Sukkula retrotransposon movements in the human genome

Buket Cakmak, Sevgi Marakli and Nermin Gozukirmizi

Department of Molecular Biology and Genetics, Faculty of Science, Istanbul University, Istanbul, Turkey

ABSTRACT
Retrotransposons, a subclass of mobile genetic elements, have been discovered in many organisms. In this study, we identified a plant-specific retrotransposon (Sukkula) in the human genome (24 different DNA samples) by using retrotransposon-based molecular marker technique: inter-retrotransposon amplified polymorphism. There were five different groups related to the ages of the subjects. The polymorphism ratios were 8%–100% among all samples, 10%–91% among females (12 subjects) and 13%–100% among males (12 subjects). Moreover, we also observed 8%–91% polymorphism ratios when comparing males to females. To the best of our knowledge, this is one of the first reports on plant-specific retrotransposons in the human genome. The obtained findings are expected to contribute to broadening the knowledge about plant retrotransposons in the human genome and their role in human genome evolution.

Introduction
Transposable elements (TEs) are DNA sequences that can move within or, occasionally, between genomes. Retrotransposons are a subclass of TEs [1]. They are dynamic elements in genomes and even cause genetic instability; they are classified as autonomous or non-autonomous based upon whether they encode proteins required for their retrotransposition or not [2–4].

Retrotransposons constitute approximately one-third of the human genome [5]. The most abundant retrotransposons are the LINE-1 (L1) element, with 0.5 million copies, and the Alu element, with over 1.1 million copies. While LINE-1 elements are autonomous, Alu elements are non-autonomous [4]. In addition to the human genome, retrotransposons have been identified in many organisms, especially in the plant kingdom, particularly in cereal genomes such as barley, maize, wheat and rice. In these cereals, the retrotransposon ratio is higher than in other plants [1,6].

Retrotransposons are found in almost every genome, and even the same retrotransposons could be found in different species [7,8]. This probably occurs as a result of horizontal transfer of genetic material. Although the transfer of genes is substantial in prokaryotic evolution, few instances of horizontal gene transfer have been reported to play an important role in eukaryotic evolution, most cases involving TEs instead [9]. Thus, there are a lot of retrotransposon studies devoted to species identification, polymorphisms among populations and even different organs within the same organism, especially in plants. In these investigations, many molecular marker techniques based on retrotransposon movements have been used to detect mobility in genomes [10]. One such marker is IRAP (inter-retrotransposon amplified polymorphism), which is based on polymerase chain reaction (PCR) amplification using primers designed to amplify in an outward direction from the conserved LTR (long terminal repeat) sequences. This way, the genomic distance between two LTRs is amplified [11]. This method has been used for investigation of specific retrotransposons in target genomes [6,7,12]. Moreover, some plant-specific retrotransposons have been found in other plants and animals [8,13].

In this study, we investigated the presence and movement of the plant-specific retrotransposon Sukkula in the human genome. Sukkula elements are non-autonomous retroelements, also named large retrotransposon derivative (LARD) elements [14].

Subjects and methods

Subjects
Sukkula retrotransposon movements were investigated in 24 individuals (12 females and 12 males, non-related to each other) within the age range of 10–79 years (Table 1). Genomic DNA was extracted from venous blood samples using a High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany).
Table 1. Demographics of the subjects enrolled in this study.

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

Bold values indicates the number of subjects only.

The procedures followed were in accordance with the current ethical standards. Spectrophotometric and electrophoretic analyses were performed to determine the quantity and quality of the isolated DNA samples.

**IRAP analysis**

IRAP-PCR was performed with the Sukkula-specific primer (3’GGAACGTGGCATCGGGCGT5’) [15]. PCR was carried out in 20-μL reaction mixtures containing 4 μL of nuclease-free dH₂O, 10 μL of 2X SapphireAmp Fast PCR Master Mix (Takara, RR350A), 2 μL of primer (10 μmol/L) and 4 μL of 20 ng/μL template genomic DNA. The amplification conditions were as follows: an initial denaturation step at 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 3 min. The reaction was completed by a final extension step at 72 °C for 10 min (T100™ Thermal Cycler, BioRad).

IRAP-PCR products were mixed with 6X loading buffer (10 mmol/L Tris-HCl, 60 mmol/L ethylenediaminetetraacetic acid (EDTA), pH 8.0, 0.3% bromophenol blue and 60% glycerol) and resolved by 2% agarose gel electrophoresis at 150 V for 2 hours in 1X Tris-Borate-EDTA buffer. Molecular weight marker (GeneRuler™ 100 bp plus, SM0321, Fermentas) was also used to determine the size of amplicons. After agarose gel electrophoresis, the gel was scanned and photographed on an ultraviolet (UV) transilluminator.

