Human Genomic Deletions Generated by SVA-Associated Events

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1. Introduction

Human diverged from chimpanzee, its most closely related species, ~six million years ago [1, 2]. Since then, the human genome has evolved independently from the chimpanzee genome, leading to human-specific insertions and deletions (INDELs). Species-specific insertion of mobile elements and subsequent genomic rearrangements are major factors causing the INDELs between human and chimpanzee genomes [3–9]. The mobile elements account for ~45% of the human genome. Among them are L1 and Alu elements, which are the most successful non-LTR (long terminal repeat) retrotransposon families in the human genome, comprising 13% and 17% of the genome, respectively [10]. A third family of retrotransposon is the SVA element, which is a composite repetitive element, composed of SINE, VNTR, and Alu. SVA elements are currently active in the human genome and are involved in the creation of novel primate genes and the development of human disease through various mechanisms including exon-trapping and 5′ transduction [9, 11–14]. The commonality of the three elements is that they use L1 enzymatic machinery to retrotranspose in their host genomes. They mobilize via a “copy and paste” mechanism; they transcribe their RNA intermediates, and the RNA intermediates integrate into new genomic regions in a process known as retrotransposition. The mechanism for this process is called target-primed reverse transcription (TPRT) [15, 16].

L1 and Alu have caused INDELs between the human and chimpanzee genomes through various mechanisms such as L1 insertion-mediated deletion [6], Alu recombination-mediated deletion [5, 7], and L1 recombination-associated deletion [4]. Genomic rearrangements that are mediated by L1 and Alu elements have been widely studied [3–8]. However, the genomic modifications caused by the SVA elements have not been studied much. A total of 2,762 SVA elements have been identified in the human genome [9]. Compared with the L1 and Alu elements, a very small number of SVA elements exist in the current human genome. The evolutionary history of the three mobile elements could explain why the number of SVA elements is much lower than that of other elements. The SVA element is the youngest retrotransposon family in primates and, thus, has had less time to propagate; the SVA element emerged in the primate genome after the split between the Old World monkeys and
apes, ~20 million years ago (mya) [9]. In contrast, L1 and Alu emerged in the mammalian genome ~100 mya [17] and in the primate genome ~60 mya [18], respectively. However, we are unable to estimate how many mobile elements will retrotranspose in the human genome during a certain time because they have been inserted into their host genome at irregular retrotransposition rates: L1 elements presented because they have been inserted into their host genome and chimpanzee sequence showed high retrotransposition rates 60 to 35 mya [20]. These two time zones are far earlier than the time when the SV A elements emerged in the primate genome. However, SV A elements have been shown to be currently retrotransposing in the human genome [9] and have the potential to rearrange the human genome.

It has been considered that the SV A elements are one of the factors modifying the human genome through various mechanisms mentioned above. However, an SV A whole genome analysis, except for SV A insertion in the human genome, has not been conducted. In this study, we carried out whole genome analysis of SV A-associated events, SRADs and SIMDs, in the human. We identified 13 SRADs and 13 SIMDs and confirmed the SRAD loci using polymerase chain reaction (PCR) display. Among the SRADs, one was an SV A recombination-mediated deletion. This study is the first whole genome analysis investigating the human genomic deletion generated by SV A-associated events and shows the possibility that SV A elements in the human genome are candidate factors able to cause genomic rearrangements and subsequently genomic variation in human populations.

2. Materials and Methods

2.1. Computational Data Mining and Manual Inspection. To identify SRADs in the human genome, we first extracted all SV A elements from the genome, based on the UCSC Table Browser utility (http://www.genome.ucsc.edu/cgi-bin/hgTables?org=Human&db=hg19&hsid=226958818&hgta_doMainPage=1). For each SV A locus, we extracted 1 kb of the flanking sequence in either direction of the genomic position and used the human sequence as a query to search against the chimpanzee genome sequence using UCSC’s BLAT utility. For each hit in the BLAT search, we extracted the human and chimpanzee sequences and annotated repeat elements existing in the sequences, utilizing RepeatMasker analysis. In the case of a candidate SIMD event, chimpanzee sequence contained extra sequences compared with the orthologous human sequence.

