

## Comparison of the Genomic Sequence of the Microminipig, a Novel Breed of Swine, with the Genomic Database for Conventional Pig

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**Abstract.** The microminipig, which weighs less than 10 kg at an early stage of maturity, has been reported as a potential experimental model animal. Its extremely small size and other distinct characteristics suggest the possibility of a number of differences between the genome of the microminipig and that of conventional pigs. In this study, we analyzed the genomes of two healthy microminipigs using a next-generation sequencer SOLiD™ system. We then compared the obtained genomic sequences with a genomic database for the domestic pig (*Sus scrofa*). The mapping coverage of sequenced tag from the microminipig to conventional pig genomic sequences was greater than 96% and we detected no clear, substantial genomic variance from these data. The results may indicate that the distinct characteristics of the microminipig derive from small-scale alterations in the genome, such as Single Nucleotide Polymorphisms or translational modifications, rather than large-scale deletion or insertion polymorphisms. Further investigation of the entire genomic sequence of the microminipig with methods enabling deeper coverage is

required to elucidate the genetic basis of its distinct phenotypic traits.

Swine provide very reliable experimental animal models because of their physiological and anatomical similarities to humans (1). The number of pigs utilized as laboratory swine every year in the European Union alone exceeds 60,000 (2, 3). The microminipig (Fuji Micra Inc., Shizuoka, Japan; brand name registered with the Japanese Ministry of Agriculture, Forestry and Fisheries) is a novel breed of swine which has emerged as a possible experimental model animal for non-clinical pharmacological/toxicological use (1, 4-15). The common maternal ancestor, or “Eve”, of the microminipig is a sow named “Catherine”, bred by mating a pot-bellied pig and another type of minipig (5). Young, mature microminipigs weigh less than 10 kg, which enables easy handling (8-10, 14). The levels of the major hematological and biochemical parameters in microminipigs have been reported as being generally similar to those in Göttingen and Yucatan minipigs (16, 17). However, the coagulatory activity, prothrombin time and activated partial thromboplastin time in microminipigs differ from those in Göttingen and Yucatan minipigs (9). The microminipig has also been reported as a suitable model for arthrosclerosis and associated pathological investigations. The microminipig resembles humans in its sensitivity to a high fat diet, and the subsequent development of atherosclerotic vascular lesions and a cholesterol-related metabolic cascade (6). The atherosclerotic lesions in microminipigs more closely resemble those in humans than do any other breed of swine.

In the present study, we analyzed the genomes of two healthy microminipigs and compared them to a reference genome for conventional pigs (*Sus scrofa*).

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## Materials and Methods

**Animals.** Two mature microminipigs (one male and one female) were used as the source of the DNA for this genomic analysis. The animals were maintained in a regular experimental environment at a temperature of  $24\pm 3^{\circ}\text{C}$  and relative humidity at  $50\pm 20\%$ , with a 12 h light/dark cycle at the breeder's facility, with a space allowance of  $0.5\text{--}1.2\text{ m}^2/\text{animal}$ . The animals were supplied with a porcine diet (Marubeni Nisshin Feed Co. Tokyo, Japan) composed of  $>13.0\%$  crude protein,  $>2.0\%$  crude fat,  $<8.0\%$  crude fiber,  $<10.0\%$  crude ash,  $>1.1\%$  calcium, and  $>0.9\%$  phosphorus. Tap water was available *ad libitum*. The animals were determined to be in good health and free of clinical signs of illness. They were not given any treatment or medication other than vaccination during the study.

**Sample collection.** A 1 ml blood sample was collected from the cranial vena cava of each animal under a fasted condition while it was conscious. The blood was treated with an anticoagulant, EDTA-2K, and stored at  $-80^{\circ}\text{C}$  until genomic DNA isolation.

**Genomic DNA isolation.** Genomic DNA was isolated from the collected blood using the PureLink Genomic DNA kits (Thermo Fisher Scientific Inc. Waltham, MA, USA) according to the manufacturer's instructions. Genomic DNA was quantified using an ND-1000 spectrophotometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). DNA quality was verified using 1% agarose gel electrophoresis.

**Genome sequencing.** The genome of each microminipig was sequenced using a SOLiD™ 4 next-generation sequencing system (Thermo Fisher Scientific Inc. Waltham, MA, USA). Two types of libraries, fragment and mate-paired, were generated. The fragment libraries were generated from 1  $\mu\text{g}$  of genomic DNA using a SOLiD fragment library construction kit. The mate-paired libraries were generated from 5  $\mu\text{g}$  of genomic DNA using a 5500 SOLiD mate-paired library construction kit. Templated beads were prepared with the SOLiD ePCR Kit V2 and XD Beads Enrichment Kit, and then deposited on a glass slide using the SOLiD XD Slide and Deposition Kit V2. Fifty base pairs at the ends of library fragments were sequenced using the SOLiD ToP Sequencing Kit and SOLiD ToP Instrument Buffer Kit. All experimental procedures were performed according to the manufacturer's instructions.

