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Origin and distribution of different retrotransposons in different taxa

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Abstract

Novel genome analysis technologies enable genomic studies of transposable elements (TEs) in different organisms. Population studies of human genome show thousands of individual TE insertions. These insertions are important source of natural human genetic variation. Researchers are beginning to develop population genomic data sets for evaluating the phenotypic impact of human TE polymorphisms. Because of the evidences of horizontal transfer of retrotransposons between different species genome, in this study we aimed to detect barley retrotransposons (*Nikita* and *BAGY2*) in the human genome. Inter retrotransposon amplified polymorphism polymerase chain reaction (IRAP PCR) were used to measure the distribution of *Nikita* and *BAGY2* retroelements in the human genome. Analyses reveals that *Nikita* and *BAGY2* are present in the human genome and show different distribution in the genome. The polymorphism ratios of retroelements suggest that *Nikita* and *BAGY2* have been active retrotransposons in the human genome.

Keywords

Transposons, Nikita, BAGY2,polymorphism, human genome

Introduction

Transposable elements (TEs) are genomic sequences that are able to move within the genome and are found in almost all organisms (Mita & Boeke, 2016). TEs are widespread components of genomes. They constitute DNA fractions that can move through the genome using a DNA intermediate or an RNA intermediate (Gonzalez et al., 2017). Initially thought to constitute only parasitic or junk DNA, TEs have now shown to grant advantages (Bennetzen & Wang, 2014). TEs are recognized as evolutionary power that shape genome structure via recombination, chromatin modifications with epigenetic mechanisms, gene capture, genomic rearrangements and exon shuffling by movements within the genome (Sharma et al., 2013; Vitte et al., 2014; Gonzalez et al., 2017). Insertion within or close to a gene could directly disrupt gene function

or generate new functions with alternative splicing, alternative promoter control and gene silencing (Xiao et al., 2008; Gonzalez et al., 2017). Recent research demonstrated that retrotransposons are found in virtually all genomes and even that the same retrotransposons could be found in different species (Cakmak et al., 2015; Elkina et al., 2015; Igiebor et al., 2016; Cakmak et al., 2017). Additionally, several retrotransposon studies showed that the same retrotransposon may exhibit polymorphism in different organs within the same organism, especially in plants (Yilmaz et al., 2014). Studies on transposons in human genome have also demonstrated that retrotransposons are altered in a variety of diseases, suggesting that misregulation of transposable elements can be detrimental (Rajan & Ramasamy, 2014). All these studies showed that retrotransposons are significant in genomes and genome evolutions.

In this study, we investigated insertion and movement of barley retrotransposons (*BAGY2* and *Nikita*) in the human genome with IRAP PCR method. This method has been used for investigation of specific retrotransposons in target genomes (Guliyev et al., 2013; Kartal et al., 2014; Yuzbasioglu et al., 2016).

Materials and methods

Genomic DNA of 24 individuals (12 females and 12 males, not related to each other) within the age range of 10–79 years were used in this study (Table 1). Genomic DNAs of samples were extracted from venous blood samples using DNA extraction kit (Roche Diagnostics GmbH, Mannheim, Germany). Primer sequences used in the IRAP PCR reactions were (5'- CGCATTTGTTCAAGCCTAAACC-3') for *Nikita* and (Forward: 5'-TCGAAAGGTCTATGATTGATCCC-3'; Reverse: 5'-CATGAAAGCATGATGCAAAATGG-3') for *BAGY2*. Primer sequences information were obtained from Rodriguez et al. (2006) and Vicient et al. (2001). PCR components and conditions were the same for *Nikita* and *BAGY2* retrotransposons. PCR was performed by using a thermal cycler (T100 TM Thermal Cycler; Bio-Rad Laboratories, Inc., Hercules, CA, USA) in a total volume of 20 µL. PCR reactions were performed with SapphireAmp® Fast PCR Master Mix (Takara, RR350A) (Table 2). PCR conditions were as follows: initial denaturation at 94°C (3 min) followed by 30 cycles of denaturation at 94°C (30 s), annealing at 51°C (30 s) and extension at 72°C (3 min). The reaction was completed by additional extension at 72°C for 10 min. IRAP PCR products were resolved by agarose gel electrophoresis for visualization and determination of polymorphism. 20 µL PCR products were mixed with 4 µL 6X loading buffer (10 mmol/LTris-HCl, 60 mmol/L EDTA, pH 8.0, 0.3% bromophenol blue, 60% glycerol). Agarose gel was prepared as 2% with the 1X TBE buffer. Samples were run at 150 V for 120 min in 1X TBE buffer after agarose gel was ready. A molecular weight marker (GeneRulerTM 100 bp plus, SM0321, Fermentas) was also loaded to determine the sizes of the amplicons. After running, the gels were photographed on a UV transilluminator.