2.2. PCR Amplification and DNA Sequence Analysis. After the manual inspection, we verified the candidate deletions by PCR assay with four different DNA templates: Homo sapiens (human), Pan troglodytes (common chimpanzee), Gorilla gorilla (gorilla), and Pongo pygmaeus (Bornean orangutan). Oligonucleotide primers for amplification of SRAD loci were mostly designed using the software Primer3 (http://frodo.wi.mit.edu/primer3/). Then, we used OligoCalc [21] to verify whether the designed primers were self-complimentary and UCSC’s insilico PCR utility (http://www.genome.ucsc.edu/cgi-bin/hgPcr?command=start) to computationally test the efficiency of the primers. In case where the primers designed by Primer3 were not desirable for actual PCR amplification, we manually aligned the human, chimpanzee, and orangutan DNA sequences using the software BioEdit v.7.1.3 [22] and designed alternative primers on the conserved region among the DNA sequences. The sequences of the oligonucleotide primers, annealing temperatures, and PCR product sizes are shown in (Supplementary Table 1 in Supplementary Materials available online at doi:10.1155/2012/807270). PCR amplification of each candidate was performed in 20 μL reaction using 10–50 ng template DNA, 200 nM of each oligonucleotide primer, and 10 μL of EmeraldAmp GT PCR Master Mix (Takara, Ohtsu, Japan). Each sample was subjected to an initial denaturation step of 5 min at 95°C, followed by 35 cycles of PCR for 30 sec of denaturation at 95°C, 30 sec at the annealing temperature, 1 to 2 min of extension at 72°C, depending on the product sizes, followed by a final extension step of 10 min at 72°C. For the case where the expected product size was >2kb, we used Ex Taq polymerase (Takara, Ohtsu, Japan), KOD (Toyobo, Osaka, Japan), and 2X EF-Taq Pre mix 2 (SolGent, Seoul, Republic of Korea) to carry out PCR in 40 μL reactions following the manufacturer’s protocol. The resulting products were loaded on 1% agarose gels, stained with ethidium bromide, and visualized using UV fluorescence (Bio-Rad, Hercules, CA, USA). In case where the chimpanzee reference genome sequence contains unsequenced region (Ns) on candidate deletion locus, we sequenced chimpanzee PCR product amplified from the locus to determine the precise deletion size in the human genome. The chimpanzee PCR product was purified from the PCR solution using the Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) and cloned into vector using the MG TOPcloner TA Core Kit (Macrogen, Seoul, Republic of Korea), according to the manufacturer’s instructions. The sequencing of the colony PCR product was conducted using dyeoxy chain-termination sequencing at Macrogen. The DNA sequence from this study has been deposited in Genbank under accession number JQ354986.
2.3. Analysis of Flanking Sequences of SVA-Associated Deletions (SADs). For flanking sequence GC content analysis, we used in-house Perl scripts to extract 10 kb of flanking sequence in either direction of each SAD locus and to calculate the percentage of GC nucleotides in the combined 20 kb of the flanking sequence. To determine whether each SAD event occurs in either intergenic or intragenic region, we used UCSC’s Genome Browser utility (http://www.genome.ucsc.edu/cgi-bin/hgGateway?org=Human&db=hg19&hgsid=244865559).

3. Results and Discussion

3.1. Whole Genome Analysis of SAD Events. We first extracted 3,608 SVA elements from the human genome and then excluded 2,206 human-specific SVA elements from the data to identify potential SRAD events in the human genome. However, the copy number of SVA elements seems to be overestimated because some of the SVA elements contained duplications of the VNTR region, and each of those elements was counted as two separate elements instead of one by RepeatMasker utility. We further examined the remaining 1,402 SVA elements, which are shared between human and chimpanzee genomes, to determine whether the elements were associated with deletions in the human genome. We identified 13 SRADs in the human genome by comparing the human and chimpanzee sequences for each locus. They seemed to occur in a low frequency compared to 73 L1 recombination-associated deletions in the human genome [4]. However, for the comparison, we need to consider their copy numbers: ∼3,000 SVAs versus ∼520,000 L1s in the human genome [9, 10]. We were not computationally able to verify the authenticity of the SRADs because most of the deletions involved portions of the SVA elements which are not shared between human and gorilla genomes. As shown in Figure 1(a), we experimentally verified the authenticity of the SRAD loci by wet-bench PCR analysis. However, we could not experimentally confirm two of the loci because they contained a high density of repetitive elements. Repetitive elements inhibit PCR amplification of their respective genomic regions. To identify potential SIMD events, we retrieved the 2,206 human-specific SVA elements and manually inspected them by the strategy described in Section 2. Through the process, we identified 13 SIMDs in the human genome. Unlike SRADs, we were able to verify the SIMDs (Figure 1(b)) and determine their deletion sizes using the chimpanzee (panTro3), gorilla (gorGor3), and orangutan (ponAbe2) reference genome sequences.