**Mapping of sequenced data to a conventional pig genome.** The 50-bp reads obtained by the SOLiD system were mapped to a *Sus scrofa* draft genomic sequence, Sscrofa10.2, as a reference using Genomics WorkBench, version 5.5.1 (CLC Bio inc. Boston, MA, USA). Before mapping, reads were trimmed to remove low-quality sequences as follows: any part of a read containing unreliable base calls (*p*-value based on the Phred score  $<0.01$ ) was removed, as were reads of less than 25 bases, and reads containing three or more ambiguous base calls. The alignment quality threshold for the mapping to the reference sequence was set at  $\geq 90\%$  similarity over  $\geq 60\%$  of the read length. Single nucleotide polymorphisms (SNPs) were detected using the SNP detection function of Genomics WorkBench (CLC Bio inc. Boston, MA, USA). The settings for quality of reads for calling SNPs were as follows: window length=11, maximum number of gaps and mismatches=2, minimum average quality of surrounding bases=15, and minimum quality of central base=20. SNPs were called when four or more reads covered

Table I. Number of reads obtained in genomic sequencing.

Type of read	Male	Female
Single <sup>a</sup>	502,663,416	461,296,093
Mate-paired	528,666,554	576,322,360

<sup>a</sup>Reads from fragment libraries (those from mate-paired libraries whose counterpart was removed in trimming are on the line below).

the nucleotide, and a minimum variant frequency of 35% was required for reporting of polymorphism.

## Results

After removal of low-quality reads, approximately  $500\times 10^6$  reads, both single and mate-paired, were obtained from each microminipig (Table I). The mapping results are shown in Table II. Coverage was high for all the chromosomes ( $>10$ -fold), except the X chromosome of the male for which it was slightly low (9.2-fold). Very high coverage was achieved for the mitochondrial genome due to its high copy number. Reads from gemones of both microminipigs mapped to the reference sequence across the whole genome ( $>95\%$ ), suggesting that microminipigs and conventional pigs share a highly similar genomic structure. Approximately 10 million polymorphisms were detected in the microminipig genomes, corresponding to only 0.4% of the reference genomic sequence (Table III). Homozygous polymorphisms were dominant. Most heterozygous polymorphisms accompanied a reference allele. Many ( $>70\%$ ) of the polymorphisms were detected in non-coding regions (Table IV). Substitutions were much more prominent than insertion or deletions and most of the substitution mutations did not affect the amino acid sequence.

## Discussion

Minipigs are regarded as a suitable experimental animal for medical studies because of their physiological and anatomical similarity to humans. However, despite continuous efforts from breeders, minipigs are not yet widely used for this purpose and one possible reason is the lack of basic data (18). Recent years have seen growth in the utilization of genomic data as sequencing becomes less expensive and data on the genomic sequences of several species become available. Genomic data for the pig have been published and extensively analyzed (19). The microminipig is the world's smallest experimental minipig and has been reported as being suitable for a model of diet-induced atherosclerosis, a possible model of thrombosis, examination of drug-induced skin lesions, a of sodium cholate-induced hepatic disease, and investigation of drug-induced pro-arrhythmia (1, 4, 6, 9, 10, 12, 14).

Table II. Results for mapping of sequenced tag from the microminipig to conventional pig genomic sequences.

