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REVIEW ARTICLE

Structural Variation of *Alu* Element and Human Disease

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Transposable elements are one of major sources to cause genomic instability through various mechanisms including *de novo* insertion, insertion-mediated genomic deletion, and recombination-associated genomic deletion. Among them is *Alu* element which is the most abundant element, composing ~10% of the human genome. The element emerged in the primate genome 65 million years ago and has since propagated successfully in the human and non-human primate genomes. *Alu* element is a non-autonomous retrotransposon and therefore retrotransposed using L1-enzyme machinery. The 'master gene' model has been generally accepted to explain *Alu* element amplification in primate genomes. According to the model, different subfamilies of *Alu* elements are created by mutations on the master gene and most *Alu* elements are amplified from the hyperactive master genes. *Alu* element is frequently involved in genomic rearrangements in the human genome due to its abundance and sequence identity between them. The genomic rearrangements caused by *Alu* elements could lead to genetic disorders such as hereditary disease, blood disorder, and neurological disorder. In fact, *Alu* elements are associated with approximately 0.1% of human genetic disorders. The first part of this review discusses mechanisms of *Alu* amplification and diversity among different *Alu* subfamilies. The second part discusses the particular role of *Alu* elements in generating genomic rearrangements as well as human genetic disorders.

Keywords: *Alu* elements, genetic disorder, genomic rearrangement, master gene model, recombination

Introduction

Transposable elements accounts for ~45% of the human genome. They are divided into DNA transposon and retrotransposons, according to their amplification mechanisms. DNA transposons mobilize through a "cut and paste" mechanism while retrotransposons propagate through "copy and paste" mechanism. Retrotransposon transcribes its RNA intermediate, and the RNA intermediate integrates into a new genomic region using a mechanism called target primed reverse transcription (TPRT). Endogenous retroviruses, long interspersed elements (LINEs), and short interspersed elements (SINEs) belong to retrotransposon. *Alu* element, one of the SINEs, is the most successful retrotransposon in primate genomes. The estimated copy number of the elements is 1.1 million and it is currently retrotranspositionally active in the human genome [1]. The full-length *Alu* element is 300 bp long and has a dimeric structure. Both of the left and right monomers were derived from 7SL RNA gene and thus they share a high level of sequence identity. *Alu* elements have an internal RNA polymerase III promoter (A and B boxes) in the 5' region and a poly (A) tail in the 3' end. The transcription of *Alu* element is initiated by internal RNA polymerase III promoter and terminated at a nearby genomic location having TTTT terminator because the element lacks a transcription terminator (Fig. 1). *Alu* elements use L1 enzyme machinery for their mobilization. L1 provides *Alu* elements with endonuclease and reverse transcriptase. L1 endonuclease recognizes consensus oligomer (5'-TTTT/AA-3') and cleaves the genomic region. A-rich region of *Alu* elements binds to the released consensus site and the elements are reverse-transcribed by L1 reverse transcriptase. The second strand of the *Alu* element is

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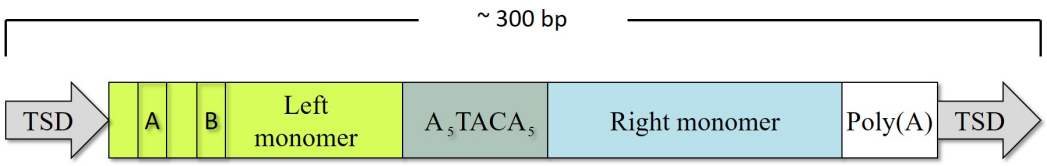

Fig. 1. Structural schema of *Alu* elements. A full-length *Alu* element is 300 bp in length and composed of left monomer, right monomer, and poly(A) tail. A and B boxes in the left monomer contain RNA polymerase III promoter binding sites. *Alu* inserts in the host genome using target site duplication (TSD) of flanking region.