The genomic positions of the SRAD and SIMD loci are described in Tables 1 and 2, respectively. In addition, their locations along human chromosomes are shown in Figure 2. Three of the SRAD loci were located on chromosome one. A previous study about SVA elements reported that the observed copy number of SVA elements is much higher than the expected copy number on human chromosome one [9].

3.2. Mechanisms Causing SRADs. We closely examined the loci to elucidate mechanisms that caused the deletions. The results showed that 12 SRADs occurred by nonhomologous end joining (NHEJ) and only one locus occurred by nonallelic homologous recombination (NAHR) between poly-A tails of two different SVA elements (Table 1). NHEJ typically involves microhomology between sequences, and Figure 3 shows the microhomology of our elements [24–26]. It was suggested that NHEJ is one of the repair mechanisms for double-strand breaks (DSBs). Thus, we suspect that SVA element involved in an NHEJ-SRAD event was present in the flanking region of DSBs and microhomology between SVA and the other elements is associated with end binding to bridge the DNA lesion. However, this mechanism is not specific to SVA elements, and other mobile elements including L1 would act in the same way as SVA [4]. At an NAHR-SRAD locus, the two closely related prerecombination SVAs found in the chimpanzee genome are seen to have recombined into a single chimeric SVA in the human genome, having resulted in the deletion of a portion of each SVA element and the intervening DNA sequences. Compared with L1 or Alu recombination mediated deletion, the frequency of the SVA recombination-mediated deletion was very low. The physical proximity and sequence similarity between repetitive elements are important factors in causing recombination between them. Therefore, the low density of SVA elements in the human genome and high sequence diversity among their VNTR regions probably resulted in the low frequency of the SVA recombination-mediated deletion in the human genome.

3.3. Characterization of SRADs. Seven different SVA subfamilies (SVA_A to SVA_F [9] and SVA2 [13]) exist, which emerged in chronological order in primate genomes. Among them, SVA2 is the oldest subfamily [13, 27]. In contrast, SVA_E and SVA_F subfamilies are human-specific and, thus, are younger than other subfamilies [9]. As shown in Table 3, we investigated subfamily of the SVA elements involved in the SRAD events by using RepeatMasker utility which is one of the most popular tools to annotate mobile elements. As for the SRADs, we expected that all of the SVA elements would belong to one of the SVA_A to SVA_D subfamilies because this study aimed to identify the SRADs mediated by the SVA elements which were inserted into the host genomes before the divergence of the human and chimpanzee lineages. However, the result showed that one event was mediated by the SVA_F element. We examined this SVA element in greater detail. The element is truncated and shared in the human, chimpanzee, gorilla, and orangutan genomes. As such, this SVA element must be inserted before the divergence of the gorilla and orangutan lineages, which means that it is not human specific. Through the pairwise sequence alignments of this SVA element and consensus sequence for each SVA subfamily, we found out that this element contains a DNA sequence which is specific to SVA2 subfamily [13]. We used RepeatMasker utility to annotate subfamily of SVA elements, but this tool does not contain the information about the SVA2 subfamily, which caused the annotation error.

The total amount of genomic sequence deleted by these SRAD events in the human genome after the divergence
of human and chimpanzee was estimated to be 15,752 bp. As shown in Table 1, the SRADs ranged in size between 113 bp and 4,980 bp with an average size of 1,212 bp. After the divergence of humans and chimpanzees, Alu elements deleted approximately 400 kb of the human genome through the recombination between the two different Alu elements [7]. The previous study found a recombination hotspot (predominant recombination breakpoint position) on the Alu element. Thus, we examined the breakpoint positions on the SVA elements responsible for the SRADs. Five SRADs and four SRADs had the breakpoint on the Alu-like and SINE-R regions, respectively, but no predominant breakpoint position was observed on the elements. However, we could not rule out that this was a false negative result due to the low sample number of SRAD events. We further examined the types of repetitive sequences involved in the breakpoints at all SRAD loci, and the results are shown in Table 4.

