg/11.25.3209

71. Wilkie SE, Morris KJ, Bhattacharya SS, Warren MJ, Hunt DM. A study of the nuclear trafficking of the splicing factor protein PRPF31 linked to autosomal dominant retinitis pigmentosa (ADRP). Biochim Biophys Acta 2006; 1762:304-11; PMID:16427773; http://dx.doi.org/10.1016/j.bbadis.2005.12.004

72. Huranova M, Hnilicova J, Fleischer B, Cvackova Z, Stanek D. A mutation linked to retinitis pigmentosa in HPRP31 causes protein instability and impairs its interactions with spliceosomal snRNPs. Hum Mol Genet 2009; 18:2014-23; PMID:19293337; http://dx.doi.org/10.1093/hmg/ddp125.

73. Wilkie SE, Vaclavik V, Wu H, Bujakowska K, Chakarova CF, Bhattacharya SS, Warren MJ, Hunt DM. Disease mechanism for retinitis pigmentosa (RP11) caused by missense mutations in the splicing factor gene PRPF31. Mol Vision 2008; 14:683-90; PMID:18431455

74. Pena V, Jovin SM, Fabrizio P, Orlowski J, Bujnicki JM, Luhrmann R, Wahl MC. Common design principles in the spliceosomal RNA helicase Brr2 and in the Hel308 DNA helicase. Mol Cell 2009; 35:454-66; PMID:19716790; http://dx.doi.org/10.1016/j.molcel.2009.08.006

75. Zhang L, Xu T, Maeder C, Bud LO, Shanks J, Nix J, Guthrie C, Pleiss JA, Zhao R. Structural evidence for consecutive Hel308-like modules in the spliceosomal ATPase Brr2. Nat Struct Mol Biol 2009; 16:731-9; PMID:19525970; http://dx.doi.org/10.1038/nsmb.1625

76. Absmeier E, Wollenhaupt J, Mozaffari-Jovin S, Becke C, Lee CT, Preussner M, Heyd F, Urlaub H, Lührmann R, Santos KF, et al. The large N-terminal region of the Brr2 RNA helicase guides productive spliceosome activation. Genes Dev 2015; 29:2576-87; PMID:26637280; http://dx.doi.org/10.1101/gad.271528.115

77. Small EC, Leggett SR, Winans AA, Staley JP. The EF-G-like GTPase Snu114p regulates spliceosome dynamics mediated by Brr2p, a DExD/H box ATPase. Mol Cell 2006; 23:389-99; PMID:16885028; http://dx.doi.org/10.1016/j.molcel.2006.05.043

78. Zhao C, Bellur DL, Lu S, Zhao F, Grassi MA, Bowne SJ, Sullivan LS, Daiger SP, Chen LJ, Pang CP, et al. Autosomal-dominant retinitis pigmentosa caused by a mutation in SNRNP200, a gene required for unwinding of U4/U6 snRNAs. Am J Hum Genet 2009; 85:617-27; PMID:19878916; http://dx.doi.org/10.1016/j.ajhg.2009.09.020

79. Cvackova Z, Mateju D, Stanek D. Retinitis pigmentosa mutations of SNRNP200 enhance cryptic splice-site recognition. Hum

Mutation 2014; 35:308-17; PMID:24302620; http://dx.doi.org/10.1002/humu.22481

80. Liu T, Jin X, Zhang X, Yuan H, Cheng J, Lee J, Zhang B, Zhang M, Wu J, Wang L, et al. A novel missense SNRNP200 mutation associated with autosomal dominant retinitis pigmentosa in a Chinese family. PLoS One 2012; 7:e45464; PMID:23029027; http://dx.doi.org/10.1371/journal.pone.0045464

81. Benaglio P, McGee TL, Capelli LP, Harper S, Berson EL, Rivolta C. Next generation sequencing of pooled samples reveals new SNRNP200 mutations associated with retinitis pigmentosa. Hum Mutation 2011; 32:E2246-58; PMID:21618346; http://dx.doi.org/10.1002/humu.21485

82. Maita H, Harada Y, Nagakubo D, Kitaura H, Ikeda M, Tamai K, Takahashi K, Ariga H, Iguchi-Ariga SM. PAP-1, a novel target protein of phosphorylation by pim-1 kinase. Eur J Biochem 2000; 267:5168-78; PMID:10931201; http://dx.doi.org.10.1046/j.1432-1327.2000.01585.x