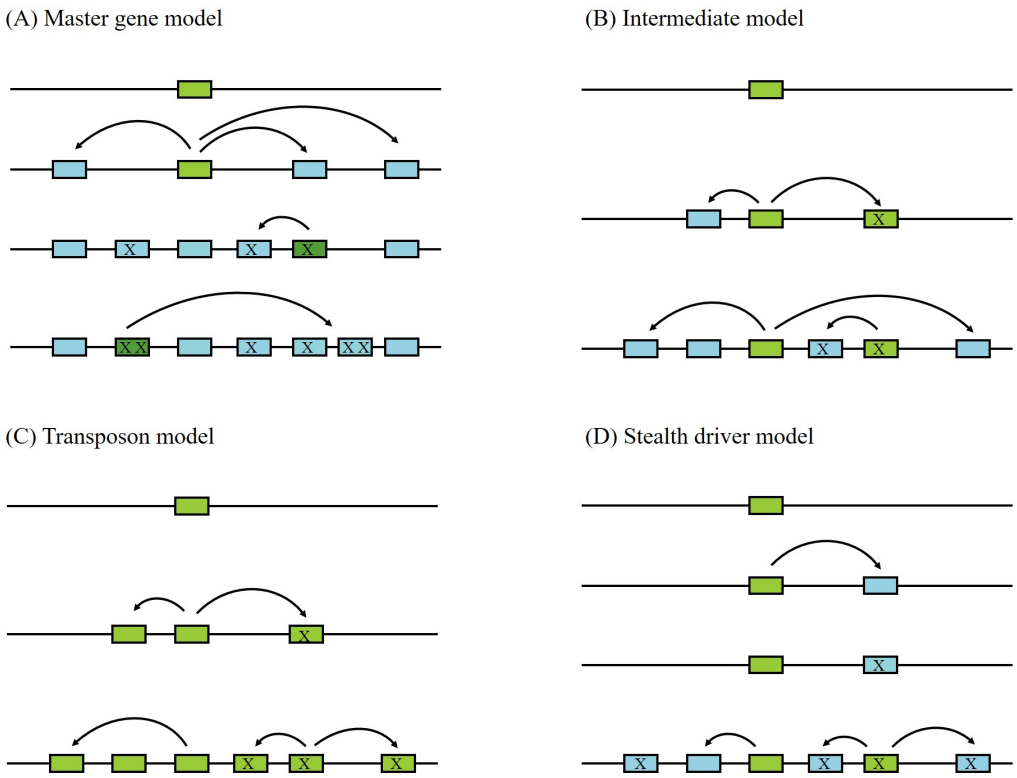

Fig. 2. Mechanisms of *Alu* amplification. This figure depicts four different mechanisms for *Alu* amplification. (A) Master gene model is a typical mechanism. All members of the same *Alu* subfamily are derived from one or a few transpositionally active *Alu* elements. When the active *Alu* element mutates but remains the transposition activity, it will produce a new *Alu* subfamily. (B) Intermediate model is literally an intermediate form of master gene and transposon models. More than a few active *Alu* elements exist in host genome and each of them actively produces its progenies. (C) Transposon model suggests that all *Alu* elements including mutated elements have transposition activities. (D) Stealth driver model suggests that old *Alu* element which stayed transcriptionally dormant for the extended period can produce a new *Alu* subfamily by retrieving a high transposition activity. Green and blue boxes indicate active and inactive *Alu* elements, respectively. X indicates a mutation and the mutated *Alu* elements represent different subfamilies.

synthesized by host DNA polymerase using the first strand of the *Alu* element as a DNA template. The newly inserted *Alu* element has 7 to 20 bp direct repeats on both sides of the element, termed target site duplications (TSDs) [2, 3].

Alu elements are divided into several subfamilies which are determined based on diagnostic nucleotides. During the past 60 million years of the primate genome evolution, old *Alu* subfamilies became retrotranspositionally dormant while new *Alu* subfamilies emerged and expanded, leading to the increased number of different *Alu* subfamilies. The generally accepted canonical mechanism for *Alu* amplification is master gene model [4] but it could not explain the recent expansion of *Alu*Yb subfamily in the human genome: *Alu*Yb subfamily was retrotranspositionally dormant for the past 20 million years but it retrieved the ability to retrotranspose and rapidly expanded in the human genome within the past a few million years, which led to a new model of *Alu* amplification, stealth model, to explain the aberrant amplification of *Alu*Yb subfamily [5].