### Table 1: Characterization of the SVA recombination-associated deletion loci.

| Locus | Genomic position (hg19) | Deletion size (bp) | Deletion mechanism | Microhomology (bp) |
|-------|------------------------|--------------------|--------------------|-------------------|
| 87    | chr1: 35413213–35414369 | 1017               | NHEJ               | 4                 |
| 273   | chr1: 181923989–181924621| 682                | NHEJ               | 0                 |
| 361   | chr1: 249205332–249205990| 4980              | NHEJ               | 5                 |
| 432   | chr2: 44288699–44288879 | 277                | NHEJ               | 7                 |
| 574   | chr2: 18465950–184658043| 113                | NHEJ               | 2                 |
| 689   | chr3: 42024110–42025361 | 4463               | NHEJ               | 2                 |
| 1505  | chr7: 55378897–55379920 | 1271               | NHEJ               | 0                 |
| 1652  | chr8: 11186832–11188441 | 305                | NHEJ               | 3                 |
| 2299  | chr11: 3418351–3419320  | 367                | NHEJ               | 2                 |
| 2335  | chr11: 46606606–46606899 | 526               | NHEJ               | 2                 |
| 2493  | chr12: 18672855–18672949 | 589               | NAHR               | 36                |
| 2836  | chr15: 20408545–20409855 | 312               | NHEJ               | 4                 |
| 2981  | chr16: 30170002–30170483 | 850               | NHEJ               | 1                 |

*NHEJ: nonhomologous end joining, NAHR: nonallelic homologous recombination.

3.4. Characterization of SIMDs. We examined subfamilies of the SVA elements involved in the SIMD events and described the result in Table 3. Interestingly, the result showed that the SVA elements involved in 9 out of 13 SIMDs belong to SVA_D subfamily. SVA_D subfamily is the largest subfamily and accounts for over 40% of SVA elements existing in the human genome [9]. Thus, we suggest that the copy number of SVA elements is proportionally related to the frequency of SIMD events. We further characterized SIMDs focusing on their deletion size. The result showed that the deletion size ranges from 14 to 8,741 bp deleting a total of 30,785 bp of human genomic sequence since the divergence of human and chimpanzee. One of the previous studies about L1 element examined L1 insertion-mediated deletions in the human genome and reported the nonrandom distribution in their deletion size, either very short (<100 bp) or relatively large (>1 kb) [6]. In contrast, the SIMDs were randomly distributed in size as shown in Table 2.
Figure 2: The 28 SVA-associated deletion loci in the human genome. Blue and red circles indicate SRAD and SIMD events, respectively. The karyotype images were created by using the idio-graphica webtool [23].

Table 2: Location and deletion size of the SVA insertion-mediated deletion loci.

| Locus | Genomic position (hg19) | Deletion size (bp) |
|-------|------------------------|--------------------|
| 242   | chr3: 14909866–149099196 | 2581               |
| 732   | chr3: 61656812–61658447  | 19                 |
| 831   | chr12: 31333177–31333788 | 347                |
| 1547  | chr7: 75581297–75582629  | 653                |
| 1667  | chr22: 35021272–35022965 | 1633               |
| 1671  | chr14: 84565214–84566566 | 8741               |
| 1755  | chr8: 122668707–122669486 | 1191               |
| 2263  | chr8: 57983370–57984835  | 14                 |
| 2508  | chr8: 145092008–145092734 | 4859               |
| 2809  | chr8: 34951832–34952650  | 2791               |
| 2817  | chr1: 160905975–160906748 | 1929               |
| 3019  | chr10: 101851975–101854321 | 5997            |
| 3577  | chr16: 67746159–67746860  | 30                 |

Table 3: SVA subfamilies involved in SVA-associated events.

| SVA subfamilies | The number of SRADs | The number of SIMDs |
|-----------------|---------------------|---------------------|
| A               | 2                   | 0                   |
| B               | 3                   | 1                   |
| C               | 3                   | 2                   |
| D               | 3                   | 11                  |
| E               | 0                   | 1                   |
| F               | 0                   | 0                   |
| SVA2            | 1                   | 0                   |