The polymorphism rates were calculated using Jaccard similarity coefficient. Well-resolved bands were scored as a binary value: ‘1’ for presence and ‘0’ for absence. The binary matrix (1/0) was used to calculate the similarity among different individuals by Jaccard’s coefficient [16]. Jaccard’s similarity index was calculated using the formula: N_AB/(N_AB + N_B + N_A), where N_AB is the number of bands shared by two samples, N_A indicates the amplified fragments in sample A and N_B represents the amplified fragments in sample B. In addition, the gel image was evaluated by using GeJ v.2.0 to construct the phylogenetic tree [17]. 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 [17].

**Results and discussion**

To determine the presence and movements of the Sukkula retrotransposon in the human genome, genomic DNA was isolated from 24 individuals from different age groups. The resulting IRAP-PCR amplification products were electrophoretically separated in a 2% agarose gel (Figure 1).

According to the band profiles, the 24 analysed samples were grouped into two clusters. The first group consisted of only one subject (No. 3). The others were clustered in the second group (Figure 2).

Polymorphism percentages among samples were calculated by comparing each sample with the other samples. Monomorphic and polymorphic bands were scored visually, and polymorphism rates were calculated among samples. The Sukkula IRAP analysis yielded 312 scorable bands in the samples: 172 monomorphic bands and 140 polymorphic ones, ranging from 350 to 1500 bp. The numbers of present (+) or absent (—) bands in the studied subjects are indicated in Table 2.

![Figure 1. IRAP-PCR results of amplification using Sukkula-specific primers. Note: M, marker (GeneRuler™ 100 bp plus, SM0321, Fermentas); NC, negative control (no template DNA). Lane numbers correspond to the subjects listed in Table 1.](image-url)
The polymorphism ratios among all samples ranged from 8% to 100%. Moreover, the polymorphism rates were 10%–91% among females (12 females) and 13%–100% among males (12 males). When males were compared to females, 8%–91% polymorphism was observed (Table 3).

Sukkula is reportedly the second most active retrotransposon in the barley genome [15]. Different studies related to Sukkula have been performed for genetic diversity analysis [18,19]. For example, Kartal-Álacam et al. [20] studied the Sukkula retrotransposon movements in 40- and 80-day-old barley callus cultures with Figure 2.

Table 2. Number of monomorphic and polymorphic bands in all subjects.

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

Table 3. Polymorphism percentages (%) of Sukkula determined by IRAP-PCR analysis.

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

Bold values indicates the number of subjects only.
two retrotransposon markers: IRAP and iPBS (inter-primer binding site). They found up to 61% and 70% polymorphism rates using IRAP and iPBS, respectively. In another study, Yigider et al. [21] also revealed Sukkula polymorphism under manganese treatment in Zea mays.

In addition to the plant genome, Alu, SVA and HerV elements have been reported as active in the human genome, although active elements are limited [22,23]. There are also some reports on the existence of plant retrotransposons in animals. Elkina et al. [13] investigated the gene pools of farm animals (horses, sheep) by using plant-specific retrotransposon markers (BARE-1 and SIRE-1). In addition, 200–500 sites of sequences homologous to plant mobile elements were found in Ovis aries and 150 sites of sequences were identified in Equus feras. The authors also reported that the fragments of mobile elements in mammals are also found in the genomes of crop plants. Our results support this investigation, demonstrating the existence and movements of non-autonomous Sukkula retrotransposons in the human genome for the first time. Although only a small number of subjects were tested in this study, the obtained results lay the ground for future larger scale analyses. It is known that retrotransposons move in living organisms in the course of evolution [24]. However, the exact nature and role of these elements in human evolution are not clear. Our results indicate the existence of plant-specific retrotransposons in different systematic groups, particularly in humans, suggesting that such TEs could have a more significant contribution to genome evolution than initially expected [9].

Conclusions

To the best of our knowledge, this study demonstrated the existence of plant-specific Sukkula retrotransposons in the human genome for the first time. Our results are expected to contribute to the knowledge about the evolutionary relationships of the retrotransposons between plants and humans. Further, larger studies are needed to gain better understanding of the role and mechanism of transposition of plant-specific retrotransposons in animals and humans.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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