Table 1. Subjects analyzed in this study

No	Average range (years)	No	Average range (years)
1	10-19	13	40-49
2	10-19	14	40-49
3	10-19	15	40-49
4	10-19	16	40-49
5	10-19	17	60-69
6	20-29	18	60-69
7	20-29	19	60-69
8	20-29	20	60-69
9	20-29	21	70-79
10	20-29	22	70-79
11	20-29	23	70-79
12	20-29	24	70-79

Table 2. PCR components

Component	Quantity (µl)
2X SapphireAmp®	10
IRAP Primer (10 µM)	2
Template DNA (20 ng)	4
dH_2O (PCR-grade)	4
Total	20

Analysis of retrotransposons polymorphism was performed based on Jaccard similarity coefficient (Jaccard, 1908). The Jaccard's similarity index was calculated using the formula: NAB/(NAB + NB+ NA), where NAB is the number of bands shared by 2 samples, NA indicates amplified fragments in sample A, and NB represents amplified fragments in sample B. In addition, the gel image was evaluated

by using GelJ v.2.0 to construct the phylogenetic tree (Heras et al., 2015). UPGMA (unweighted pair-group method with arithmetic mean) clustering method with Jaccard's coefficient was used to cluster the subjects based on band distances on gel images.

Results and Discussion

Nikita and *BAGY2* retrotransposons were identified in the human genome for the first time by IRAP analysis. Photographed PCR products are as in the Figure 1 and Figure 2. Percentages of polymorphism among samples were calculated according to the Jaccard coefficient. *Nikita* retrotransposon polymorphism ratios ranged from 0% to 36% (Table 3).

Moreover, the polymorphism rates were 0% - 36% among females (12 females) and among males (12 males). Similarly, *BAGY2* retrotransposon polymorphism ratios ranged from 0% to 27% (Table 4). When males and females were compared; polymorphism percentages were 0%-27% among females and 0%-21% among males.

The study group consisted of samples of different age groups. When the groups were compared according to age, the age-associated polymorphism for *Nikita* and *BAGY2* retrotransposons was not observed. Clustering analysis was performed with UPGMA clustering method for *Nikita* and *BAGY2* profiles in the samples. According to the band profiles of *Nikita*, the 24 analyzed samples were

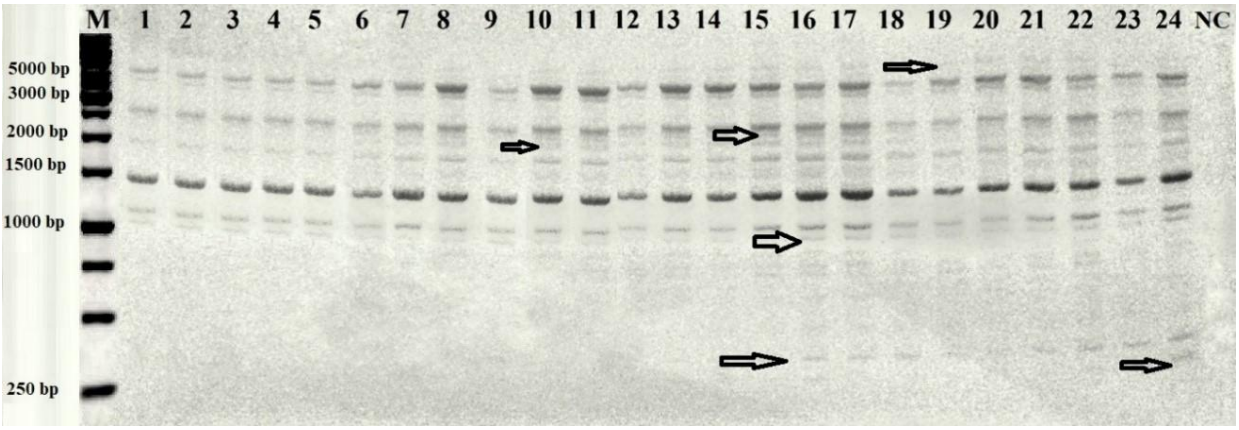

Figure 1. *Nikita* IRAP PCR results. Lane numbers correspond to the subjects listed in Table 1. M, marker (GeneRuler™ 1 kb Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA); NC, negative control (no template DNA). Arrows indicate polymorphic bands.

