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Novel brain-expressed noncoding RNA, *HSTR1*, identified at a human-specific variable number tandem repeat locus with a human accelerated region

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ABSTRACT

The evolutionary conserved genomic sequences that have acquired significantly increased number of nucleotide substitutions specifically in the human lineage, called human accelerated regions (HARs), have been identified as candidate genomic regions that have contributed to the evolution of human-specific traits. A number of HARs were indeed shown to have novel enhancer activity and be associated with human-specific brain development and with cognition and social behavior. It is therefore of great importance to investigate the details of genomic function of each HAR to understand the roles of HARs as critical contributors to the genetic basis of human evolution. In this study, we identified a previously unannotated brain-expressed noncoding RNA gene, *HSTR1*, at a human-specific tandem repeat locus. Notably, the 5′ flanking sequence of *HSTR1* showed the signature of HARs and the dramatic human-specific enhancement of promoter activity, providing the evidence of positive selection to increase the expression level of *HSTR1* during human evolution. We also revealed that the tandem repeat number in *HSTR1* was highly variable among individual alleles and affected the stability of *HSTR1* RNA, suggesting variation in the activity of *HSTR1* between human individuals. Our work thus provides a novel candidate gene that potentially contributed to the evolution of the human brain. It may also underpin some of the variation between human brains.

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1. Introduction

The highly sophisticated human brain evolved ~8 million years after divergence of humans from their closest non-human primate relatives, the chimpanzee and bonobo [1]. Considering that protein-coding genes are highly conserved between human and chimpanzee, changes in noncoding genomic regions are thought to be major contributors to the genetic basis of human evolution [2,3]. As candidate genomic regions reflecting potential roles in the evolution of human-specific traits, DNA sequences with dramatically increased nucleotide substitution rates in the human lineage, called human accelerated regions (HARs) were first identified in 2006, followed by several similar studies [4–9]. Most HARs are located in noncoding regions and some HARs associated with noncoding RNA genes have the ability to regulate gene expression [10]. Interestingly, a number of studies reported that noncoding, but not coding, sequences in human genes regulating neurodevelopment showed positive selection [11–14]. Indeed, there is evidence that some noncoding HARs function as developmental enhancers and biallelic point mutations in specific HARs disrupt cognition and social behavior [15,16]. It is therefore of great importance to investigate the details of genomic function of each HAR to understand the roles of HARs as the genetic basis of the evolution of the human brain [17].

We previously identified newly emergent human-specific CpG islands that did not share sequence similarity within any orthologous chromosomes with the chimpanzee, gorilla and orangutan [18]. We found an interesting locus in which a CpG island emerged by human-specific creation of tandem repeats that contained CpG sites in the repeat unit but also by a number of human-specific nucleotide substitutions to create CpG sites in the flanking region

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of the tandem repeat. This finding thus prompted us to further investigate the genomic function hidden in this locus and its potential contribution to evolution of the human brain. In this study, we identified a novel brain-expressed noncoding RNA gene, *HSTR1* (Human-Specific Tandem Repeat 1), and characterized the functions of the tandem repeat and the evolutionary-accelerated region that is associated with regulation of *HSTR1*.

2. Materials and methods

2.1. Quantitative reverse transcription PCR (RT-qPCR)

Total RNA was extracted from human cell lines using Trizol (Thermo Fisher Scientific) as instructed by the manufacturer. Normal human tissue total RNA was obtained from Clontech (636643, 636527, 636584) and BioChain (R1234142-50, R1234062-50, R1234051-50, R1234149-50, R1234078-50, R1234066-50, R1244039-50, R1244078-50, R1244051-50, R1244062-50, R1244066-50, R1244149-50). Total RNA was treated with DNase I to remove genomic DNA (Promega, M6101). cDNA was synthesized using Transcriptor First Strand cDNA Synthesis Kit (Roche) with an oligo dT primer. No detection of *β-ACTIN* amplification from the minus RT controls was confirmed for any of the RNA samples examined in this study. qPCR was carried out in triplicate in 10 μl volumes containing 25 ng cDNA, 5 nM of the primers and FastStart Essential DNA Green Master (Roche) using LightCycler 96 (Roche). The amplification efficiency was calculated from the standard curve. GAPDH was used as the reference gene and the data was analyzed using Microsoft Excel.

2.2. Luciferase reporter assay

The genomic regions of interest to examine promoter activity were cloned upstream of the luciferase gene in the pGL3 Luciferase Reporter Vector (Promega). These vectors were transfected into HEK293T cells using polyethylenimine (PEI). Luciferase measurements were performed using Dual-Glo Luciferase Assay System (Promega) as instructed by the manufacturer.

