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Defects of pre-mRNA splicing causing retitinis pigmentosa

Defekty v pre-mRNA sestřihu způsobující retintis pigmentosa

Bachelor´s thesis

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Poděkování

Chtěla bych především poděkovat svému školiteli Davidu Staňkovi za odborné vedení, konzultace a cenné rády během procesu psání této práce. Chtěla bych taky poděkovat celému Oddělení biologie RNA za sdílení znalostí a přijetí do kolektivu. Ráda bych též vyjádřila svoji vděčnost rodině a blízcím lidem za nekonečnou podporu a motivaci.

Prohlášení

Prohlašuji, že jsem závěrečnou práci zpracovala samostatně a že jsem uvedla všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

V Praze, 29.04.2024   Yelyzaveta Pakhomova

Abstrakt

Retinitis pigmentosa je genetická porucha postihující sítnici. Progrese onemocnění vede ke ztrátě zraku. Tato práce se zaměřuje na příčinu autozomálně dominantní retinitis pigmentosa. Konkrétněji, je nastíněna druhá největší zodpovědná skupina mutací. Výše uvedená skupina genových mutací je zodpovědná za tvorbu mutantních variant jejich odpovídajících sestřihových proteinů. Tyto proteiny a důsledky jejich mutací jsou v práci shrnuty a prezentovány. Pro každý mutovaný protein je uveden přehled dopadu mutace na sítnici. Dotyčné proteiny jsou: PRPF8, PRPF31, PRPF3, PRPF4, PRPF6, SNRNP200, DHX38 (výjimka, způsobující autozomálně recesivní retinitis pigmentosa), PAP-1, CWC27 (výjimka, způsobující autozomálně recesivní retinitis pigmentosa). Přehled literatury umožnil tezi dospět k závěru, že sestřihové proteiny s vysokou pravděpodobností hrají rozhodující roli ve zdraví sítnice. Kromě toho jsou stručně představeny některé další pozoruhodné poznatky. Například, zjištění týkající se nedostatku údajů o některých mutacích. Dalším příkladem takového zjištění je, že stále zůstává neznámé, proč tyto mutace způsobují takový tkáňově specifický fenotyp.

Klíčová slova: sestřih, retinitis pigmentosa, snRNP, sítnice, autozomálně dominantní retinitis pigmentosa, sestřihové proteiny

Abstract

Retinitis pigmentosa is a genetic disorder affecting the retina. The progression of the disease leads to vision loss. This thesis concentrates on the causation of autosomal dominant retinitis pigmentosa. More specifically, the second biggest responsible mutation group is outlined. The above mentioned gene-mutations group is responsible for the formation of mutant variants of their corresponding splicing proteins. These proteins and consequences of their mutations are reviewed and presented in the thesis. The outline of mutation impact on the retina is presented for each mutated protein. The proteins in question are: PRPF8, PRPF31, PRPF3, PRPF4, PRPF6, SNRNP200, DHX38 (an exemption causing an autosomal recessive retinitis pigmentosa), PAP-1, CWC27 (an exemption causing an autosomal recessive retinitis pigmentosa). The literature review allowed the thesis to conclude that splicing proteins are highly likely to play a critical role in retina's health. In addition, some other noteworthy findings are briefly presented. For example, findings regarding lack of data about some of the mutations. Another example of such finding is that it still remains unknown why these mutations cause such a tissue-specific phenotype.

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Glossary

RP	Retinitis pigmentosa
adRP	Autosomal dominant retinitis pigmentosa
arRP	Autosomal recessive retinitis pigmentosa
xlRP	X-linked retinitis pigmentosa
mRNA	Messenger RNA
RPE	Retinal pigmented epithelium
snRNPs	Small nuclear ribonucleoproteins
snRNA	Small nuclear RNA
WT	Wild type
iPSCs	Induced pluripotent stem cells
SS	Splice site
PPIase	Peptidyl-prolyl cis-trans isomerase

Introduction

Retinitis pigmentosa (RP) is an inherited neurodegenerative disease characterized by night blindness, followed by progressive mid-peripheral vision loss and subsequently complete loss of vision. RP is genetically heterogeneous with all three patterns of Mendelian inheritance occurring: autosomal dominant (adRP), autosomal recessive (arRP), and X-linked (xlRP) (Inglehearn, 1998). adRP forms around 30% of cases with most of the genes expressed specifically in the retina. However, the second biggest group of mutations causing adRP is attributed to proteins involved in splicing (Hartong et al., 2006).

Splicing is a process required for mature messenger RNA (mRNA) formation. Pre-mRNA is a precursor of mRNA that is synthesized during the process of transcription and contains both non-coding intervening regions (introns) and expressing sequences (exons). Subsequently, coding regions are spliced to form mature RNA (Berget et al., 1977). Splicing is catalyzed by a ribonucleoprotein complex called spliceosomes (Brody & Abelson, 1985; Frendewey & Keller, 1985).

Given that mRNA processing is a crucial step in regulation of gene expression, mutations altering the splicing pattern can be a cause of various disorders, including retinitis pigmentosa (Linder et al., 2015; López-Bigas et al., 2005). Notably, despite being synthesized ubiquitously within the organism, mutations in splicing proteins associated with retinitis pigmentosa specifically affect the retina without causing additional systemic issues. The investigation on how mutations in splicing proteins are involved in formation of such a tissue-specific phenotype can shed light on RP pathogenesis, providing a potential avenue for developing the most effective treatment at different stages.