Some of *Alu* elements amplified and spread in genic regions,

contributing to human genetic diversity [2, 4, 6]. *Alu* element is able to disrupt gene function either by inserting into exonic regions or causing alternative splicing of the genes. In addition, they could cause genomic deletions through insertion-mediated deletion or recombination-associated deletion. The homologous recombination (HR) between *Alu* elements has associated with genomic duplications, genomic conversion as well as genomic deletions in the human genome. The genomic changes could affect gene expression and lead to abnormal proteins resulting in genetic diseases [7-11].

Amplification and Diversity of *Alu* Elements

Alu elements emerged in the primate genome, approximately 65 million years ago (mya). Since then, they have successfully amplified and diversified in primate genomes. However, their amplification rate has been fluctuated over the time. The majority of *Alu* elements were inserted in the primate genome ~40 mya and one new *Alu* insertion occurred in every birth at the time of the most successful amplification [1], which is much higher than an averaged amplification rate, one insertion every 21 new births during the past 60 million years [10, 12]. *Alu* elements are divided into different subfamilies according to key diagnostic nucleotides on them. Therefore, *Alu* elements sharing the diagnostic nucleotides are grouped into the same subfamily. Major *Alu* lineages are *Alu*J, *Alu*S, and *Alu*Y which are distinguished from each other, based on 18 diagnostic nucleotides on their sequences [6]. Among the three major lineages, *Alu*Y lineage is the youngest and *Alu*J lineage is the oldest. During the long time, *Alu*J subfamilies have accumulated more random mutations on them and thus their mutation rates are much higher than the younger subfamilies [2].

There are four different models suggested for *Alu* expansion. The first model is master gene model which well explains how new *Alu* subfamily is created. It suggests that new *Alu* subfamily is generated by point mutation(s) of retrotranspositionally hyperactive *Alu* element. During the primate evolution, the master genes accumulated diagnostic nucleotide changes, leading to different subfamilies at different times. Thus, all *Alu* subfamilies were derived from a series of sequential master genes which accumulated diagnostic base changes, and all members of an *Alu* subfamily were propagated from a few master genes representing the subfamily. Because *Alu* element contains a high content of CpG dinucleotide, point mutation frequently occurs on them. In general, the master genes produce a high number of its

