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Characterization of single nucleotide polymorphisms for a forward genetics approach using genetic crosses in C57BL/6 and BALB/c substrains of mice

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Abstract: Forward genetics is a powerful approach based on chromosomal mapping of phenotypes and has successfully led to the discovery of many mouse mutations in genes responsible for various phenotypes. Although crossing between genetically remote strains can produce F_2 and backcross mice for chromosomal mapping, the phenotypes are often affected by background effects from the partner strains in genetic crosses. Genetic crosses between substrains might be useful in genetic mapping to avoid genetic background effects. In this study, we investigated single nucleotide polymorphisms (SNPs) available for genetic mapping using substrains of C57BL/6 and BALB/c mice. In C57BL/6 mice, 114 SNP markers were developed and assigned to locations on all chromosomes for full utilization for genetic mapping using genetic crosses between the C57BL/6J and C57BL/6N substrains. Moreover, genetic differences were identified in the 114 SNP markers among the seven C57BL/6 substrains from five production breeders. In addition, 106 SNPs were detected on all chromosomes of BALB/cAJcl and BALB/cByJJcl substrains. These SNPs could be used for genotyping in BALB/cJ, BALB/cAJcl, BALB/cAnNCrlCrlj, and BALB/cCrSlc mice, and they are particularly useful for genetic mapping using crosses between BALB/cByJJcl and other BALB/c substrains. The SNPs characterized in this study can be utilized for genetic mapping to identify the causative mutations of the phenotypes induced by N-ethyl-N-nitrosourea mutagenesis and the SNPs responsible for phenotypic differences between the substrains of C57BL/6 and BALB/c mice.
Key words: BALB/c, C57BL/6, genotyping, single nucleotide polymorphism, substrain

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Introduction

Mouse models are important tools for understanding the phenotypes of human diseases, genes responsible for diseases, and molecular mechanisms for the development of disease phenotypes. The approaches to developing mouse models include reverse genetics and forward genetics [1–4]. Reverse genetics is a powerful approach based on gene targeting and genome editing. This approach has successfully produced null mutations in almost all genes and mimics mutations associated with several human diseases. Although reverse genetics is a major approach in mouse genetics, forward genetics is a powerful approach based on chromosomal mapping of phenotypes and has led to the discovery of numerous mutations in genes responsible for various spontaneous phenotypes [4]. Moreover, the combination of forward genetics and large-scale N-ethyl-N-nitrosourea (ENU) mutagenesis studies has uncovered the functions of unknown genes and mutations [1, 5–7].

To identify genes associated with spontaneous and ENU-induced phenotypes using the classical forward genetic approach, researchers first produced experimental populations of chromosomal recombinants—F_2 and backcross (N_2) mice—by crossing between the mutant and wild-type mice. Wild-type mice must be chosen from other strains with different genetic backgrounds and mutants, and they are preferred over genetically diverse strains with mutants. This is because the mutations map to a chromosomal region via genotyping of the polymorphic markers, such as microsatellites and single nucleotide polymorphisms (SNPs), between both parental strains. Therefore, interspecific, intersubspecific, and interstrain genetic crosses have been used for classical chromosomal mapping of mutants of interest because of the availability of many polymorphic markers for genotyping in F_2 and N_2 mice from these genetic crosses [8].

However, partner strains that show highly similar phenotypes should be chosen to create a genetic cross in cases involving the mapping of causative mutations for mild phenotypes, which are easily affected by the genetic background of the partner strain. Crosses with similar phenotypic strains are effective for the mapping of behavioral and sensorial traits. Since there are individual differences in these phenotypic traits, phenotypic noise caused by modifiers in the genetic background of the partner strain should be reduced. Substrains may be candidate partners for genetic crosses. Genetically different substrains have been established in most classical inbred strains [9–12]. Although there are far fewer genomic polymorphisms between substrains than between strains, previous studies have successfully identified the mutations associated with phenotypes via linkage and quantitative trait loci (QTL) analysis using intersubstrain genetic crosses [13–17].