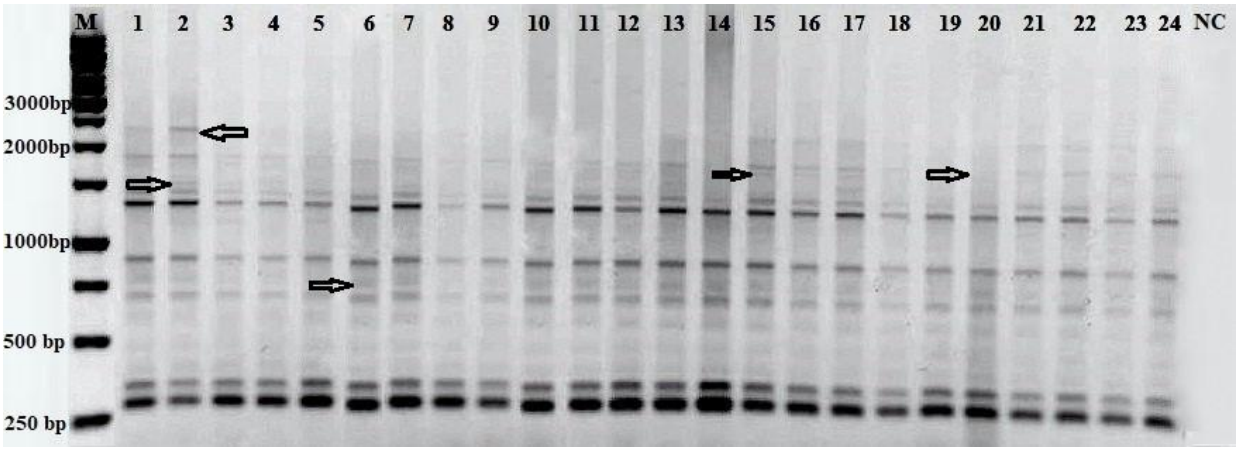

Figure 2. *BAGY2* IRAP PCR results. Lane numbers correspond to the subjects listed in Table 1. M, marker (GeneRuler™ 100 bp plus Fermentas; Thermo Fisher Scientific, Inc., Waltham, MA, USA); NC, negative control (no template DNA). Arrows indicate polymorphic bands.

Table 3. Polymorphism rates (%) of *Nikita* determined by Jaccard coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	-																							
2	0	-																						
3	0	0	-																					
4	0	0	0	-																				
5	0	0	0	0	-																			
6	**22**	**22**	**22**	**22**	**22**	-																		
7	**22**	**22**	**22**	**22**	**22**	**0**	-																	
8	**30**	**30**	**30**	**30**	**30**	**10**	**10**	-																
9	**13**	**13**	**13**	**13**	**13**	**30**	**30**	**20**	-															
10	**30**	**30**	**30**	**30**	**30**	**10**	**10**	**0**	**20**	-														
11	**30**	**30**	**30**	**30**	**30**	**10**	**10**	**0**	**20**	**0**	-													
12	**30**	**30**	**30**	**30**	**30**	**10**	**10**	**0**	**20**	**0**	0	-												
13	30	30	30	30	30	10	10	0	20	0	0	0	-											
14	30	30	30	30	30	10	10	0	20	0	0	0	0	-										
15	36	36	36	36	36	18	18	9	27	9	9	9	9	9	-									
16	36	36	36	36	36	18	18	9	27	9	9	9	9	9	0	-								
17	**36**	**36**	**36**	**36**	**36**	**18**	**18**	**9**	**27**	**9**	**9**	**9**	**9**	**9**	**0**	**0**	-							
18	**36**	**36**	**36**	**36**	**36**	**18**	**18**	**9**	**27**	**9**	**9**	**9**	**9**	**9**	**0**	**0**	**0**	-						
19	**36**	**36**	**36**	**36**	**36**	**18**	**18**	**9**	**27**	**9**	**9**	**9**	**9**	**9**	**0**	**0**	**0**	**0**	-					
20	**30**	**30**	**30**	**30**	**30**	**27**	**27**	**18**	**20**	**18**	**18**	**18**	**18**	**18**	**9**	**9**	**9**	**9**	**9**	-				
21	30	30	30	30	30	27	27	18	20	18	18	18	18	18	9	9	9	9	9	0	-			
22	30	30	30	30	30	27	27	18	20	18	18	18	18	18	9	9	9	9	9	0	0	-		
23	13	13	13	13	13	30	30	36	22	36	36	36	36	36	27	27	27	27	27	20	20	20	-	
24	30	30	30	30	30	27	27	18	20	18	18	18	18	18	9	9	9	9	9	0	0	0	20	-