Therefore, the aim of the thesis is to investigate existing studies about known mutations in splicing proteins to further understand the disease's pathogenesis and how mutated splicing proteins affect the retina. To fulfill this purpose, it is important, firstly, to give an overview of RP symptoms and the splicing process.

Overview of retinitis pigmentosa

RP is an inherited neurodegenerative disease. It is a rod-cone dystrophy with rods being affected first in most cases (Birch et al., 1999). Degeneration of the photoreceptors affects the retina pigmented epithelium (RPE) layer. RPE cells are detached from the Bruch membrane and migrate to the inner retina where they encircle the retinal blood vessels and form epithelial layers known as bone spicule pigments. Suggested stimulus of detachment is a close contact of RPE cells and inner retina vessels due to degeneration of the photoreceptor layer that separates them. The thick extracellular matrix layer between these cells and vascular endothelium resembles the Bruch membrane in its organization. Thickening of this layer leads to attenuation of the vascular lumina, causing symptoms with close resemblance to choriocapillaris (Fig. 1) (Jaissle et al., 2010; Li et al., 1995; Lucas, 1956; Verhoeff, 1930).
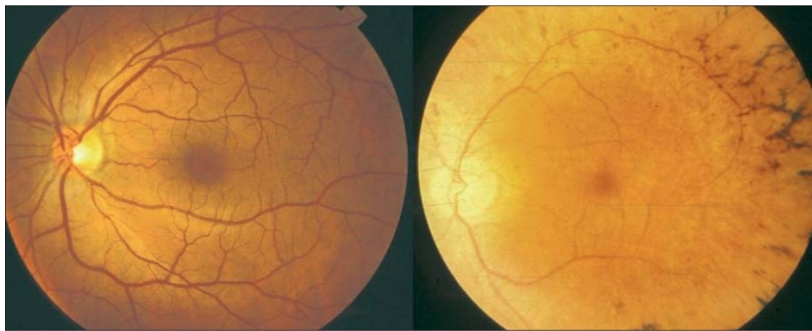

Figure 1. Eye of a healthy individual (left) and a patient with
retinitis pigmentosa (right) (Hartong et al., 2006)

Retinitis pigmentosa is a progressive disease with the first symptoms being commonly observed in adolescence. Its progression can be divided into three stages: early, mid and late stage (Hamel, 2006; Li et al., 1995):

- The main symptom of the early stage of the disease is night blindness caused by the degeneration of rods abundant on the periphery of the retina. However, visible changes of the retinal layer are rare. Thus, the diagnosis is rarely established at this stage.
- The mid stage is characterized by a more intense manifestation of night blindness due to the progression of retinal degradation. Furthermore, patients are able to notice the loss of peripheral vision and experience photophobia during the day. Bone spicule pigments are now visible, blood vessels become thin and fenestrated.

- In the late stage the gradual loss of peripheral vision evolves into tunnel vision accompanied by strong photophobia. Pigment deposits are observed in all areas of the retinal layer, blood vessels are thin and attenuated. Optic nerve head is waxy and pale. Further degeneration of the retina can lead to total loss of central vision due to degeneration of macular photoreceptors.

As was mentioned previously, RP is genetically heterogeneous. The majority of RP cases can be divided into three groups based on inheritance pattern: adRP, arRP and xlRP (Hartong et al., 2006). adRP forms 30-40% of all cases with mutations in the *rhodopsin* gene responsible for 25% of RP cases. Interestingly, mutations in splicing proteins form the second biggest group of mutations after the ones in *rhodopsin* of adRP cases (Colombo et al., 2021; Hartong et al., 2006).

RP's worldwide prevalence on average varies between 1:3000 – 1:5000. The prevalence was studied in several countries: 1:3700 in United States (Boughman et al., 1980); 1:3600 in Singapore (Teo et al., 2021); 1:4869 in Birmingham, United Kingdom (Bundey & Crews, 1984); 1:3026 in Denmark (Haim, 2002). Retinitis pigmentosa has been one of the major causes of blindness in Kuwait (20%) and in Denmark (29% in age group 20-64 y.o) (Al-Merjan et al., 2005; Buch et al., 2004). Study of prevalence in the Czech Republic has not been performed yet.