To perform linkage and QTL analyses using intersubstrain genetic crosses, it is important to set up polymorphic markers between substrains of mice without large gaps on the chromosomes. Therefore, we investigated the SNPs that are available for immediate use in linkage and QTL analyses using substrains. In this study, we chose substrains of C57BL/6 and BALB/c mice as the research targets. These are very common inbred mice, and several substrains have been established. Moreover, there are potential mutants available as human disease models because several large-scale ENU mutagenesis studies have been carried out based on ENU-treated male mice from both strains [18–21]. Therefore, marker sets based on SNPs between substrains can be a useful tool for identifying new phenotypes associated with human diseases.

Materials and Methods

Mice and ethics statement

The C57BL/6 and BALB/c substrains used in this study were purchased from each their respective production breeders (Table 1). This study was reviewed and approved by the Institutional Animal Care and Use Com-

Table 1. C57BL/6 and BALB/c substrains used in this study

Strain	Substrain	Breeder
C57BL/6	C57BL/6J	The Jackson Laboratory via Charles River Laboratories Japan
	C57BL/6JJcl	CLEA Japan
	C57BL/6JJmsSlc	Japan SLC
	C57BL/6NTac	Taconic Biosciences via CLEA Japan
	C57BL/6NJcl	CLEA Japan
	C57BL/6NCrl	Charles River Laboratories Japan
	C57BL/6NCrSlc	Japan SLC
BALB/c	BALB/cAJcl	CLEA Japan
	BALB/cByJJcl	CLEA Japan
	BALB/cAnNCrlCrlj	Charles River Laboratories Japan
	BALB/cCrSIc	Japan SLC

mittee of the RIKEN Tsukuba Branch and the Animal Care and Use Ethics Committee of the Tokyo Metropolitan Institute of Medical Science.

Preparation of PCR templates

All PCR templates were obtained from the pinna and/or tail samples of adult mice euthanized by carbon dioxide inhalation. The pinna (diameter: 2 mm) and tail (length: less than 2 mm) samples were incubated in 50 μl of lysis buffer solution (50 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 100 mM NaCl; and 1% sodium dodecyl sulfate) with 0.5 μl of proteinase K solution (20 mg/ml) at 37°C overnight and then incubated at 95°C for 10 min. The solution samples were diluted with water (1:200) and used as PCR templates.

Validation of SNPs between C57BL/6J and C57BL/6N substrains

To prepare novel SNP markers, we selected SNPs between the C57BL/6J and C57BL/6N substrains, based on published data [16, 22–24]. For several SNPs, the flanking sequences including the SNPs were amplified by PCR using AmpliTaq Gold DNA Polymerase (Thermo Fisher Scientific, Waltham, MA, USA) or TaKaRa Ex Taq (Takara Bio, Inc., Kusatsu, Japan) and the primers listed in Supplementary Table 1 according to the manufacturer's protocol. The SNPs were validated via Sanger sequencing of the PCR products. The PCR products were purified using the ExoSAP-IT (Thermo Fisher Scientific) and sequenced using an Applied Biosystems 3130xl Genetic Analyzer and 3730xl DNA Analyzer (Thermo Fisher Scientific) by the Genetic Analysis Division of Fasmac Co., Ltd. (Atsugi, Japan).

SNP genotyping

A set of validated SNPs was used to design TaqMan assays using a custom TaqMan SNP Genotyping Assay (Thermo Fisher Scientific; Supplementary Table 2). PCR template solutions (1 μl) were dispensed into each well of 384-well plates and dried using a speed-vacuum drying system. The reaction mixtures for genotyping using TaqMan Genotyping Master Mix (Thermo Fisher Scientific) and the custom TaqMan assays were dispensed into 384-well plates and amplified via PCR following the manufacturer's instructions. The PCR products were analyzed using the TaqMan Genotyper software with an ABI PRISM 7900HT Sequence Detection System (Thermo Fisher Scientific) or QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific) according to the manufacturer's instructions.