Table 4. Polymorphism rates (%) of *BAGY2* determined by Jaccard coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	-																							
2	8	-																						
3	21	14	-																					
4	15	8	8	-																				
5	8	15	15	8	-																			
6	**8**	**15**	**15**	**8**	**0**	-																		
7	**21**	**14**	**0**	**8**	**15**	**15**	-																	
8	**8**	**15**	**15**	**8**	**0**	**0**	**15**	-																
9	**15**	**8**	**8**	**0**	**8**	**8**	**8**	**8**	-															
10	**15**	**8**	**8**	**0**	**8**	**8**	**8**	**8**	**0**	-														
11	**15**	**8**	**8**	**0**	**8**	**8**	**8**	**8**	**0**	**0**	-													
12	**15**	**8**	**8**	**0**	**8**	**8**	**8**	**8**	**0**	**0**	**0**	-												
13	8	15	15	8	0	0	15	0	8	8	8	8	-											
14	8	15	15	8	0	0	15	0	8	8	8	8	0	-										
15	21	**27**	14	21	15	15	14	15	21	21	21	21	15	15	-									
16	21	**27**	14	21	15	15	14	15	21	21	21	21	15	15	0	-								
17	**27**	**20**	**7**	**14**	**21**	**21**	**7**	**21**	**14**	**14**	**14**	**14**	**21**	**21**	**7**	**7**	-							
18	**8**	**15**	**15**	**8**	**0**	**0**	**15**	**0**	**8**	**8**	**8**	**8**	**0**	**0**	**15**	**15**	**21**	-						
19	**8**	**15**	**15**	**8**	**0**	**0**	**15**	**0**	**8**	**8**	**8**	**8**	**0**	**0**	**15**	**15**	**21**	**0**	-					
20	**8**	**15**	**15**	**8**	**0**	**0**	**15**	**0**	**8**	**8**	**8**	**8**	**0**	**0**	**15**	**15**	**21**	**0**	**0**	-				
21	21	**27**	14	21	15	15	14	15	21	21	21	21	15	15	0	0	7	15	15	15	-			
22	15	21	21	15	8	8	21	8	15	15	15	15	8	8	8	8	14	8	8	8	8	-		
23	21	**27**	14	21	15	15	14	15	21	21	21	21	15	15	0	0	7	15	15	15	0	8	-	
24	21	14	14	8	15	15	14	15	8	8	8	8	15	15	14	14	7	15	15	15	14	8	14	-

grouped into two clusters. The first group consists of 1, 23 and 24, while the second group includes other samples (Figure 3). According to the band profiles of *BAGY2* the 24 analyzed samples were grouped into two clusters. The first group consists of 1-5, 14-17 and 19 while the second group consists of other samples (Figure 4).

TEs are amongst the most variable fractions of genomes and they could change overall architecture of functional genes (Fedoroff & Bennetzen, 2013). TEs are abundant in almost all living organisms and could have significant contribution to genome evolution (Schaack et al., 2010). Closely related species have similar TE content. As an example,

barley retrotransposons such as *BARE-1*, *Sukkula* and *SIRE1* have been shown to have active homologues in genomes of other cereals (Bonchev & Parisod, 2013; Cakmak et al., 2015; Cakmak et al., 2017). Recent studies have shown existence of plant retrotransposons in animals (Elkina et al., 2015; Cakmak et al., 2017). The study conducted by Elkina and colleagues have shown that *SIRE1* and *BARE-1* are present in the genome of farm animals and have different distribution within genomes. In the previous study of our research group, we determined *SIRE1* retrotransposon in the human genome (Cakmak et al., 2017).