Splicing

The spliceosome is a dynamic and highly complex ribonucleoprotein machinery, which is responsible for the process of pre-mRNA splicing. This process is essential for the generation of mature mRNA transcripts before they can be translated into proteins. The spliceosome is composed of several small nuclear ribonucleoproteins (snRNPs), each consisting of a small nuclear RNA (snRNA) molecule complexes with specific proteins. These snRNPs include U1, U2, U4, U5, and U6 snRNPs, which participate in different stages of spliceosome assembly and catalysis. In addition to snRNPs, the spliceosome contains numerous non-snRNP protein factors that aid in spliceosome assembly, stabilization, and catalysis (Fig. 2) (Will & Luhrmann, 2011).
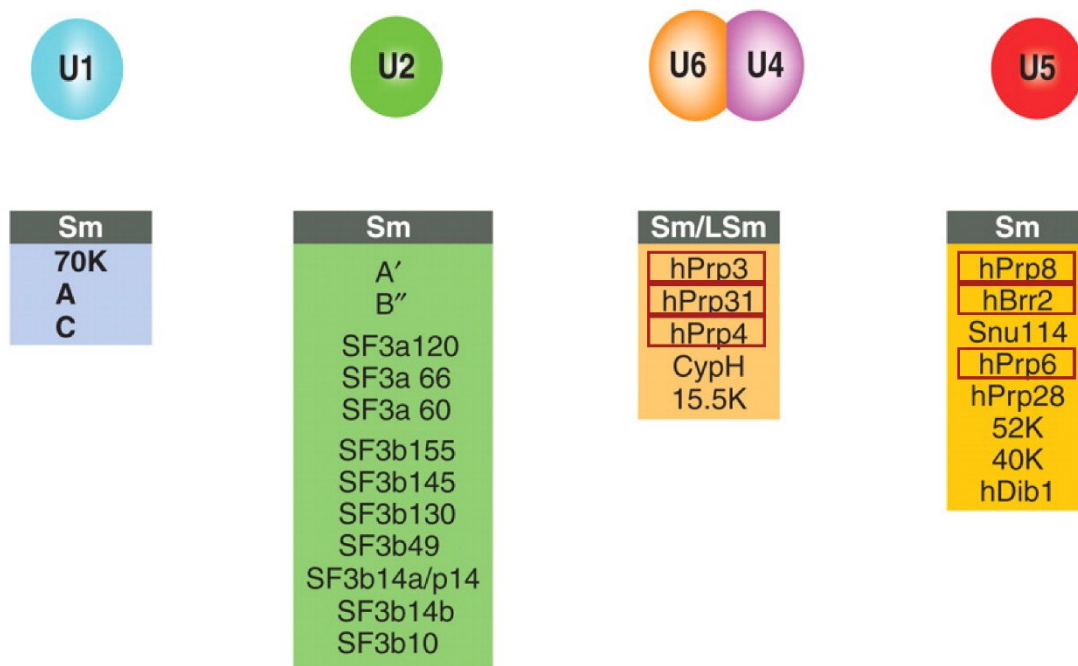

Figure 2. Protein composition the major human spliceosomal snRNPs. RP-linked proteins marked with red frames (edited from Will & Luhrmann, 2011).

The assembly of the spliceosome involves a series of steps that culminate in the precise excision of introns and ligation of exons via the formation of the E complex, pre-spliceosome A complex and pre-catalytic B complex, followed by the formation of the catalytically active spliceosome (B* complex and C complex) (Fig. 3) (Wahl et al., 2009; Will & Luhrmann, 2011). The process begins with the recognition of the pre-mRNA molecule by the U1 snRNP. U1 snRNP binds to the 5' splice site at the junction of the exon and intron. This step is followed by the recruitment of U2 snRNP to the branch point sequence located near the 3' end

4

of the intron. Subsequently, the U4/U6-U5 tri-snRNP is formed by the association of U4, U6, and U5 snRNPs and recruited to complex A to form complex B (Wahl et al., 2009; Will & Luhrmann, 2011). The pre-catalytic complex undergoes a series of rearrangements to form the catalytically active spliceosome. This involves the displacement of the U1 snRNP from the 5' splice site by U6, interaction of U6 and U2 and the release of U4 snRNP from the U4/U6-U5 tri-snRNP complex (Wahl et al., 2009; Will & Luhrmann, 2011).