Detection of SNPs between BALB/cA and BALB/cByJ substrains

To obtain information on SNPs between BALB/cAJcl (BALB/cA) and BALB/cByJJcl (BALB/cByJ) mice, next-generation sequencing (NGS) technology was used. For sequencing, genomic DNA was extracted from the spleens of female BALB/cA and BALB/cByJ mice using a QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany). Whole genome sequencing and listing of mutations were performed by Hokkaido System Science Co., Ltd. (Hokkaido, Japan). Paired-end reads of 150 bp were generated with HiSeq X with HiSeq X Ten Reagent Kit v2.5 (Illumina, San Diego, CA, USA) to achieve more than 30× genome coverage. The sequence reads were mapped on the reference genome sequence (mouse genome reference assembly, GRCm38.p6) and analyzed using Samtools/BCFtools version 1.2 [25] and SnpEff version 4.1g [26]. The quality of a given base call is measured as a Phred quality score, which indicates the probability that the base is correctly called. The scores generally range from 2 to 40, with higher scores indicating greater confidence in the call. The candidate SNPs between BALB/cA and BALB/cByJ were confirmed via Sanger sequencing.

Results

Construction of marker sets for genetic mapping of genetic crosses between C57BL/6J and C57BL/6N substrains

We previously developed 101 primer and probe sets (Supplementary Table 2) for TaqMan assays and succeeded in linkage mapping of several phenotypes using these sets [14–17]. To place markers on chromosomal regions for linkage mapping at equal intervals without the absence of markers in the long-range chromosomal region, SNPs were screened from databases. Thirteen SNPs were identified as available for linkage mapping in genetic crosses between the C57BL/6J and C57BL/6N substrains via Sanger sequencing (Supplementary Tables 1 and 2). The locations of all markers for TaqMan assays are shown in Fig. 1. The markers were assigned at 20.58 Mb intervals on the chromosomes on average in the case of choosing either one from the neighboring markers (*D2SNP38*/*D2SNP39*, *D3SNP39*/*D3SNP48*, *D4SNP38*/*D4SNP42*, *D12SNP38*/*D12SNP41*, *D17SNP34*/*D17SNP32*, and *D19SNP23*/*D19SNP26*) located within 10 Mb (Fig. 1 and Supplementary Table 2). Although the SNP markers were absent in several long-range genomic regions (e.g., *D6SNP32–D6SNP33*, 50.18 Mb, and *DXSNP58–DXSNP52*, 48.43 Mb), we predicted that the marker sets for identifying the loci linked with pheno-