2.3. Determination of the number of tandem repeat units

Genomic DNA was extracted from human cell lines using Trizol (Thermo Fisher Scientific) or DNeasy Blood and Tissue Kit (QIAGEN). Human tissue genomic DNA was obtained from BioChain (D1234035, D1234062, D1234086, D1234142, D1234149, D1234260, D1234274, D1234200, D1244035, D1244142, D1244149, D1244272). Twenty-five cycles of PCR were performed using the primer pairs designed in the 5′ and 3′ flanking regions of the *HSTR1* tandem repeat. The PCR products were analyzed by MultiNA microchip gel electrophoresis system (Shimadzu) and the number of tandem repeat units was estimated based on the length of PCR products.

2.4. RNA stability analysis

The full-length *HSTR1*, having 1, 6 and 15 repeat units, were cloned into pCI-neo Mammalian Expression Vector (Promega). The expression vectors were transfected into MCF7 cells using the NEPA21 electroporator (NEPAGENE). After the culture with

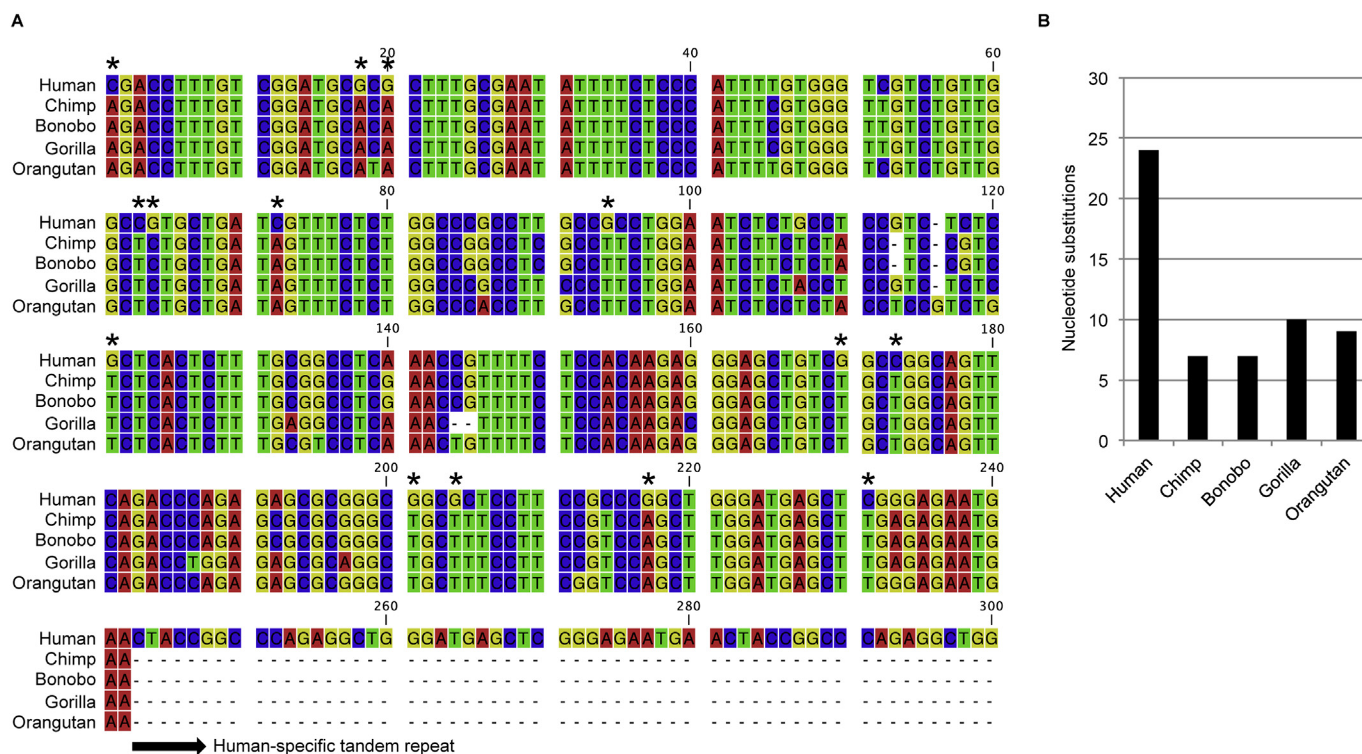

Fig. 1. Multiple sequence alignment and nucleotide substitutions in the flanking region of a human-specific tandem repeat. (**A**) Human-specific enrichment of nucleotide substitutions creating CpG sites in multiple sequence alignment of the flanking sequence of a human-specific tandem repeat. The asterisks indicate human-specific nucleotide substitutions that created CpG sites in the human sequence. The arrow represents the start of human-specific tandem repeat. The alignment was created using "CLC Sequence Viewer 8" software. (**B**) The number of nucleotide substitutions in the sequence of human, chimpanzee, bonobo, gorilla and orangutan estimated from the predicted ancestral sequence.

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