The catalytically active spliceosome catalyzes two sequential transesterification reactions. The 2'-OH group of the branch point adenosine attacks the phosphate at the 5' splice site leading to the formation of a lariat intermediate and the release of the 5' exon. Then the 3' OH of the 5' exon attacks the phosphate at the 3' splice site, resulting in the joining of the exons. The ligated exons and the spliced intron lariat are released. The spliceosome disassembles, completing the splicing process (Wahl et al., 2009; Will & Luhrmann, 2011).
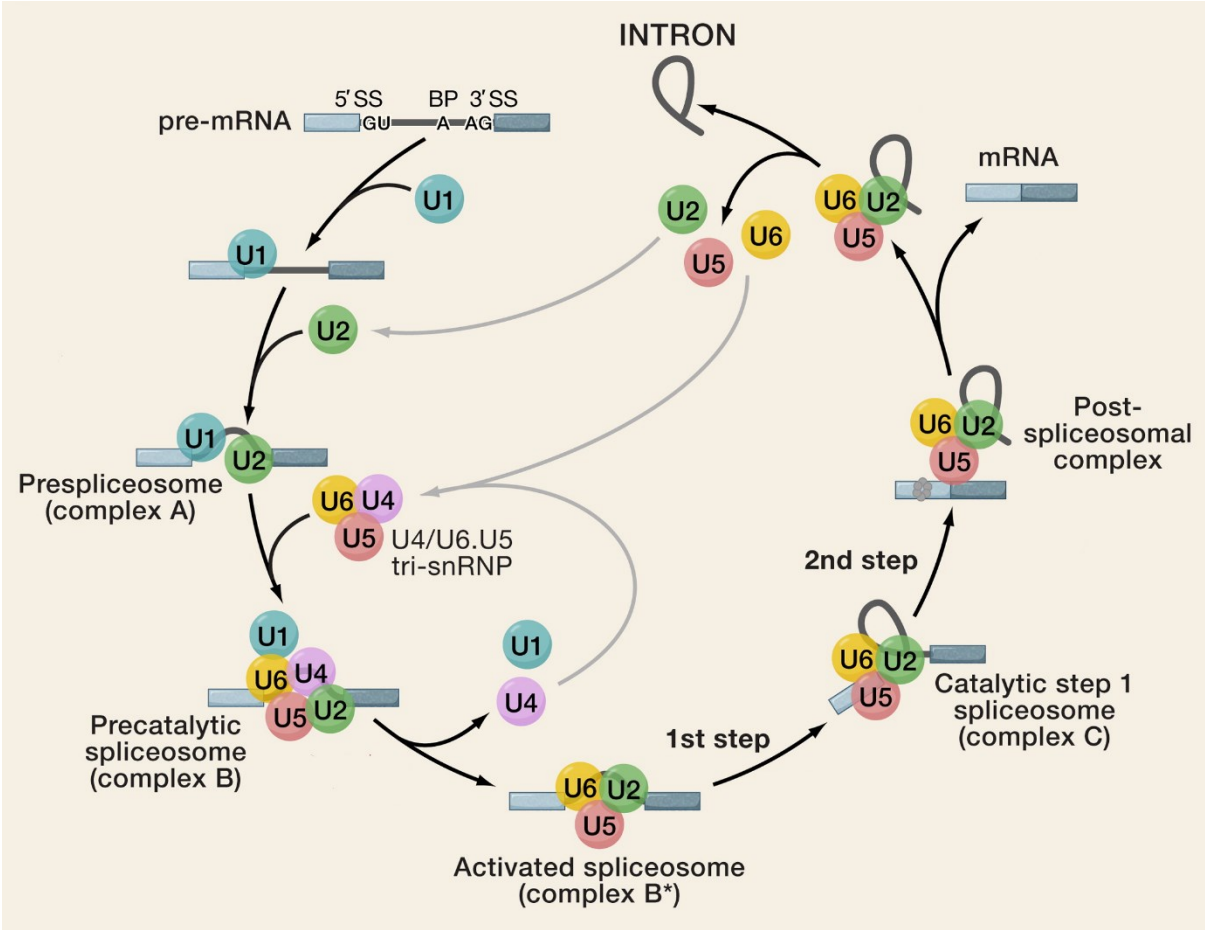

Figure 3. The stepwise cycle of the spliceosomal snRNPs during the removal of an intron from a pre-mRNA (edited from Wahl et al., 2009).

U4/U6-U5 tri-snRNP specific proteins

PRPF3

PRPF3 is a U4/U6 specific splicing protein, which is associated with RP formation. PRPF3 is evolutionarily conserved from yeast to humans, especially on its C-terminus. Within U4/U6 complex it interacts with PRPF4 and plays one of the major roles in spliceosome activation and formation of spliceosome catalytic core (Lauber et al., 1997; Nottrott et al., 2002; Wang et al., 1997). Inactivation of PRPF3 homologue in yeast leads to the failure of U4/U6-U5 complex formation. Subsequently, an active spliceosome is not formed out of the pre-spliceosome suggesting that PRPF3 is important for stabilization of the U4/U6 complex and formation of the U4/U6-U5 complex (Anthony et al., 1997).

Three mutations in *PRPF3* gene were firstly identified to cause adRP: Thr494Met, Pro493Ser and Ala489Asp (Chakarova, 2002). It is noteworthy that all these mutations are clustered in exon 11 in the C-terminal domain. This domain is especially important for the binding of U4 and U6 (Liu et al., 2015).

The Thr494Met mutation is the most studied. First, localization of wild-type (WT) and Thr494Met-mutated PRPF3 in HeLa cells was tested. In both cases PRPF3 showed similar localization in the nucleus. During the next step the localization of PRPF3 in 661W cell-line (model for in vitro studies of photoreceptor cells) and murine eye was tested. Wild-type PRPF3 localized in the nucleus with a dot-like pattern; however, the Thr494Met-mutated PRPF3 formed big aggregates inside the nucleus in these tissues. A similar pattern was observed in photoreceptors (Comitato et al., 2007). Aggregation of adRP mutants was also noticed in connection with another splicing protein PRPF31 (viz chapter PRPF31).

Another study performed by Gonzalez-Santos et al. revealed that WT PRPF3 is phosphorylated by casein kinase II and the Thr494Met mutation led to the reduction of phosphorylation. Moreover, the Thr494Met mutant had impaired interaction with the U4/U6 complex and PRPF4, leading to partial destabilization of the U4/U6 complex. Researchers have also hypothesized that the altered level of phosphorylation can lead to mis-localization of PRPF3 in the photoreceptor nucleus as mentioned in the previous paragraph (Gonzalez-Santos et al., 2008).

More recently two novel adRP mutations outside the exon 11 were identified: c.1345C > G (Arg449Gly) in exon 10 and c.1532A > C (His511Pro) in exon 12. Researchers suggested that, based on the spliceosome structure, these mutations can affect PRPF3 binding to U4/U6 and thus the stability and formation of the U4/U6-U5 tri-snRNP complex. Patients with these mutations show typical RP symptoms with an early onset of the disease (Zhong et al., 2016).

The knockout of both *PRPF3* alleles in zebrafish causes worse retinal development, suggesting that normal PRPF3 function is important for retinal development (Graziotto et al., 2008).

PRPF4

PRPF4 is a splicing protein, which is required for splicing pathway in the early stages (Banroques & Abelson, 1989). As was mentioned before (viz chapter PRPF3), it interacts with PRPF3 within the U4/U6 complex.