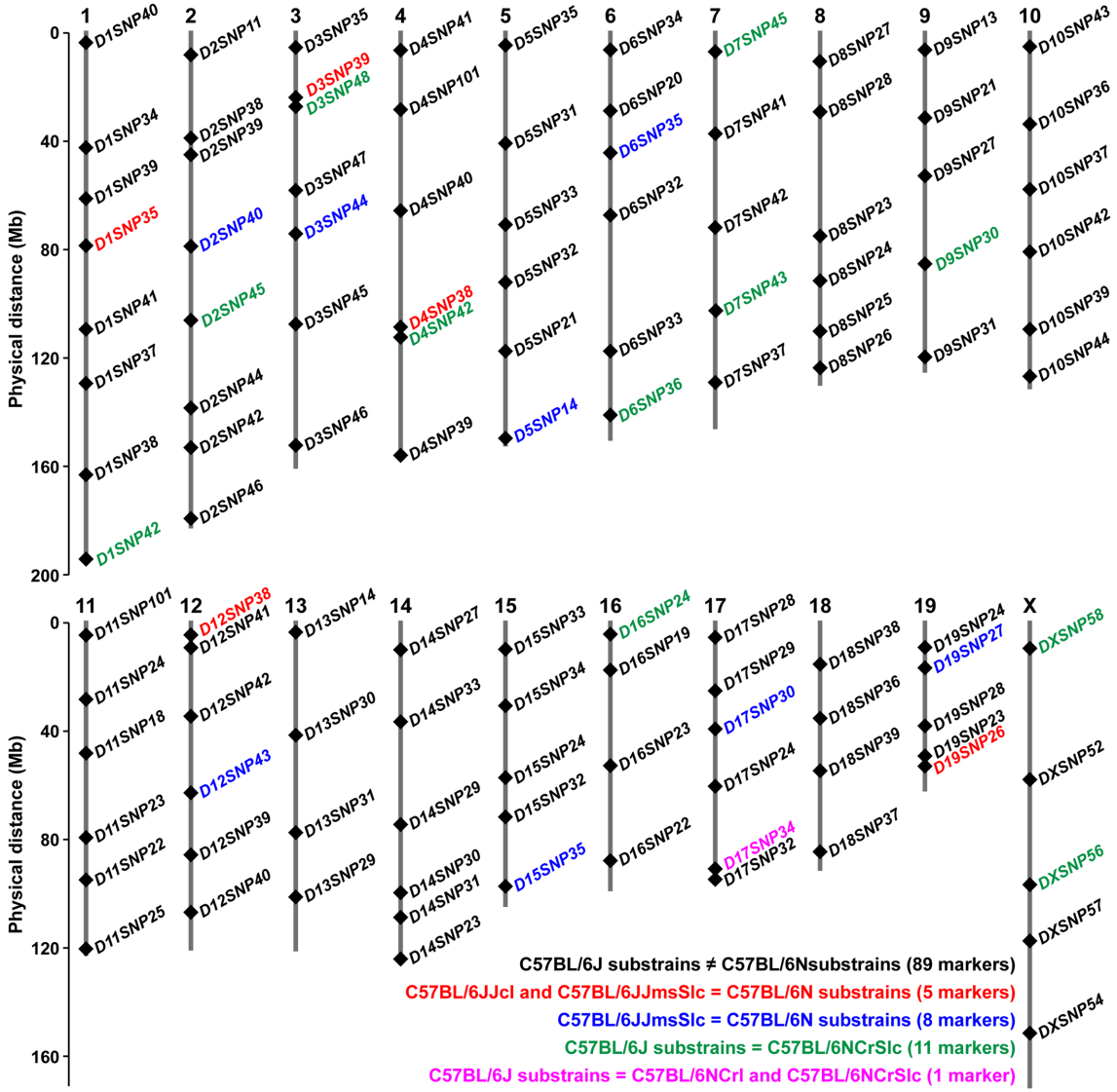

Fig. 1. Chromosomal map of the 114 SNP markers for genotyping of genetic crosses between the C57BL/6J (C57BL/6J, C57BL/6JJcl, and C57BL/6JJmsSlc) and C57BL/6N (C57BL/6NTac, C57BL/6NJcl, C57BL/6NCrl, and C57BL/6NCrSlc) substrains. The vertical gray lines and black diamonds indicate chromosomes and locations of SNP markers, respectively. The SNP markers common between C57BL/6J and C57BL/6N substrains are labeled in black. The intrasubstrain polymorphisms were detected in 25 markers, which are labeled in red, blue, green, and magenta.

types could be assigned to all chromosomes.

The marker sets were designed based on the SNPs between C57BL/6J mice from The Jackson Laboratory and C57BL/6NTac mice from Taconic Biosciences. C57BL/6J is the most commonly used substrain in biomedical research, and its genomic sequence was first published as a reference genome sequence by the Mouse Genome Sequencing Consortium (MGSC) [27, 28]. C57BL/6NTac was selected by the Knockout Mouse Project and the International Mouse Phenotyping Consortium as a background strain to produce genetically engineered mutants [29–31]. However, previous studies have reported that there are several SNPs at the intrasubstrain level in both C57BL/6J and C57BL/6N mice from each breeder [22, 32]. Therefore, we investigated SNPs in C57BL/6J and C57BL/6N mice purchased from three major breeders (Table 1) in Japan using TaqMan assays. SNPs were detected in 25 markers, and SNPs in 13 markers were detected within the C57BL/6J substrains. The genotypes of 13 markers in C57BL/6JJmsSlc mice and 5 markers in C57BL/6JJcl mice were identical to those of all C57BL/6N mice (Fig. 1 and Table 2). In C57BL/6N mice, SNPs were detected in 12 markers and were identical to those of all C57BL/6J mice (Fig. 1 and Table 2). Twelve SNPs were detected in C57BL/6NCrSlc mice, and only one SNP was detected in C57BL/6NCrl mice. There were no SNPs in C57BL/6NTac and C57BL/6NJcl mice.

