Whole genome comparison of donor and cloned dogs

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Cloning is a process that produces genetically identical organisms. However, the genomic degree of genetic resemblance in clones needs to be determined. In this report, the genomes of a cloned dog and its donor were compared. Compared with a human monozygotic twin, the genome of the cloned dog showed little difference from the genome of the nuclear donor dog in terms of single nucleotide variations, chromosomal instability, and telomere lengths. These findings suggest that cloning by somatic cell nuclear transfer produced an almost identical genome. The whole genome sequence data of donor and cloned dogs can provide a resource for further investigations on epigenetic contributions in phenotypic differences.

Dogs are one of the invaluable animal models in biomedical fields, because they exhibit 333 genetic diseases that are similar to human’s1. In 2005, the clone of a male Afghan hound, named ‘Snuppy’, was generated2,3 by somatic cell nuclear transfer (SCNT), which is a form of cloning that transfers the nucleus from a somatic cell into an oocyte. Snuppy has grown up without any detectable abnormality to date. He and other cloned dogs also seem to be normally fertile, as artificial insemination with two cloned female dogs resulted in 10 healthy puppies being born in 20094.

Cloned offspring can be exposed to different environments, whereas identical twins usually grow up under very similar conditions right from birth. Therefore, cloning by SCNT is an invaluable model to study the effect of the environment on the phenotype. However, it has not been confirmed that their whole length genomes are indeed identical. Fortunately, the full reference genome of a dog has already been assembled5 and is publicly available. Here we carried out whole genome sequencing of the cloned dog and its nuclear donor dog (Supplementary Fig. S1), in order to compare them with the dog assembly. To investigate the level of genomic difference in the dogs, we compared it with the genomes of human monozygotic twins (ethnic Korean, female), which serve as an example of natural cloning and were assumed to be of identical genetic make-up6. We carried out a genome-wide analysis in terms of single nucleotide variation (SNV), copy number variation (CNV), structural variation (SV), and telomere lengths (Fig. 1 and Table 1).

Results

Whole genome sequencing of donor and cloned dogs. The DNA of a male cloned dog (Snuppy, 7.5 years old) and a male donor dog (Tai, 10.5 years old) was sequenced using Illumina HiSeq2000 (Supplementary Table S1 and Methods). On average, 56 gigabases per sample (~20 × depth) were produced (Supplementary Table S2) and were mapped to the dog reference genome (CanFam3.1) at a mapping rate of over 98% (Supplementary Table S3). In both dogs, on average, about 4.4 million SNVs and 1.1 million small insertions and deletions (indels) were identified (Supplementary Tables S4 and S5). When the variations were compared, 8,534 SNVs (8,337 autosomal, 115 sex chromosomes, and 82 mitochondrial) and 6,872 small indels (6,789 autosomal, 82 sex chromosomes, and 1 mitochondrial) from the cloned dog were detected as somatic (i.e., post-cloning de novo) variations (Supplementary Table S6). These are comparable to those of the monozygotic twin genomes (9,129 somatic (post-twinning de novo) SNVs and 3,509 somatic indels) that have been analyzed by the same methods. Additionally, the mutation rate of the cloned dog (3.77 mutations/Mb) was comparable to those of the donor...
Figure 1 | Overview of somatic alterations in cloned dog and monozygotic twin. (a) Difference of alterations in cloned and donor dogs. (b) Difference of alterations in monozygotic twins. From the outside, each layer represents reference chromosomes (grey), mapping depth of cloned and donor dogs (red and blue, respectively), the number of somatic SNVs (green), and the number of somatic indels (yellow). The difference of structural variations (SVs) is shown as black lines in the center (the short-lines indicate insertion, deletion, and intra-chromosomal translocation; the long-lines across the centers indicate inter-chromosomal translocation).

dog (3.84 mutations/Mb) and twins (3.36–3.57 mutations/Mb) (Supplementary Fig. S2 and Supplementary Table S7). The number of mitochondrial somatic variations of the cloned dog was higher than that of the twin (zero mitochondrial somatic variation), and this was expected as the cloned dog’s mitochondrial DNA was transmitted by an oocyte donor. The somatic variation patterns of nucleotide substitution is an important element in disease research, such as cancers7, and we found that the variation patterns of the dogs and twins showed a high level of similarity (bias into the transitions of A > G and C > T) (Supplementary Fig. S3). These results suggest that the SCNT did not cause any altering of the mutation rates and patterns.

Identification of de novo mutation in cloned dog. Notably, only six somatic autosomal nonsynonymous SNVs (nsSNVs in DNAJC14, KNTC1, ZNF683, KA768, ESCO1, and ENSCAFG00000030636 genes) were found in the cloned dog. While occurring in different genes, an identical number of nsSNVs was found in the monozygotic twin (PRB3, TMCS, DISP1, SALL4, SPATS1, and C9orf139 genes; Supplementary Table S8). Additionally, the cloned dog and twin did not show any insertion or deletion in coding regions. Upon in-depth analyses using computational prediction (PolyPhen2)8 among the genes containing nsSNVs, only ESCO1 (K811E) in the cloned dog and SPATS1 (G8R) and C9orf139 (D49N) in the monozygotic twin were predicted to be function altered (probably or possibly damaging). Interestingly, the ESCO1 gene, which belongs to a conserved family of acetyltransferases, is involved in sister chromatid cohesion in the S phase of the mitotic cell cycle8. Also, the KNTC1 gene has an nsSNV (E1204D, neutral) in the cloned dog, which is known to be an essential component of the mitotic checkpoint and prevents cells from prematurely exiting mitosis in M phase10. Although these mutations occurred in the genes that are associated with the cell cycle, all of the somatic nsSNVs were heterozygous variations, perhaps indicating proper function of the genes. Furthermore, there was no experimental evidence that the cloning caused any abnormality in the cell cycle, as the cultured cell lines derived from the donor and cloned dogs grew without any obvious differences.