83. Keen TJ, Hims MM, McKie AB, Moore AT, Doran RM, Mackey DA, Mansfield DC, Mueller RF, Bhattacharya SS, Bird AC, et al. Mutations in a protein target of the Pim-1 kinase associated with the RP9 form of autosomal dominant retinitis pigmentosa. Eur J Hum Genet 2002; 10:245-9; PMID:12032732; http://dx.doi.org/10.1038/sj/ejhg/5200797

84. Wahl MC, Luhrmann R. SnapShot: Spliceosome Dynamics I. Cell 2015; 161:1474-e1; PMID:26046445; http://dx.doi.org/10.1016/j.cell.2015.05.050

85. Maita H, Kitaura H, Keen TJ, Inglehearn CF, Ariga H, Iguchi-Ariga SM. PAP-1, the mutated gene underlying the RP9 form of dominant retinitis pigmentosa, is a splicing factor. Exp Cell Res 2004; 300:283-96; PMID:15474994; http://dx.doi.org/10.1016/j.yexcr.2004.07.029

86. Sullivan LS, Bowne SJ, Birch DG, Hughbanks-Wheaton D, Heckenlively JR, Lewis RA, Garcia CA, Ruiz RS, Blanton SH, Northrup H, et al. Prevalence of disease-causing mutations in families with autosomal dominant retinitis pigmentosa: a screen of known genes in 200 families. Invest Ophthalmol Visual Sci 2006; 47:3052-64; PMID:16799052; http://dx.doi.org/ 10.1167/iovs.05-1443

87. Bischof JM, Chiang AP, Scheetz TE, Stone EM, Casavant TL, Sheffield VC, Braun TA. Genome-wide identification of pseudogenes capable of disease-causing gene conversion. Hum Mutation 2006; 27:545-52; PMID:16671097; http:// 10.1002/humu.20335

88. Cordin O, Hahn D, Alexander R, Gautam A, Saveanu C, Barrass JD, Beggs JD. Brr2p carboxy-terminal Sec63 domain modulates Prp16 splicing RNA helicase. Nucleic Acids Res 2014; 42:13897-910; PMID:25428373; http://dx.doi.org/10.1093/nar/gku1238.

89. Cordin O, Hahn D, Beggs JD. Structure, function and regulation of spliceosomal RNA helicases. Curr Opin Cell Biol 2012; 24:431-8; PMID:22464735; http://dx.doi.org/10.1016/j.ceb.2012.03.004.

90. Hogg R, de Almeida RA, Ruckshanthi JP, O'Keefe RT. Remodeling of U2-U6 snRNA helix I during pre-mRNA splicing by Prp16 and the NineTeen Complex protein Cwc2. Nucleic Acids Res 2014; 42:8008-23; PMID:24848011; http://dx.doi.org/10.1093/nar/gku431.

91. Ajmal M, Khan MI, Neveling K, Khan YM, Azam M, Waheed NK, Hamel CP, Ben-Yosef T, De Baere E, Koenekoop RK, et al. A missense mutation in the splicing factor gene DHX38 is associated with early-onset retinitis pigmentosa with macular coloboma. J Med Genetics 2014; 51:444-8; PMID:24737827; http://dx.doi.org/10.1136/jmedgenet-2014-102316.

92. Cao H, Wu J, Lam S, Duan R, Newnham C, Molday RS, Graziotto JJ, Pierce EA, Hu J. Temporal and tissue specific regulation of RP-associated splicing factor genes PRPF3, PRPF31 and PRPC8–implications in the pathogenesis of RP. PLoS One 2011; 6:e15860; PMID:21283520; http://dx.doi.org/10.1371/journal.pone.0015860.

93. Pleiss JA, Whitworth GB, Bergkessel M, Guthrie C. Transcript specificity in yeast pre-mRNA splicing revealed by mutations in core spliceosomal components. PLoS Biol 2007; 5:e90; PMID:17388687; http://dx.doi.org/10.1371/journal.pbio.0050090

94. Lotti F, Imlach WL, Saieva L, Beck ES, Hao le T, Li DK, Jiao W, Mentis GZ, Beattie CE, McCabe BD, et al. An SMN-dependent U12 splicing event essential for motor circuit function. Cell 2012; 151:440-54; PMID:23063131; http://dx.doi.org/10.1016/j.cell.2012.09.012

95. Winton MJ, Igaz LM, Wong MM, Kwong LK, Trojanowski JQ, Lee VM. Disturbance of nuclear and cytoplasmic TAR DNA-binding protein (TDP-43) induces disease-like redistribution, sequestration, and aggregate formation. J Biol Chem 2008; 283:13302-9; PMID:18305110; http://dx.doi.org/10.1074/jbc.M800342200.