Table 2. SNPs among the C57BL/6 substrains within the markers developed in this study

SNP marker	Chr	dbSNP_ID	C57BL/6J				C57BL/6N			
			Ref	J	JJcl	JJmsSlc	Ntac	NJcl	NCrl	NCrSlc
D1SNP40	1	rs32685032	A	A	A	A	T	T	T	T
D1SNP34	1	rs31362610	T	T	T	T	C	C	C	C
D1SNP39	1	rs13475886	T	T	T	T	C	C	C	C
D1SNP35	1	rs32481241	T	T	C	C	C	C	C	C
D1SNP41	1	rs30928757	T	T	T	T	C	C	C	C
D1SNP37	1	rs6327099	T	T	T	T	C	C	C	C
D1SNP38	1	rs6341208	A	A	A	A	T	T	T	T
D1SNP42	1	rs242712390	C	C	C	C	A	A	A	C
D2SNP11	2	rs13476337	A	A	A	A	T	T	T	T
D2SNP38	2	rs33064547	A	A	A	A	G	G	G	G
D2SNP39	2	rs33488914	A	A	A	A	G	G	G	G
D2SNP40	2	rs33162749	C	C	C	T	T	T	T	T
D2SNP45	2	rs227312316	G	G	G	G	A	A	A	G
D2SNP44	2	rs13476801	T	T	T	T	C	C	C	C
D2SNP42	2	rs29818510	C	C	C	C	G	G	G	G
D2SNP46	2	rs29673978	T	T	T	T	C	C	C	C
D3SNP35	3	rs13476956	C	C	C	C	T	T	T	T
D3SNP39	3	rs13477019	T	T	A	A	A	A	A	A
D3SNP48	3	rs237712466	T	T	T	T	G	G	G	T
D3SNP47	3	rs13477132	G	G	G	G	C	C	C	C
D3SNP44	3	rs31154737	A	A	A	T	T	T	T	T
D3SNP45	3	rs31321678	A	A	A	A	G	G	G	G
D3SNP46	3	rs31594267	A	A	A	A	C	C	C	C
D4SNP41	4	rs32143059	G	G	G	G	A	A	A	A
D4SNP101	4	rs13477622	T	T	T	T	C	C	C	C
D4SNP40	4	rs13477746	T	T	T	T	C	C	C	C
D4SNP38	4	rs3680956	A	A	G	G	G	G	G	G
D4SNP42	4	rs245725397	C	C	C	C	T	T	T	C
D4SNP39	4	rs6397070	T	T	T	T	C	C	C	C
D5SNP35	5	rs247844351	C	C	C	C	T	T	T	T
D5SNP31	5	rs33508711	C	C	C	C	T	T	T	T
D5SNP33	5	rs13478320	C	C	C	C	A	A	A	A
D5SNP32	5	rs33249065	A	A	A	A	G	G	G	G
D5SNP21	5	rs3662161	A	A	A	A	G	G	G	G
D5SNP14	5	rs33208334	T	T	T	C	C	C	C	C
D6SNP34	6	rs30450019	A	A	A	A	G	G	G	G
D6SNP20	6	rs30764547	T	T	T	T	A	A	A	A
D6SNP35	6	rs30892442	A	A	A	C	C	C	C	C
D6SNP32	6	rs6157367	T	T	T	T	A	A	A	A
D6SNP33	6	rs13478995	C	C	C	C	G	G	G	G
D6SNP36	6	rs235068709	G	G	G	G	T	T	T	G
D7SNP45	7	rs242748489	G	G	G	G	A	A	A	G
D7SNP41	7	rs31221380	A	A	A	A	C	C	C	C
D7SNP42	7	rs32060039	C	C	C	C	G	G	G	G
D7SNP43	7	rs243575509	C	C	C	C	T	T	T	C
D7SNP37	7	rs13479522	A	A	A	A	G	G	G	G
D8SNP27	8	rs13479605	C	C	C	C	A	A	A	A
D8SNP28	8	rs13479672	T	T	T	T	C	C	C	C
D8SNP23	8	rs32729089	T	T	T	T	A	A	A	A
D8SNP24	8	rs33601490	T	T	T	T	C	C	C	C
D8SNP25	8	rs33219858	T	T	T	T	C	C	C	C
D8SNP26	8	rs32577205	A	A	A	A	G	G	G	G
D9SNP13	9	rs33672596	A	A	A	A	G	G	G	G
D9SNP21	9	rs13480122	T	T	T	T	C	C	C	C
D9SNP27	9	rs29644859	T	T	T	T	G	G	G	G
D9SNP30	9	rs260373537	T	T	T	T	C	C	C	T
D9SNP31	9	rs30431245	T	T	T	T	C	C	C	C
D10SNP43	10	rs50477269	A	A	A	A	G	G	G	G
D10SNP36	10	rs13480575	T	T	T	T	C	C	C	C
D10SNP37	10	rs13480619	T	T	T	T	C	C	C	C
D10SNP42	10	rs13459122	A	A	A	A	T	T	T	T
D10SNP39	10	rs13480759	C	C	C	C	T	T	T	T
D10SNP44	10	rs236992510	G	G	G	G	A	A	A	A