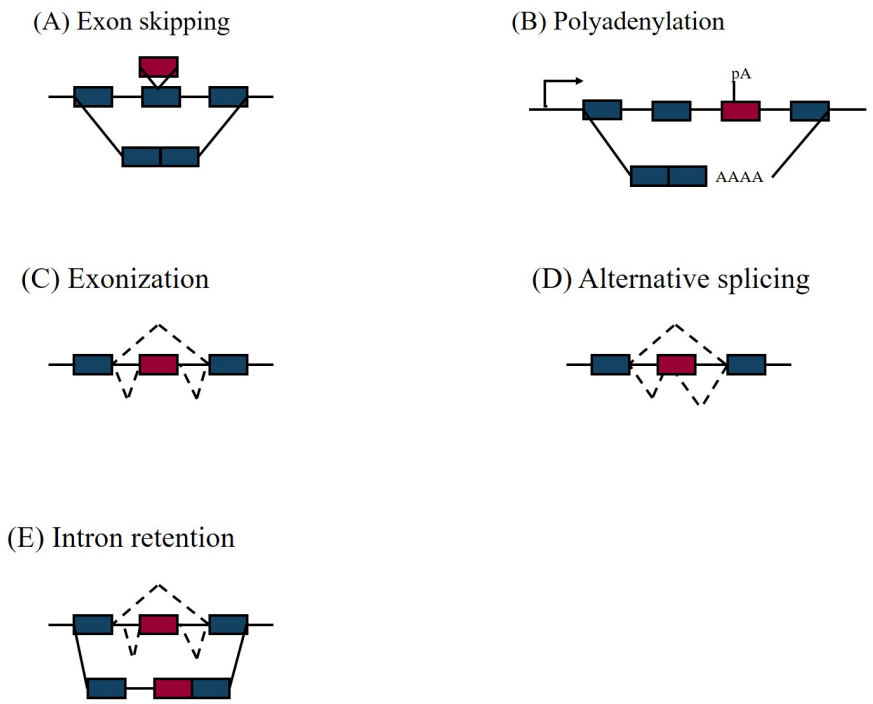

Fig. 3. Impact of *Alu* insertion on alternative transcription. *Alu* element inserting in the genic region could alter the expression level of the respective gene. (A) *Alu* insertion in the exonic region. Exons could be skipped and deleted, called exon skipping. (B) *Alu* insertion in the genic region, *Alu* element is able to provide a polyadenylation signal. Thus, *Alu* element could induce the premature termination of gene transcription. (C–E) *Alu* element is also able to provide cryptic splicing sites, leading to alternative gene transcripts. Blue and red boxes indicate exon and *Alu* element. Arrow shows promoter and the dash line indicates alternative splicing form.

progenies (Fig. 2A) [4, 6]. However, some *Alu* elements have low retrotranspositional activity and their amplification is not well explained by the master gene model. Unlike the master gene model, transposon model suggests that all elements have a similar capability of generating new copies (Fig. 2C). The third model is intermediate model which is literally the intermediate of master gene and transposon models. The third model suggests that there are much more than a few hyperactive *Alu* elements (Fig. 2B) [4, 13]. Recently, Han *et al*. [5] introduced the forth model called stealth model. It follows the concept of the master gene model but explains aberrant amplification of *Alu* elements. Hyperactive *Alu* elements get mutated and lose its retrotranspositional capability relatively quickly by selection. In contrast, *Alu* elements with a low retrotransposition activity are able to retain its retrotransposition activity and produce the short-lived hyperactive *Alu* elements over an extended period of time. It was introduced to explain the recent remarkable expansion of old *Alu* elements, *Alu*Yb lineage, in the human genome (Fig. 2D) [5].

Alu Insertion

Alu element is a primate-specific retrotransposon and has played an important role in primate genomic diversity. During the past six million years, 5,530 *Alu* elements newly inserted into the human genome [14, 15]. Most of them were propagated through classical insertion, in that, *Alu* elements are inserted into the human genome using TPRT mechanism. The hallmark of the classical *Alu* insertions is TSDs flanking both ends of an *Alu* element. However, *Alu* elements integrated through non-classical insertions are deficient of TSDs but have instead 1 to 7 bp microhomologous sequences on their pre-insertion site. When chromosomal double strand break (DSB) happens, *Alu* element is able to integrate into the genomic region through the HR between the elements and the chromosomal break site [14, 16]. Through the *de novo* insertion event, *Alu* elements have modified the human genome in a species-specific manner. In addition, they could disrupt genes by inserting into their exonic regions (Fig. 3A). *Alu* elements locating in the intronic region could also regulate gene function by promoting alternative splicing of the genes; the elements have multiple splice donor/accept sites (Fig. 3D). The alternative splicing generates *Alu* exonization or/and intron retention in respective transcripts, which could disrupt or modify the function of the genes (Fig. 3C and 3E) [8, 17]. As mentioned above, *Alu* elements have a relatively high point mutation rate and can obtain splicing sites by the point mutations after the insertion. The *Alu* elements provide cryptic splicing sites and recognize these sites by splicing factors (Fig. 3C and 3D). Ornithine aminotransferase (OAT) gene encodes mitochondrial enzyme ornithine δ-aminotransferase, which converts ornithine to glutamate. The deficiency of this enzyme results in autosomal recessive eye disease. The third intron of OAT gene contains the right monomer of *Alu* element which is residues, 279 to 138, of antisense *Alu* element. The antisense *Alu* monomer provides cryptic splicing sites by the point mutation, cytosine to guanine. The cryptic splicing site causes an alternative splicing of the gene, disrupting the function of OAT gene. Consequently, patients suffer from gyrate atrophy of the choroid and retina by producing abnormal proteins [18]. *Alu* elements can influence gene function through RNA editing which is a post-transcriptional alteration. Adenosine deamination by an enzyme, adenosine deaminase acting on RNA (ADAR) results in inosine, which in turn interpreted as guanosine by translation or spliceosome machinery. Adenosine to inosine (A-to-I) is the most frequent RNA editing in humans. A-to-I RNA editing occurs within a long duplex of RNA sequence because ADAR works only on double strand RNA structures. Due to the dimeric structure, *Alu* element in RNA sequences forms the stem loop structure, leading to A-to-I RNA editing [19]. In addition, two *Alu* elements located in close to each other can make a stem loop structure, which result in A-to-I editing and the edited *Alu* element could subsequently bring out novel alternative splicing site [19-23]. On the other hand, the intronic *Alu* elements could lead to deletion of nascent exons, which is called exon skipping. It therefore disrupts open reading frames of the human genes (Fig. 3A). Ganguly *et al*. [24] have reported that intronic insertion of *Alu*Yb9 causes exon skipping and leads to hemophilia A disease. Hemophilia A is an X-linked disorder caused by exon skipping of exon 19 in Factor VIII gene. *Alu*Yb9 locates in the intron 18 of the gene and causes the skipping of the exon19 from the gene transcript.