Chromosome	Reference		Male		Female	
	Accession no.	Ungapped sequence (nt) <sup>a</sup>	Mapped, n (%)	Coverage (Fold)	Mapped, n (%)	Coverage (fold)
1	NC_010443	279,896,939	271,602,078 (97.0%)	15.8	273,602,940 (97.8%)	15.3
2	NC_010444	145,622,229	141,079,155 (96.9%)	16.1	142,291,290 (97.7%)	15.9
3	NC_010445	129,199,842	124,473,578 (96.3%)	16.0	126,057,752 (97.6%)	16.1
4	NC_010446	129,354,738	125,496,217 (97.0%)	15.9	126,333,974 (97.7%)	15.5
5	NC_010447	99,266,972	95,851,896 (96.6%)	15.1	96,711,922 (97.4%)	15.0
6	NC_010448	139,057,429	133,900,404 (96.3%)	16.1	135,761,058 (97.6%)	16.4
7	NC_010449	121,265,440	117,425,461 (96.8%)	15.5	118,570,148 (97.8%)	15.7
8	NC_010450	132,685,595	128,772,353 (97.1%)	15.6	129,568,302 (97.7%)	14.7
9	NC_010451	139,481,936	135,354,407 (97.0%)	16.0	136,439,370 (97.8%)	15.7
10	NC_010452	71,117,837	68,958,896 (97.0%)	16.5	69,393,898 (97.6%)	16.1
11	NC_010453	77,952,427	75,329,819 (96.6%)	15.1	75,852,402 (97.3%)	14.5
12	NC_010454	56,396,794	53,806,789 (95.4%)	14.9	54,812,994 (97.2%)	15.6
13	NC_010455	195,576,489	190,542,165 (97.4%)	16.1	191,526,741 (97.9%)	15.3
14	NC_010456	140,658,398	136,359,054 (96.9%)	15.9	137,682,543 (97.9%)	16.1
15	NC_010457	140,667,008	136,451,253 (97.0%)	16.0	137,200,533 (97.5%)	15.3
16	NC_010458	78,715,191	76,421,420 (97.1%)	18.3	76,903,840 (97.7%)	17.2
17	NC_010459	62,133,331	59,944,688 (96.5%)	15.5	60,630,251 (97.6%)	15.8
18	NC_010460	55,636,783	53,718,189 (96.6%)	16.7	54,326,484 (97.6%)	17.1
X	NC_010461	127,502,287	117,150,783 (91.9%)	9.2	124,361,526 (97.5%)	14.9
Y	NC_010462	1,333,869	1,260,308 (94.5%)	11.5	-	-
Mitochondria	NC_000845	16,613	16,589 (99.9%)	350.8	16,599 (99.9%)	501.6
Total		2,323,521,534	2,243,915,502 (96.6%)		2,268,611,157 (97.6%)	

<sup>a</sup>Number of nucleotides other than N (ambiguous base calling).

Table III. Number of polymorphisms detected in microminipigs.

Haplotype	Male	Female
Ungapped reference sequence, n (%)	2,323,538,147 (100%)	2,323,538,147 (100%)
Polymorphisms, n (%)	9,304,315 (0.40%)	9,971,157 (0.43%)
Homozygous, n (%)	6,524,720 (0.28%)	6,756,821 (0.29%)
Heterozygous, n (%)		
Reference allele	2,762,916 (0.12%)	3,193,232 (0.14%)
No reference allele	16,679 (0.001%)	21,104 (0.001%)

Table IV. Type of polymorphisms detected in microminipigs.

Haplotype				Male	Female
Coding, n (%)	Substitution	Single	Amino acid change	24,971 (0.3%)	27,538 (0.3%)
			Silent	2,256,696 (24.3%)	2,451,870 (24.6%)
		Multiple	Amino acid change	1,311 (0.01%)	1,425 (0.01%)
			Silent	64,200 (0.7%)	66,971 (0.7%)
	Insertion/deletion		288,434 (3.1%)	288,043 (2.9%)	
	Non-coding, n (%)	Substitution	Single	5,762,332 (61.9%)	6,228,965 (62.5%)
Multiple			177,586 (1.9%)	182,156 (1.8%)	
Insertion/deletion		728,785 (7.8%)	724,189 (7.3%)		
Total				9,304,315 (100%)	9,971,157 (100%)

The microminipig possesses a number of characteristics which distinguish it from the domestic pig, in addition to its smaller body size. Reference ranges for hematological parameters and lipid profiles in the microminipig have been reported (7-9); however, data on its genomic sequence have not been previously investigated. In this study, we analyzed the genomic sequence of microminipigs and compared the results with a reference genomic sequence for conventional pig.

On average, reads from the microminipig genomes covered 96.6% and 97.6% of the reference pig genome (Table II). Except for Y chromosome of the female, all chromosomes showed very high coverage, indicating that the genomic sequence of the microminipig is very similar to that of a conventional pig. The small body size and other distinctive characteristics of the microminipig may not have derived from large-scale insertion or deletion polymorphism in its chromosomal structure.

In the present study, we provided mapping of the microminipig genome sequences to a reference genomic database for the domestic pig. The high degree of similarity between the genomic sequences of the microminipig and conventional pig may indicate that the distinct characteristics of microminipigs derive from small-scale alterations in the genome, such as SNPs or translational modifications, rather than any large-scale deletion or insertion polymorphism. We conclude that further analyses of the entire genomic sequence of the microminipig with methods enabling deeper coverage and long read are needed to elucidate the genetic basis of its distinctive characteristics.

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## Conflicts of Interest

There is no conflicts of interest in this report.

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