Table 2. (continued)

SNP marker	Chr	dbSNP_ID	C57BL/6J				C57BL/6N			
			Ref	J	JJcl	JJmsSlc	Ntac	NJcl	NCrl	NCrSlc
D11SNP101	11	rs3659787	G	G	G	G	A	A	A	A
D11SNP24	11	rs259140591	C	C	C	C	T	T	T	T
D11SNP18	11	rs13481014	T	T	T	T	C	C	C	C
D11SNP23	11	rs13481117	G	G	G	G	T	T	T	T
D11SNP22	11	rs29411641	T	T	T	T	G	G	G	G
D11SNP25	11	rs49027247	T	T	T	T	C	C	C	C
D12SNP38	12	rs29158719	A	A	G	G	G	G	G	G
D12SNP41	12	rs29142759	A	A	A	A	G	G	G	G
D12SNP42	12	rs29487143	G	G	G	G	C	C	C	C
D12SNP43	12	rs29133146	A	A	A	C	C	C	C	C
D12SNP39	12	rs13481569	G	G	G	G	A	A	A	A
D12SNP40	12	rs13481634	A	A	A	A	C	C	C	C
D13SNP14	13	rs30039194	A	A	A	A	T	T	T	T
D13SNP30	13	rs3722313	T	T	T	T	C	C	C	C
D13SNP31	13	rs29802434	G	G	G	G	C	C	C	C
D13SNP29	13	rs3702296	A	A	A	A	G	G	G	G
D14SNP27	14	rs31187642	G	G	G	G	A	A	A	A
D14SNP33	14	rs31133670	A	A	A	A	G	G	G	G
D14SNP29	14	rs30264676	T	T	T	T	A	A	A	A
D14SNP30	14	rs31059846	A	A	A	A	G	G	G	G
D14SNP31	14	rs31273189	A	A	A	A	G	G	G	G
D14SNP23	14	rs31233932	C	C	C	C	T	T	T	T
D15SNP33	15	rs257670740	T	T	T	T	C	C	C	C
D15SNP34	15	rs261563123	C	C	C	C	T	T	T	T
D15SNP24	15	rs3702158	A	A	A	A	G	G	G	G
D15SNP32	15	rs31858887	T	T	T	T	C	C	C	C
D15SNP35	15	rs31921278	A	A	A	G	G	G	G	G
D16SNP24	16	rs219190959	C	C	C	C	T	T	T	C
D16SNP19	16	rs4165065	T	T	T	T	C	C	C	C
D16SNP23	16	rs4187179	T	T	T	T	C	C	C	C
D16SNP22	16	rs4214728	T	T	T	T	C	C	C	C
D17SNP28	17	rs4137196	T	T	T	T	C	C	C	C
D17SNP29	17	rs29512740	C	C	C	C	A	A	A	A
D17SNP30	17	rs33334258	G	G	G	A	A	A	A	A
D17SNP24	17	rs13483055	T	T	T	T	C	C	C	C
D17SNP34	17	rs229426697	C	C	C	C	T	T	C	C
D17SNP32	17	rs33132419	T	T	T	T	C	C	C	C
D18SNP38	18	rs13483221	C	C	C	C	T	T	T	T
D18SNP36	18	rs13483296	A	A	A	A	T	T	T	T
D18SNP39	18	rs13483369	A	A	A	A	C	C	C	C
D18SNP37	18	rs29690544	T	T	T	T	C	C	C	C
D19SNP24	19	rs31112038	G	G	G	G	C	C	C	C
D19SNP27	19	rs30709918	T	T	T	C	C	C	C	C
D19SNP28	19	rs30953636	G	G	G	G	T	T	T	T
D19SNP23	19	rs3724876	G	G	G	G	T	T	T	T
D19SNP26	19	rs30447436	C	C	T	T	T	T	T	T
DXSNP58	X	rs250603596	T	T	T	T	A	A	A	T
DXSNP52	X	rs6368704	A	A	A	A	G	G	G	G
DXSNP56	X	rs259126064	G	G	G	G	T	T	T	G
DXSNP57	X	rs213430510	A	A	A	A	T	T	T	T
DXSNP54	X	rs31266096	A	A	A	A	G	G	G	G