Alu elements could regulate gene function by providing canonical polyadenylation signal, AATAAA (Fig. 3B) [25, 26]. *Alu* elements contain three potential polyadenylation sites and two of them are active in the human genome. One of the previous studies on *Alu* elements has reported that ~10,000 *Alu* elements are identified within the 3' untranslated region (UTR) of protein coding human genes. Among them, 107 *Alu* elements retain active polyadenylation site. Interestingly, 99% of polyadenylation-active *Alu* elements locate in the forward direction although the elements exist in the 3' UTR of human genes randomly, regarding the insertion direction. In addition, old *Alu* subfamily has more active polyadenylation sites than younger subfamilies [27, 28]. An example of *Alu* polyadenylation site affecting gene function is calcium-sensing receptor (CaSR) which is a member of G protein-coupled receptor. The gene involves in regulating

extracellular level of calcium ion. *Alu* element locates in the exon 7 of the gene and pre-terminates transcripts of CaSR by providing a stop codon signal. Patients having the missense mutations show symptoms of familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism [29-31].

Recombination between *Alu* Elements

A total of 515 *Alu*-mediated deletion events have been identified in the human genome. The deletion events occurred either through *Alu* insertion-mediated deletions (AIMD) or *Alu* recombination-mediated deletions (ARMD): 24 AIMDs and 492 ARMDs. AIMDs and ARMDs have deleted 11,206 and 396,420 base pairs of the human genome, respectively, after the divergence of the human and chimpanzee lineages (~6 mya) [14, 32-34]. *Alu* element has been frequently involved in the genomic recombination because of its two characteristics: a high level of sequence identity among them and its abundance in the human genome. Among the 492 ARMD events, 197 ARMD events were occurred by the recombination between *Alu* elements belonging to different subfamilies. Although *Alu*J subfamily is more abundant than *Alu*Y subfamily in the human genome, *Alu*Y subfamily is more associated with ARMD events because *Alu*Y subfamily retains a higher sequence identity among its members than *Alu*J subfamily does. *Alu* element accumulates random mutations over time so that older *Alu* elements have more mutations than younger elements [33].

DNA DSB is one of the most dangerous events of DNA damage. In the human genome, it could be repaired by retrotransposons using two different mechanisms: nonhomologous end-joining mediated deletion (NHEJ) and HR [34]. NHEJ does not need a homologous template for repair of DSBs while HR requires sequence homologies on either side of the break for the repair (Fig. 4A). DSB repair by NHEJ mechanism is initiated by an enzyme complex including Ku70/80 heterodimer. After the enzyme complex binds to either side of DSB, it functions as a docking site for other NHEJ enzymes such as DNA ligase [35, 36]. Nonallelic homologous recombination (NAHR) is one of HRs. It occurs between two DNA sequences which are not alleles but share a high sequence similarity from one another. Major NAHR hotspots for several human diseases locate at repeat elements including *Alu* element. During meiosis, *Alu* elements can misalign and the subsequent crossover could lead to genetic rearrangements of duplication, deletion, and translocation [37, 38]. NAHR is proceeded either by interchromosomal recombination, in which occurs between different chromosomes or intrachromosomal recombination, in which recombination occurs via crossing over within the same chromosome. Interchromosomal recombination results in a deletion or duplication depending the orientation of the DNA sequences. Intrachromosomal recombination results in a deletion or inversion (Fig. 4B).