Until 2011, PRPF4 was not linked to RP. However, Linder et al. suggested that PRPF4 may be involved in the disease's development. They revealed that silencing of PRPF4 leads to retina-specific phenotype in the zebrafish model. The same effect was shown to be a result of the reduced expression of PRPF31 that is known to be involved in RP (Linder et al., 2011).

In a case study of a Chinese family, it was later discovered that the Pro315Leu missense mutation in *PRPF4* can be a cause of adRP. Examination of the patient's fibroblasts revealed that the level of other splicing factors in the cells was upregulated. Based on this fact a conclusion was drawn that this mutation caused a dysregulation of PRPF4 and a compensational response of other splicing factors. In the zebrafish model, the Pro315Leu mutation induced significant deformities of the embryo. In this case the retina was primarily affected, including the loss of photoreceptors, which is consistent with RP disease progression (Chen et al., 2014).

A heterozygous 18 bp deletion in *PRPF4* promoter was detected in another RP patient. This is significant since the region of *PRPF4* gene affected by the deletion contains putative binding sites for transcription factors. Consistently, this deletion downregulated the promoter activity of *PRPF4* and was associated with significantly reduced expression of PRPF4 in patient's blood (Chen et al., 2014).

Another missense mutation is known: c.575G>A (Arg192His). The mutation affects a highly conserved arginine residue. This mutation decreases the ability of PRPF4 to bind to PRPF3 and thus affects its incorporation to the tri-snRNP complex (Linder et al., 2014). Other researchers have also suggested Pro187Ala as a possible adRP mutation. However, further research is needed, as its pathogenicity cannot be both validated and excluded (Benaglio et al., 2014).

According to the above-mentioned findings it is possible to conclude that mutations in *PRPF4* can cause adRP via both haploinsufficiency and dominant-negative effect (Chen et al., 2014).

SNRNP200

SNRNP200 gene encodes the U5-200kDa RNA helicase responsible for unwinding of U4/U6 complex. With its C-terminus it interacts with the Jab domain of PRPF8, which is another spliceosomal protein linked to adRP. This interaction has a regulatory function. The interaction inhibits SNRNP200 helicase activity and prevents premature U4/U6 unwinding (Laggerbauer et al., 1998; Mozaffari-Jovin et al., 2013a; Zhang et al., 2009). Knock down of the SNRNP200 leads to compensatory response and upregulation of splicing proteins such as PRPF8, PRPF6, PRPF31, PRPF3, and downregulation of some important retinal proteins, suggesting its role in retinal health (Liu et al., 2015).

It was estimated that mutations in *SNRNP200* might be responsible for 1,6% of adRP cases (Benaglio et al., 2011; Bowne et al., 2013). Most of the mutations are missense and dominant with some exceptions (Gerth-Kahlert et al., 2019; Liu et al., 2012).

While several mutations have been identified in *SNRNP200*, only two of them have been intensively studied – Ser1087Leu and Arg1090Leu. The mutated proteins are normally incorporated into the spliceosome (Cvačková et al., 2014). Equivalent mutation in yeast was shown to compromise the unwinding of U4 and U6 (Zhao et al., 2009). However, connection between slower U4/U6 unwinding and the RP formation needs further examination as its effect on splicing kinetics is a subject of discussion (Cvačková et al., 2014).

More recently another mutation, Arg2030Cys, was found. This mutation can possibly affect the retina by impairing its development. Specifically, the mutation can have a negative effect on the retinal stem cells and retinal progenitor cells located in the area of ciliary marginal zone with high SNRNP200 expression level (Zhang et al., 2021).

PRPF6

PRPF6 is a spliceosomal protein that works as a bridging factor between U5 and U4/U6 complexes. It interacts with large U5-specific proteins (SNRNP200, PRPF8) and U4/U6 specific proteins (PRPF31, PRPF3) (Liu et al., 2006). PRPF6 depletion leads to a significant decrease in stability of tri-snRNP complexes (Galisson & Legrain, 1993).

The first RP-related mutation identified in *PRPF6* was Arg729Trp missense substitution that affects a highly conserved residue. In lymphoblasts with such mutation a colocalization of PRPF6 with Cajal bodies-specific protein was observed, suggesting a dysregulation of tri-snRNP formation or recycling (Tanackovic et al., 2011). Cajal bodies are nuclear structures that were previously shown to contain accumulated tri-snRNP intermediates when the snRNP assembly is inhibited (Novotný et al., 2015).

RPE cells with another *PRPF6* mutation Arg900His were derived from induced pluripotent stem cells (iPSCs) established by reprogramming of blood cells of a patient with adRP. In mutated RPE cells the researchers were able to observe an impaired morphological structure and a decrease in expression of RPE-specific markers. The cell polarity and barrier function were also damaged (Liang et al., 2022).

Another *PRPF6* adRP mutation Thr743Ile was also identified. However, this variant requires further studying, as its significance remains unknown (Ruiz-Justiz et al., 2023).