Table 4: Types of repetitive sequences involved in the breakpoints of SRAD loci.

| Locus | Left break point | Right break point |
|-------|------------------|-------------------|
| 87    | Unique sequence  | SVA_B(1)          |
| 273   | L1MA5A           | SVA_B(2)          |
| 361   | SVA_B(2)         | Unique             |
| 432   | SVA2(6)          | Unique             |
| 574   | SVA_A(1)         | LIPAS             |
| 689   | SVA_C(1)         | ERVL-E-int         |
| 1505  | SVA_D(2)         | Unique sequence   |
| 1652  | AluIr4           | SVA_C(5)          |
| 2299  | Simple repeat (TA)| SVA_D(1)        |
| 2335  | Alu5x1           | SVA_D(3)          |
| 2493  | SVA_A(4)         | SVA_A(4)          |
| 2836  | SVA_C(1)         | Unique             |
| 2981  | SVA_A(2)         | AluSg4            |

Each number in the parenthesis indicates the localization of the break point in the SVA element (1): Alu-like, (2): VNTR, (3): SINE-R, (4): poly-A tail, (5): hexamer repeats, and (6): DNA sequence specific to SVA2.)

t-test showed that these two estimates were not significantly higher than the genome-wide GC content. We further examined whether the SAD loci resided on the intergenic region or intragenic region. The results showed that five SRAD and seven SIMD events occurred in the intragenic region of the human genome. Among the seven SIMD events, one deleted two exons in a human gene, but none of SRADs involved exons. An SRAD event that is deleterious to the host genome would have a very low probability of becoming fixed in the genome. However, this does not mean that the SRAD events are unable to cause human disease or genomic instability. In fact, neurofibromatosis, a genetic disorder of the nervous system, was caused by SRAD events in humans; an SVA element existing in intron 4 of the human neurofibromatosis type 2 (NF2) gene caused intragenic deletions in the genes of two patients with neurofibromatosis [29].

3.6. SIMD as an Agent Affecting Human-Chimpanzee Divergence. One of previous studies about transcriptional structures of chimpanzee sperm development-associated genes reported that the transcript of chimpanzee tMDC II (metalloproteinase-like, disintegrin-like and cysteine-rich domain) gene has different structure compared with that of human [30]. In addition, another study found out that human
**4. Conclusion**

Since the release of the human and chimpanzee genome reference sequences, various mechanisms responsible for the magnitude of retrotransposon-mediated genomic rearrangements have been proposed. In this study, we identified SRAD and SIMD events in the human genome through a genome-wide analysis. Our results showed that the events have deleted ∼46.5 kb of human genomic sequence, since the divergence of human and chimpanzee lineages. Among the deletions, one SIMD is responsible for non-functional human *tMDC II* gene by causing a loss of two exons in the gene. Through those mechanisms, all SVA elements existing in the human genome may have the potential to cause genomic deletions that could result in human disease. This study is the first whole genome analysis to investigate human genomic deletions generated by SRAD and SIMD events and suggests the possibility that those SVA elements play a role in lineage-specific changes in the human and other hominid genomes.

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**References**

[1] M. Goodman, W. J. Bailey, K. Hayasaka, M. J. Stanhope, J. Slightom, and J. Czelusniak, "Molecular evidence on primate phylogeny from DNA sequences," *American Journal of Physical Anthropology*, vol. 94, no. 1, pp. 3–24, 1994.

[2] M. Goodman, C. A. Porter, J. Czelusniak et al., "Toward a phylogenetic classification of primates based on DNA evidence complemented by fossil evidence," *Molecular Phylogenetics and Evolution*, vol. 9, no. 3, pp. 585–598, 1998.

[3] P. A. Callinan, J. Wang, S. W. Herke, R. K. Garber, P. Liang, and M. A. Batzer, "Alu retrotransposition-mediated deletion," *Journal of Molecular Biology*, vol. 348, no. 4, pp. 791–800, 2005.