Genetic Disorder Caused by *Alu* Elements

During the past 6 million years, *Alu* elements have modified the human genome in a species-specific manner and also caused human disease through *de novo* insertion or the recombination between them. Genomic rearrangements induced by *Alu* insertion account for approximately 0.1% of

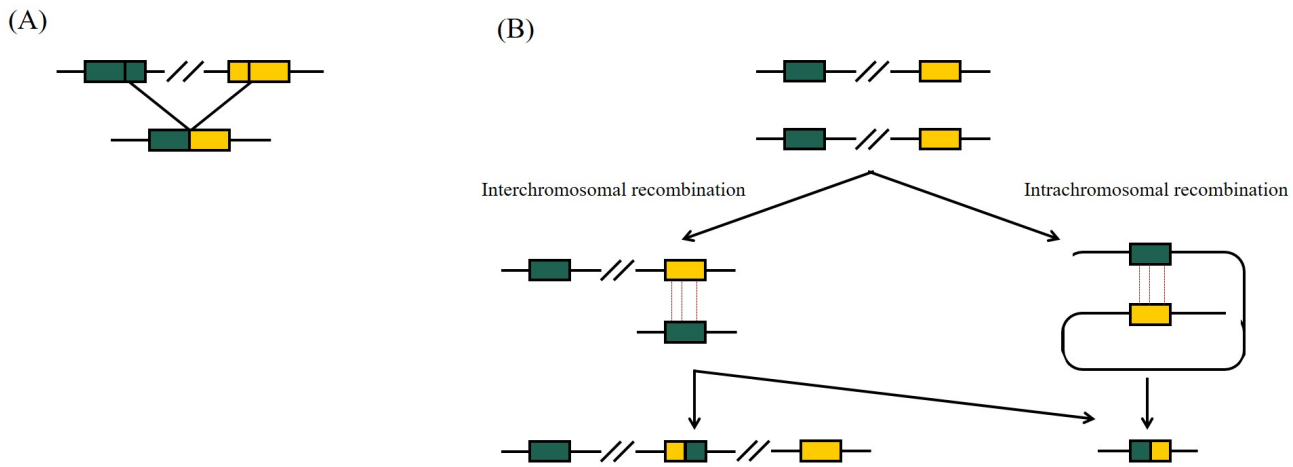

Fig. 4. Mechanisms of *Alu* recombination-mediated deletions. (A) Nonhomologous end-joining mediated deletion mechanism. After DNA double strand breaks, non-homologous templates are ligated by *Alu* element. (B) Nonallelic homologous recombination mechanism, interchromosomal recombination occurs between two different *Alu* elements which locate on different chromosomes and mediates genomic duplication or deletion. Intrachromosomal recombination occurs between two different *Alu* elements which locate on the same chromosome and mediates genomic deletion. Green and yellow boxes represent *Alu* elements. The red dot line indicates homologous sequences.

Table 1. A list of *Alu*-mediated genetic disorder in recent studies

Gene	Position	Subfamily	Mechanism	Disease	Reference
ACE	Chr 17	*Alu*Ya5	Insertion	Alzheimer's disease	[39]
ALMS1	Chr 2	*Alu*Ya5	Insertion	Alström syndrome	[40]
BMPR2	Chr 2	*Alu*Y *Alu*S	ARMD_NAHR NHEJ	Pulmonary arterial hypertension	[41]
CDSN	Chr 6	*Alu*S	ARMD_NHEJ	Peeling skin disease	[42]
COL4A5	Chr X	*Alu*Y	Insertion	Alport syndrome	[43]
FA	Chr X	*Alu*Y	ARMD_NAHR	Fanconi anemia	[44]
GBA1	Chr 1	*Alu*Sx	ARMD_NAHR	Gaucher disease	[45]
GGA	Chr 17	*Alu*S	ARMD_NAHR	Pomp disease	[46]
GLA	Chr X	*Alu*	Insertion mediated deletion	Fabry disease	[47]
MUTYH	Chr 1	*Alu*Yb8	Insertion	Breast cancer/gastric cancer	[48]
PMP22	Chr 17	*Alu*Y/*Alu*Sc	ARMD_NAHR	Charcot-Marie-Tooth disease	[49]
SOX10	Chr 22	*Alu*S	FoSTes/MMBIR	Waardenburg syndrome type 4	[50]
SPAST	Chr 2 Chr 2	*Alu*Y/*Alu*S *Alu*Y	FoSTes/MMBIR	Hereditary spastic paraplegia	[51]
SPG11	Chr 15 Chr 15	*Alu*Y/*Alu*S *Alu*S	ARMD_NAHR	Spastic paraplegias	[52]
STK11	Chr 19	*Alu*Y	ARMD_NAHR	Peutz-Jeghers syndrome	[53]