PRPF8

PRPF8 is a U5-specific large protein. It is located in the center of the spliceosomal core and plays a central role during the process of splicing (Achsel et al., 1998). PRPF8 contacts all three sequences crucial for splicing: 5' splice site (SS), branch point and 3' SS, and supports spliceosome assembly (Brown & Beggs, 1992; Teigelkamp et al., 1995; Turner et al., 2006). PRPF8 also interacts with other snRNPs and splicing proteins, such as SNRNP200 (viz chapter SNRNP200). As was mentioned previously, PRPF8 is a regulator of SNRNP200 having both stimulatory and inhibitory activity (Maeder et al., 2009; Mozaffari-Jovin et al., 2012, 2013a). RP mutations in *PRPF8* are clustered in the Jab1/MPN domain important for regulation of SNRNP200 activity. Considering the fact that mutations in *SNRNP200* are also linked to RP, it is possible to speculate that impaired regulation of SNRNP200 helicase

activity can be a cause of the RP. These mutations can be divided into three groups (Mozaffari-Jovin et al., 2013b, 2014):

1. Group I residues located in the globular Jab1 region. It was shown, that deletion of this region in *S.cerevisiae* impaired growth and reduced expression of *PRPF8*. Some mutations of this region caused increased levels of dissociated U4/U6 and U5 snRNPs (Bellare et al., 2006). Therefore, these mutations may destabilize the PRPF8 (Zhang et al., 2007).

2. Group II residues located in the proximal part of the C-terminal tail (R2310 and F2314).

3. Group III residues (Gln2321stop, Tyr2334, and frameshift mutations) located in the C-terminal tail.

Group II and group III residues are located in the region responsible for protein-protein interactions and can therefore affect the binding between PRPF8 and its partners suggesting that splicing products specific for the retina functions might be sensitive to these changes (Zhang et al., 2007).

Most of the *PRPF8* RP-causing mutations reduce the formation of snRNPs due to insufficient binding to SNRNP200. However, there are some exemptions that do not affect snRNPs assembly (Phe2314Leu and Tyr2334Asn), Therefore, these mutations are an interesting target for further studying (Malinová et al., 2017). Tyr2334Asn mutation was studied closely in a mouse model. It was observed that cerebellar granule cells in mutated mice were specifically degenerated, while other cerebellar cells remained unaffected. The mutation also causes deregulation of mostly granule cell specific circular RNAs in cerebellum, followed by neurodegeneration. The same study shows that the levels of splicing proteins in cerebellum and retina are decreasing with age. The reduction was even more significant in the case of the Tyr2334Asn mutation. Thus, the main hypothesis of how Tyr2334Asn mutation affects specific cell types is that the reduced level of splicing proteins makes those cells more sensitive to their mutations and provoke neurodegeneration (Krausová et al., 2023).

Interestingly, residues 2309 and 2310, in which mutations can cause adRP, are absolutely conserved throughout the tested species (*D. melanogaster*, *A. thaliana*, *C. elegans*, *S. pombe*, *T. bruceii*). Other residues that fall under group I (2301, 2304, 2314) and linked to adRP, are also highly conserved (McKie, 2001).

There is some evidence that *PRPF8* mutations can perform incomplete penetrance (Maubaret et al., 2011). Other studies have also shown the early onset of the disease during the first or second decade of life with a possibility of more severe symptoms in case of *PRPF8* mutations (Tarttelin et al., 1996; Towns et al., 2010).

PRPF31

PRPF31 is a pre-mRNA splicing factor essential for assembly of mature spliceosomal complexes, and specifically PRPF31 is required for formation of the U4/U6-U5 complex where it interacts with PRPF6 and this interaction is critical for tri-snRNP stability (Makarova, 2002; Weidenhammer, 1996; Weidenhammer et al., 1997).

Knockdown of PRPF31 protein leads to inhibition of U4/U6 and U5 association and to accumulation of U4/U6 complex in Cajal bodies (Schaffert et al., 2004). At the same time, it was also shown that adRP caused by *PRPF31* mutation has an opposite affect and leads to U5 accumulation in Cajal bodies (Georgiou et al., 2022). However, in both cases, these accumulations may be caused by dysfunction of tri-snRNP formation.

In 2001 it was found that seven mutations in *PRPF31* are responsible for adRP phenotype. Among them, mutations inactivating splice sites, both donor and acceptor, missense, frameshift mutations and duplication were observed (Vithana et al., 2001). More than 100 mutations have been additionally identified in the following years (Wheway et al., 2020).

Interestingly enough, *PRPF31* mutations may perform variant haploinsufficiency. It was shown that asymptomatic carriers have higher levels of wild-type mRNA than the affected, which was also supported by Western blot analysis of protein levels. These findings suggest an existence of low-expressed WT allele, which in combination with the mutated one causes adRP phenotype (Vithana et al., 2003). Another study proposed a differentiating mechanism based on the existence of modifier genes that modulate *PRPF31* expression (Frio et al., 2008; Venturini et al., 2012). One of the suggested modulators was *CNOT3* (Venturini et al., 2012). However, some researchers were unable to observe a correlation between the *CNOT3* genotype with the penetrance of the disease (Vithana et al., 2001; Wheway et al., 2020). Another suggested modulator is MSR1 repeat element (Rose et al., 2016).

There are several hypotheses about how mutations in *PRPF31* affect the retina. One of them is that reduced levels of PRPF31 causes mis-splicing of genes involved in phototransduction

and deregulation of genes responsible for photoreceptor cell development (Pormehr et al., 2020). Mutated PRPF31 can also impair splicing of important photoreceptor-specific genes: *rhodopsin*, *peripherin-2* and *fascin* (Mordes et al., 2007; Yuan et al., 2005).