Chromosomal instability analysis. Chromosomal instability, such as CNV and SV, is important in disease research11. The analysis showed that there was no CNV difference between the donor and cloned dogs, with the exception of three CNV differences in mitochondrial DNA that were caused by a different oocyte. This was fewer than the human twins who had only two CNV differences in the autosome (Supplementary Table S9). This result indicates that the clone had almost identical genomic structure to that of the nuclear donor. Additionally, we found 903 and 778 SV signals from the donor dog and cloned dog, respectively. Among them, only 12 SVs (1.5%) were identified as somatic SVs (Supplementary Table S10). This is much fewer than that of the monozygotic twin (394 somatic SVs, 25.1%). Four out of the 12 somatic SVs in the cloned dog were located in the intron regions of HPSS, AGPS, and FAM73A (insertions) genes, and only one exon region of the unknown gene (ENSCAFG00000015277) suffered from inter-chromosomal translocation (Supplementary Table S11). On the other hand, 116 of the twin’s genes were affected by the
somatic SVs. In short, these chromosomal instability analyses revealed that the degree of similarity in the cloned dog is higher than that of the twin, especially when considering the age effect as the human equivalent biological age of the dogs was higher than the twins’ age (40 to 70 years compared with 20 years, respectively).

Telomere length of donor and cloned dogs. Telomeres protect the ends of chromosomes and are reduced in length in most mammalian cell types during replication. When telomeres reach a critically short length, a DNA damage signal is initiated, inducing cell senescence. Telomere length is one of the major issues in cloned animals because telomeres act as a safeguard at the ends of chromosomes and are reduced in length in most mammalian cell types. The shorter the length, the more likely a DNA damage signal is initiated, inducing cell senescence. The length of cloned cattle and mice showed the same or longer telomeres than those of the normal calves. Moreover, previous reports suggest that telomere length is a major factor involved in the development of cloned animals. Therefore, we estimated the telomere lengths of the donor and cloned dog using whole genome sequencing data (see Methods).

For the genome-wide analysis, the raw read sequences of the tail of the first read and the head of the index adapter are identical. B. More than 6 repeats) by the number of total reads as described in a previous report. To identify somatic variations, variations from the donor dog genome using VarScan2 ver. 2.3.4 with default options. In the same manner, the somatic variations of monozygotic twins were identified by filtering variations from one twin genome by the mutations from the other twin genome. The somatic variations were filtered out using the FreeBayes algorithm with a posterior likelihood of P > 0.05. All somatic variations altering amino acid sequences were checked by expert lab personnel using the tview command of SAMTools. Snpeff28 was used to annotate the variations.

Methods
Sample preparation and whole genome sequencing. Genomic DNA was extracted from blood collected from the jugular vein of both the cloned and original donor dogs from Seoul National University of Korea with the PAXgene Blood DNA Kit (Qiagen, Valencia, CA, USA), following the manufacturer’s instructions. The human monozygotic twins’ DNA came from the Korean Personal Genome Project (KPGP, available at http://opengenome.net). A library of ~ 200 bp insert size was constructed.

Telomere length estimation. Relative telomere lengths of the cloned dog and donor dog were estimated by dividing the number of reads having ‘TTAGGG’ repeat (from 1 to 6 repeats) by the number of total reads as described in a previous report17. To normalize bias from sequencing quality, other repeats, such as ‘GGGATT’, were also used as controls. Southern blotting is also used to validate the telomere lengths in experiments. Mean telomere length was determined by mean terminal restriction fragment (TRF) length analysis with a Tol-TAGGG Telomere Length Assay (Roche, Mannheim, Germany). The isolated genomic DNA (5 μg) was digested with restriction enzymes, Hinf I and Rsa I, and hybridized in 10 μl of DIG Easy Hyb containing 50 pmol of end-labeled, telomere-specific probe for 16 hours at 42°C. Membranes were washed three times in 50 ml of 0.5 × standard saline citrate (SSC; 1 × SSC; 0.15 M NaCl, 0.015 M Sodium Citrate) for 15 mins at room temperature. The signals were visualized by chemiluminescence using a Cronex Lightemissence Kit (Roche) and exposed to x-ray film (Hyperlfilm, Amersham Pharmacia Biotech.). The signals were scanned and analyzed using Gel Doc software (Bio-rad, Hercules, CA).

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Author contributions

H.K., H.-M.K., S.I. and Y.S.C. performed the bioinformatic analysis of whole genome sequence data. J.Y.C. performed the genome sequencing. H.-M.K., Y.S.C., H.K., S.K., B.S., G.J. and J.B. wrote the manuscript. J.B., B.C.L. and G.J. conceived and designed this study.

Additional information

Supplementary information accompanies this paper at http://www.nature.com/scientificreports.

Accession codes: Whole genome sequence data of the cloned dog and donor dog used for this analysis are available at Short Read Archive (SRA) under accession code SRP025974.

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