96. Patel A, Lee HO, Jawerth L, Maharana S, Jahnel M, Hein MY, Stoynov S, Mahamid J, Saha S, Franzmann TM, et al. A liquid-to-solid phase transition of the ALS protein FUS accelerated by disease mutation. Cell 2015; 162:1066-77; PMID:26317470; http://dx.doi.org/10.1016/j.cell.2015.07.047.

97. Shinde V, Kotla P, Strang C, Gorbatyuk M. Unfolded protein response-induced dysregulation of calcium homeostasis promotes retinal degeneration in rat models of autosomal dominant retinitis pigmentosa. Cell Death Dis 2016; 7:e2085; PMID:26844699; http://dx.doi.org/10.1038/cddis.2015.325.

98. Martinez-Gimeno M, Gamundi MJ, Hernan I, Maseras M, Milla E, Ayuso C, García-Sandoval B, Beneyto M, Vilela C, Baiget M, et al. Mutations in the pre-mRNA splicing-factor genes PRPF3, PRPF8, and PRPF31 in Spanish families with autosomal dominant retinitis pigmentosa. Invest Ophthalmol Visual Sci 2003; 44:2171-7; PMID:12714658

99. Towns KV, Kipioti A, Long V, McKibbin M, Maubaret C, Vaclavik V, Ehsani P, Springell K, Kamal M, Ramesar RS, et al. Prognosis for splicing factor PRPF8 retinitis pigmentosa, novel mutations and correlation between human and yeast phenotypes. Hum Mutation 2010; 31:E1361-76; PMID:20232351; http://dx.doi.org/10.1002/humu.21236.

100. McKie AB, McHale JC, Keen TJ, Tarttelin EE, Goliath R, van Lith-Verhoeven JJ, Greenberg J, Ramesar RS, Hoyng CB, Cremers FP, et al. Mutations in the pre-mRNA splicing factor gene PRPC8 in autosomal dominant retinitis pigmentosa (RP13). Hum Mol Genet 2001; 10:1555-62; PMID:11468273; http://dx.doi.org/10.1093/hmg/10.15.1555

101. Ziviello C, Simonelli F, Testa F, Anastasi M, Marzoli SB, Falsini B, Ghiglione D, Macaluso C, Manitto MP, Garrè C, et al. Molecular genetics of autosomal dominant retinitis pigmentosa (ADRP): a comprehensive study of 43 Italian families. J Medical Genetics 2005; 42:e47; PMID:15994872; http://dx.doi.org/10.1136/jmg.2005.031682

102. De Erkenez AC, Berson EL, Dryja TP. Novel Mutations in the PRPC8 Gene, Encoding a Pre-mRNA Splicing Factor in Patients with Autosomal Dominant Retinitis Pigmentosa. Invest Ophthalmol Visual Sci 2002; 43:791

103. Waseem NH, Vaclavik V, Webster A, Jenkins SA, Bird AC, Bhattacharya SS. Mutations in the gene coding for the pre-mRNA splicing factor, PRPF31, in patients with autosomal dominant retinitis pigmentosa. Invest Ophthalmol Visual Sci 2007; 48:1330-4; PMID:17325180; http://dx.doi.org/10.1167/iovs.06-0963

104. Jin ZB, Mandai M, Yokota T, Higuchi K, Ohmori K, Ohtsuki F, Takakura S, Itabashi T, Wada Y, Akimoto M, et al. Identifying pathogenic genetic background of simplex or multiplex retinitis pigmentosa patients: a large scale mutation screening study. J Medical Genetics 2008; 45:465-72; PMID:18310263; http://dx.doi.org/10.1136/jmg.2007.056416.

105. Pan X, Chen X, Liu X, Gao X, Kang X, Xu Q, Chen X, Zhao K, Zhang X, Chu Q, et al. Mutation analysis of pre-mRNA splicing genes in Chinese families with retinitis pigmentosa. Mol Vision 2014; 20:770-9; PMID:24940031

106. Li N, Mei H, MacDonald IM, Jiao X, Hejtmancik JF. Mutations in ASCC3L1 on 2q11.2 are associated with autosomal dominant retinitis pigmentosa in a Chinese family. Invest Ophthalmol Visual Sci 2010; 51:1036-43; PMID:19710410; http://dx.doi.org/10.1167/iovs.09-3725.