PRPF31 double knockout in zebrafish model can also cause accumulation of DNA damage and aberrant mitosis in retina progenitor cells leading to apoptosis, suggesting its possible retina-specific role (Li et al., 2021).

Several studies reported a presence of cytoplasmic aggregates in RPE and retinal cells containing *PRPF31* mutants (Georgiou et al., 2022; Valdés-Sánchez et al., 2020). In these aggregates other splicing proteins linked to RP were also detected together with proteins involved in pathways like protein folding, stress response, and visual cycle proteins. Misfolded proteins were also found to form aggregates suggesting a possible dysfunction in the degradation system (Georgiou et al., 2022). *PRPF31* mutations were also shown to change alternative splicing (Valdés-Sánchez et al., 2020).

Other splicing proteins

PAP-1 (RP9)

PAP-1 is a splicing protein, which splicing activity is controlled by PIM-1 serine/threonine kinase (Maita et al., 2000). PAP-1 is not an essential component of spliceosomes; however, PAP-1 is able to modulate splicing under some conditions (Maita et al., 2005). PAP-1 also interacts with other splicing proteins - SC35 and U2AF35. However, its direct function in splicing remains unknown (Maita et al., 2004).

So far two adRP missense mutations were studied in PAP-1 – His137Leu and Asp170Gly – located in residues that are highly conserved (Keen et al., 2002). It was found that in cells transfected with PAP-1 D170G-mutated the level of dephosphorylated protein is higher. It was shown that PAP-1 alters splicing of E1A minigene pre-mRNA. Interestingly, PAP-1 containing the D170G mutation loses its ability to alter the splicing pattern of an E1A pre-mRNA. Thus, this allowed the researchers to conclude that less efficient phosphorylation of PAP-1 causes changes in splicing crucial for photoreceptors function, which can lead to RP (Maita et al., 2004). However, an increased level of dephosphorylation of PAP-1 in PAP-1 His137-transfected cells was not observed suggesting another mechanism of RP development (Maita et al., 2004).

Following the above-mentioned, it was discovered that PAP-1 interacts with PRPF3, suggesting a new possible mechanism of how the mutation affects splicing. However, adRP *PAP1* mutations do not disrupt binding of these proteins (Maita et al., 2005).

It is important to mention that several papers reported the existence of *PAP-1* pseudogene carrying mutations. However further studies in this direction are needed (Bischof et al., 2006; Sullivan et al., 2006).

The fact that direct function and involvement of PAP-1 into the splicing process is unknown and that the mutations are not studied properly, it raises a question whether *PAP-1* mutations are indeed involved in adRP formation and in what way.

CWC27

CWC27 is another spliceosomal protein that is associated with RP. It is a peptidyl-prolyl cis-trans isomerase (PPIase), which consists of N-terminal cyclophilin type PPIase domain and elongated C-terminus. However, the PPIase domain in humans lacks isomerase activity, even though it maintains its proline-binding activity (Davis et al., 2010; Ulrich & Wahl, 2014).

Molecular function of CWC27 in human splicing still remains unknown, however mutated *CWC27* gene knock-in in a mouse model leads to splicing changes such as intron retention, exon skipping or differential splice site usage suggesting its involvement in the splicing process. Regarding its proline-binding activity, it is possible to suggest that CWC27 provides protein-protein interactions in the spliceosome (Bertrand et al., 2022; Ulrich & Wahl, 2014). Indeed, it was found that CWC27 interacts with other proteins within the spliceosome (Busetto et al., 2020). The mouse model also showed the RP phenotype starting from the age of 3 months with photoreceptor dysfunction, which is followed by photoreceptor degradation at the age of 4 months (Bertrand et al., 2022).

Unlike mutations of other splicing proteins, mutations of *CWC27* can cause syndromic recessively inherited RP. Among such mutations is, for example, Arg119* located in the PPIase proline-binding pocket domain of CWC27 leading to truncated protein. The patient with this mutation had not only RP, but other clinical syndromes: hypoplastic/agenesic teeth, several skeletal defects, hypergonadotropic hypogonadism, and cataracts. Other study shows that in case of syndromic RP the common symptoms are craniofacial defects, short stature, brachydactyly and neurological defects (Brea-Fernández et al., 2019; Xu et al., 2017).

Another interesting observation is correlation between the position of the mutation and the severity of the phenotype: more N-terminal CWC27 truncations leads to higher severity of RP. However, this hypothesis needs further confirmation and exploration (Xu et al., 2017).

DHX38

DHX38 is a DEAH-box helicase, which is important for both transesterification reactions of splicing. One of its suggested functions is proofreading of 5' splice site cleavage and assisting in branch point splicing during the pre-mRNA splicing process (Koodathingal et al., 2010; Tseng et al., 2011). Furthermore, DHX38 is involved in spliceosome remodeling between two reactions of splicing (Hogg et al., 2014; Schwer & Guthrie, 1991). DHX38 also interacts with

another spliceosomal helicase associated with RP, SNRNP200, which unwinds the U4/U6 complex (viz chapter SNRNP200) (Cordin et al., 2014).

Three mutations in *DHX38* are known to cause RP - Gly332Asp, Arg324Gln and Ala857=. Unlike other splicing factors mutations, they cause arRP, which is characterized by its early onset (Ajmal et al., 2014; Al-Johani et al., 2021; Latif et al., 2018).