[4] K. Han, J. Lee, T. J. Meyer, P. Remedios, L. Goodwin, and M. A. Batzer, "L1 recombination-associated deletions generate..."
human genomic variation,” Proceedings of the National Academy of Sciences of the United States of America, vol. 105, no. 49, pp. 19366–19371, 2008.
[5] K. Han, J. Lee, T. J. Meyer et al., “Alu recombination-mediated structural deletions in the chimpanzee genome,” PLoS Genetics, vol. 3, no. 10, pp. 1939–1949, 2007.
[6] K. Han, S. K. Sen, J. Wang et al., “Genomic rearrangements by LINE-1 insertion-mediated deletion in the human and chimpanzee lineages,” Nucleic Acids Research, vol. 33, no. 13, pp. 4040–4052, 2005.
[7] S. K. Sen, K. Han, J. Wang et al., “Human genomic deletions mediated by recombination between Alu elements,” American Journal of Human Genetics, vol. 79, no. 1, pp. 41–53, 2006.
[8] S. K. Sen, C. T. Huang, K. Han, and M. A. Batzer, “Endonuclease-independent insertion provides an alternative pathway for L1 retrotransposition in the human genome,” Nucleic Acids Research, vol. 35, no. 11, pp. 3741–3751, 2007.
[9] H. Wang, J. Xing, D. Grover et al., “SVA elements: a hominid-specific retroposon family,” Journal of Molecular Biology, vol. 354, no. 4, pp. 994–1007, 2005.
[10] E. S. Lander, L. M. Linton, B. Birren et al., “Initial sequencing and analysis of the human genome,” Nature, vol. 409, no. 6822, pp. 860–921, 2001.
[11] E. M. Ostertag, J. L. Goodier, Y. Zhang, and H. H. Kazazian Jr., “SVA elements are nonautonomous retrotransposons that cause disease in humans,” American Journal of Human Genetics, vol. 73, no. 6, pp. 1444–1451, 2003.
[12] J. Xing, H. Wang, V. P. Belancio, R. Cordaux, P. L. Deininger, and M. A. Batzer, “Emergence of primate genes by retrotransposon-mediated sequence transduction,” Proceedings of the National Academy of Sciences of the United States of America, vol. 103, no. 47, pp. 17608–17613, 2006.
[13] A. Damert, J. Raiz, A. V. Horn et al., “5′-transducing SVA retrotransposon groups spread efficiently throughout the human genome,” Genome Research, vol. 19, no. 11, pp. 1992–2008, 2009.
[14] D. C. Hancks, A. D. Ewing, J. E. Chen, K. Tokunaga, and H. H. Kazazian, “Exon-trapping mediated by the human retrotransposon SVA,” Genome Research, vol. 19, no. 11, pp. 1983–1991, 2009.
[15] D. D. Luan, M. H. Korman, J. L. Jakubczak, and T. H. Eickbush, “Reverse transcription of R2Bm RNA is primed by a nick at the chromosomal target site: a mechanism for non-LTR retrotransposition,” Cell, vol. 72, no. 4, pp. 595–605, 1993.
[16] G. J. Cost, Q. Feng, A. Jacquier, and J. D. Boeke, “Human L1 element target-primed reverse transcription in vitro,” EMBO Journal, vol. 21, no. 21, pp. 5899–5910, 2002.
[17] A. F. A. Smit, G. Toth, A. D. Riggs, and J. Jurka, “Ancestral, mammalian-wide subfamilies of LINE-1 repetitive sequences,” Journal of Molecular Biology, vol. 246, no. 3, pp. 401–417, 1995.
[18] E. Ziętkiewicz, C. Richer, D. Sinnett, and D. Labuda, “Monophyletic origin of Alu elements in primates,” Journal of Molecular Evolution, vol. 47, no. 2, pp. 172–182, 1998.
[19] H. Khan, A. Smit, and S. Boissinot, “Molecular evolution and tempo of amplification of human LINE-1 retrotransposons since the origin of primates,” Genome Research, vol. 16, no. 1, pp. 78–87, 2006.
[20] K. Johanning, C. A. Stevenson, O. O. Oyeniran et al., “Potential for retroposition by old Alu subfamilies,” Journal of Molecular Evolution, vol. 56, no. 6, pp. 658–664, 2003.
[21] W. A. Kibbe, “OligoCalc: an online oligonucleotide properties calculator,” Nucleic Acids Research, vol. 35, pp. W43–W46, 2007.