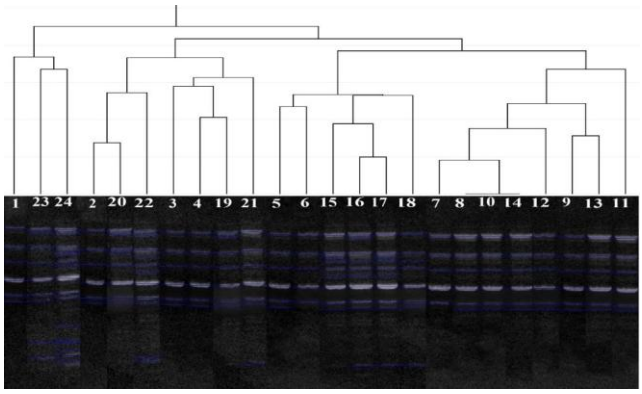

Figure 3. Clustering of subjects based on IRAP PCR amplification using Nikita primers (UPGMA analysis). Lane numbers correspond to the subjects listed in Table 1

Another study conducted by Metzger and colleagues stated that a selfish endogenous retroelement "Steamer" belonged to soft-shell clam family, also founded within the genome of 19 different bivalve species. Researchers also compared sequences with the National Center for Biotechnology Information sequence database, and determined that Steamer-like elements are present in the genomes of completely unrelated organisms, including zebrafish, sea urchin, acorn worms, and coral (Metzger et al., 2018). Hou and colleagues (2018) also investigated horizontal transposable element transfers (HTTs) in sequenced genomes of seven species of Rosales. They analyzed phylogenetic relationships of RT sequences and LTR sequences. All these results demonstrated that LTR retrotransposons still have potential transposition activity in host genomes. According to these findings, we investigated *Nikita* and *BAGY2* barley retrotransposons in the human genome in this study. Previous studies stated that *Nikita* and *BAGY2* are active retrotransposons in barley genome (Bayram et al., 2012; Kartal et al., 2014; Gozukirmizi et al., 2016). Thus, we expected it to be active in the human genome as well. Our results show that *Nikita* and *BAGY2* are active elements, and have different insertion pattern in the human genome as expected. We also investigated whether the detected polymorphism is related to age and gender. Our study group included subjects of different age range and both sexes (10-79 age, 12 females and 12 males, shown in the Table 1). When band profiles of *Nikita* and *BAGY2* were compared to each other, we did not find any age and gender related polymorphism. This finding corresponds to our previous work, determination of *Sukkula* retrotransposon in the human genome (Çakmak et al., 2017).

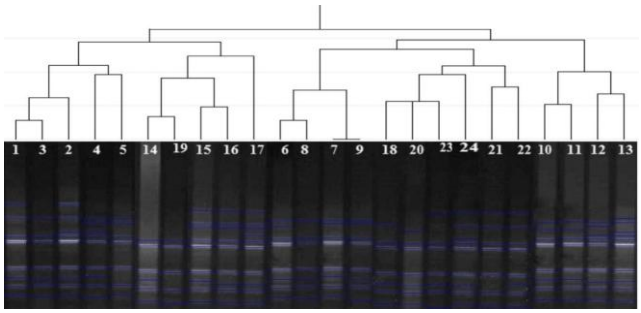

Figure 4. Clustering of subjects based on IRAP PCR amplification using *BAGY2* primers (UPGMA analysis).Lane numbers correspond to the subjects listed in Table 1

Additionally, we analyzed the results using UPGMA method. According to the UPGMA results, the samples are re-organized based on band pattern similarity. When investigating dendograms of *Nikita* and *BAGY2*, re-organized samples were aligned randomly. In this study, we aimed to investigate whether retroelement polymorphisms could occur based on different age groups and different gender or not. According to the UPGMA results, we could see that polymorphism of *Nikita* and *BAGY2* retroelements were not associate with the age or gender. When Table 3 and Table 4 were examined, it could be seen that polymorphism percentages were not increase or decrease in proportion with age groups and gender. Polymorphisms occurred individual specific. UPGMA results support the IRAP PCR analysis results. To sum up, *Nikita* and *BAGY2* are active retroelements in the human

genome and movements of these retrotransposons are individual specific. Polymorphism of *Nikita* and *BAGY2* are not associate age or gender. Recently, a few cases of plant retrotransposon insertion polymorphism have been studied in the animal genomes (Elkina et al., 2015; Çakmak et al., 2017). Insertion polymorphism of plant retrotransposons seems to be a very rare event. Our results are expected to contribute the knowledge about plant retrotransposon polymorphisms in different species.

Conclusions

This study and previous studies demonstrated that plant specific transposons may be integrated into animal and human genomes by horizontal transfer. Further, detailed studies are needed to gain better understanding of the mechanism and role of transposition of plant-specific retrotransposons in animals and humans.

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