Two of mutations mentioned above (Gly332Asp, Arg324Gln) are located in the N-terminus of *DHX38* gene, which is important for nuclear localization of DHX38 and its interaction with the spliceosome (Wang & Guthrie, 1998). The most studied *DHX38* mutation is Gly332Asp. It was shown that this mutation does not affect neither DHX38 stability nor its interaction with the spliceosome but some suboptimal introns are specifically sensitive to this substitution. However, how this mutation affects the retina has to be investigated further (Obuća et al., 2022).

Even though it was known that DHX38 is linked to arRP, its role in retina and retinal development was unknown. However, in 2023, Sun et al. discovered that *DHX38* knockout in zebrafish causes chromosome nondisjunction, mitotic arrest, and apoptosis of retinal progenitor cells. They have also shown that DHX38 deficiency causes accumulation of DNA damage resulting in reduction of DNA synthesis. These findings were also confirmed for human cells. Thus, DHX38 is important for maintenance of retinal progenitor cells differentiation and homeostasis, and regulation of the mitosis process (Sun et al., 2023).

Discussion and conclusion

Retinitis pigmentosa is a genetic progressive disorder that affects retina function, leading to complete vision loss. Mutations in splicing proteins form the second biggest group of mutations causing adRP, but the reason for how and why these mutations affect the retina is an area of active research.

One of the possible explanations of sensitivity of the retina to changes in splicing is based on the knowledge that RP-linked splicing factors have higher expression compared to other tissues (Cao et al., 2011). Expression of the *PRPF3*, *PRPF8* and *PRPF31* genes in retina, especially in the photoreceptor layer, is higher than in other tissues in adult mice (Cao et al., 2011). Thus, mutations of *PRPF3*, *PRPF8* and *PRPF31* can cause a reduction of splicing activity, which leads to cumulative effect and, as a result, a tissue specific consequence for the retina, especially a photoreceptor layer (Cao et al., 2011). However, in heterozygous mice with one *PRPF3* allele knocked out retina degeneration as well as changes in expression levels of retinal specific genes, such as *rhodopsin* or *rod cGMP-gated channel alpha* subunit, were not observed (Graziotto et al., 2008). The same was observed in case of *PRPF8* knockout in a mouse model (Krausová et al., 2023). One of the suggested explanations is that the mice's life span is too short for the cumulative effect of only one altered allele to be observable. This hypothesis has not been tested yet, since the homozygous *PRPF3* knocked-out mice dies before embryonic day 14 (Graziotto et al., 2008). This upregulation of splicing factors may be a consequence of higher volumes of spliced RNA in the retina tissue (Tanackovic et al., 2011). Other study also shows that the level of splicing proteins significantly decreases with age in WT mice cerebellum. The reduction is even more significant in mice with *PRPF8* mutation found in RP patients (Krausová et al., 2023). Hence, one of the possible hypotheses is that due to higher load on splicing machinery in the retina possible defects are more significant there compared to other tissues, especially considering a possible reduction of splicing proteins expression with age.

Additional supporting evidence for this theory can be found in cancer studies. During the study patients were treated with splicing inhibitors. Treated patients started to experience vision loss (Eskens et al., 2013; Hong et al., 2014). The splicing inhibitor used in both studies is E7107. It blocks proper A complex assembly of spliceosome via targeting the U2-assoceated SF3b complex and blocking an ATP-dependent remodeling of the U2 snRNP, required for proper binding of U2 to pre-mRNA (Folco et al., 2011). The observed vision loss

was acute and progressive. The suggested mechanism of the following side effect was optic neuritis (Hong et al., 2014). The shared symptom among the effected patients was central scotoma (Eskens et al., 2013; Hong et al., 2014). Even though the exact mechanism of how E7107 splicing inhibitor causes vision loss remains unclear, the above-mentioned studies are emphasizing the importance of proper splicing process for eye health.

Another discussed mechanism is based on the possibility of existence of the unknown retina-specific function of the above-mentioned proteins. Mutations of these proteins might also impair splicing of retina-specific transcripts. While those mechanisms have not been extensively explored it is still worthwhile to consider the possibility of their occurrence.

It is important to note that while these factors may possibly contribute to the retina-specific phenotype of splicing mutations, the exact mechanism may vary depending on the splicing protein and specific mutation, as was described throughout the work.

Some treatment options can be potentially used in the future:

1. Adeno-associated virus (AAV)-mediated augmentation of the mutated gene that has already been proposed for cases with mutated *PRPF31*. This method was also proposed for treating adRP caused by mutations in the *rhodopsin* gene (Brydon et al., 2019; Cideciyan et al., 2018; Millington-Ward et al., 2011).
2. Human embryonic stem cell–derived retinal pigment epithelium implantation suggested for treating another blindness-causing disease. This method can be theoretically modified for treating RP as well (da Cruz et al., 2018; Kashani et al., 2018).
3. The Argus® II Retinal Prosthesis System – vision prosthetic device, the idea of which is to functionally replace the degenerated photoreceptors (Luo & da Cruz, 2016).
4. Usage of antisense oligonucleotides. The idea of this method is to modulate gene expression or function of the target gene (Xue & MacLaren, 2020).

To conclude, the combination of these possible treatments with protein and mutation-specific approach, based on knowledge about mechanism of impact of discussed mutations can help to develop treatment for RP caused by mutations in splicing proteins.

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