ARMD, *Alu* recombination-mediated deletions; NAHR, nonallelic homologous recombination; NHEJ, nonhomologous end-joining mediated deletion; FoSTeS/MMBIR, fork stalling and template switching/microhomology-mediated break-induced replication.

human diseases and genomic deletions by ARMD are responsible for approximately 0.3% of human genetic disorders [10, 32, 33]. There are many *Alu* elements which are closely related to human diseases (Table 1) [39-53]. Alström syndrome is a rare genetic disease which is caused by *Alu* insertion in *ALMS1* gene. *ALMS1* is composed of 23 exons and encodes centrosome and basal body-associated protein which plays an important role in microtubule organization, especially form and maintain cilia. The protein is associated with insulin resistance, hypogonadism, and heart disease. Therefore, Alström syndrome has several symptoms such as blindness and obesity. Most mutations causing Alström syndrome occur in exons 8, 10, and 16 of *ALMS1* [54]. The mutation of *ALMS1* caused by *Alu* element was first discovered in 2013. *Alu*Ya5 element exists in the exon 16 of *ALMS1*, which disrupts open reading frame, leading to frameshift mutation [40]. Pulmonary arterial hypertension (PAH) is also caused by *Alu* element, which locates in *BMPR2* gene. *BMPR2* locates on chromosome 2 and has 13 exons. Exons 1–3 encoding an extracellular domain were deleted by the recombination between two different *Alu*Y elements in PAH patients. In another PAH patient, the exon 10 of the gene was deleted by *Alu*Sx involved-nonhomologous recombination. The recombination took place between the *Alu* element in the intron 9 and a unique sequence in the intron 10 of the gene [41]. Fork stalling and template switching/microhomology-mediated break-induced replication model (FoSTeS/MMBIR) has been often proposed in cases where the complexity of the genomic rearrangement is not able to be explained by using classic recombination mechanisms. Recently, *Alu* element was reported to be associated with a human genomic deletion responsible for Waardenburg syndrome type 4 (WS4). WS4 is a rare neural crest disorder. Three *Alu* elements are involved in the large deletion within SOX10 regulatory sequences in patients with WS4. The deletion could be explained by a two-step FoSTeS/MMBIR mechanism mediated *via* the 4-bp and 13-bp microhomology found at the *Alu*1/*Alu*3 and *Alu*3/*Alu*2 breakpoints, respectively. The deletion of *SOX10* regulatory sequences was also identified in patients with Hirschsprung disease and peripheral demyelinating neuropathy–central dysmeylinating leukodystrophy [50].

Conclusion

In this review, we discuss the amplification of *Alu* elements and its impact on human genomic rearrangements and human disease. Four different models: master gene, intermediate, transposon, and stealth models have been suggested to explain the *Alu* amplification during primate evolution. Among them, master gene model is generally accepted to explain the diversity of *Alu* subfamilies. Since the divergence of human and chimpanzee, *Alu* elements have caused various genetic/genomic rearrangements in the human genome through human-specific insertion, deletion, and recombination. *Alu* elements could repair DSBs in the

human genome using microhomology between the element and the break point, leading to *de novo Alu* insertion. In spite of the positive effect, *Alu* element is considered to be one of major factors to cause human genomic instability because many *Alu* elements are associated with various human diseases. The recombination between *Alu* elements has deleted human genic regions and subsequently disrupted gene function, leading to human diseases. The abundance of *Alu* elements in the human genome and a high level of sequence identity among them predispose them to be a tremendous and unpredictable factor to cause genomic instability and the related human diseases. Characterization of *Alu* amplification in the human genome and elucidating the mechanisms which *Alu* elements could utilize to cause human diseases may help us understand *Alu*-associated pathogenesis and predict *Alu*-associated human diseases.

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