SNPs with the reference genome sequence (genome assembly: GRCm38.p6) of C57BL/6J (Ref) are highlighted in gray. J, C57BL/6J; JJcl, C57BL/6JJcl; JJmsSlc, C57BL/6JJmsSlc; NTac, C57BL/6NTac; NJcl, C57BL/6NJcl; NCrl, C57BL/6NCrl; and NCrSlc, C57BL/6NCrSlc.

Identification of SNPs for genetic mapping of the genetic crosses between BALB/cA and BALB/cByJ substrains

Next, we performed NGS whole-genome sequencing to screen SNPs used for genetic mapping of the genetic crosses between the BALB/cA and BALB/cByJ substrains. In total, 912,105,404 and 636,697,536 reads of BALB/cA and BALB/cByJ mice, respectively, were mapped to the reference genome sequence. A large number of SNPs were detected in BALB/cA and BALB/cByJ mice via this analysis. However, the quality of the sequence data was low because the read coverage was poor

in many genomic regions, including these SNPs. Therefore, candidate SNPs for genotyping between BALB/cA and BALB/cByJ mice were filtered according to several criteria that included read coverage, allele frequency, and genotype information (Supplementary Table 3). In addition, we selected SNPs that were assigned dbSNP IDs from the filtered SNPs to increase the reliability of SNPs because a large amount of SNP data generated using oligonucleotide arrays in BALB/cByJ mice have previously been reported [33]. Finally, 632 candidate SNPs were screened between BALB/cA and BALB/cByJ mice (Supplementary Table 3). To construct the marker sets for genetic mapping using F_2 and N_2 progeny between the BALB/c substrains, we used Sanger sequencing to validate SNPs selected based on the genomic locations. One hundred six SNPs were detected on all chromosomes in BALB/cA and BALB/cByJ mice (Supplementary Table 4). Figure 2 shows the locations of the 106 SNPs. Most of the SNPs were assigned locations on all chromosomes, as planned. The average interval between the SNPs was 21.18 Mb when either SNP was chosen from the neighboring markers (*D10SNP302*/*D10SNP303*, *D16SNP304*/*D16SNP305*, *D18SNP301*/*D18SNP302*, *D19SNP301*/*D19SNP302*, *D19SNP302*/*D19SNP303*, and *DXSNP303*/*DXSNP304*) located within 10 Mb. However, we could not detect the SNPs in the long-range genomic region (~39.12 Mb) of the centromere on chromosome 1 because of difficulty of designing primer sets for Sanger sequencing to validate them. This was attributed to the presence of repetitive sequenc-

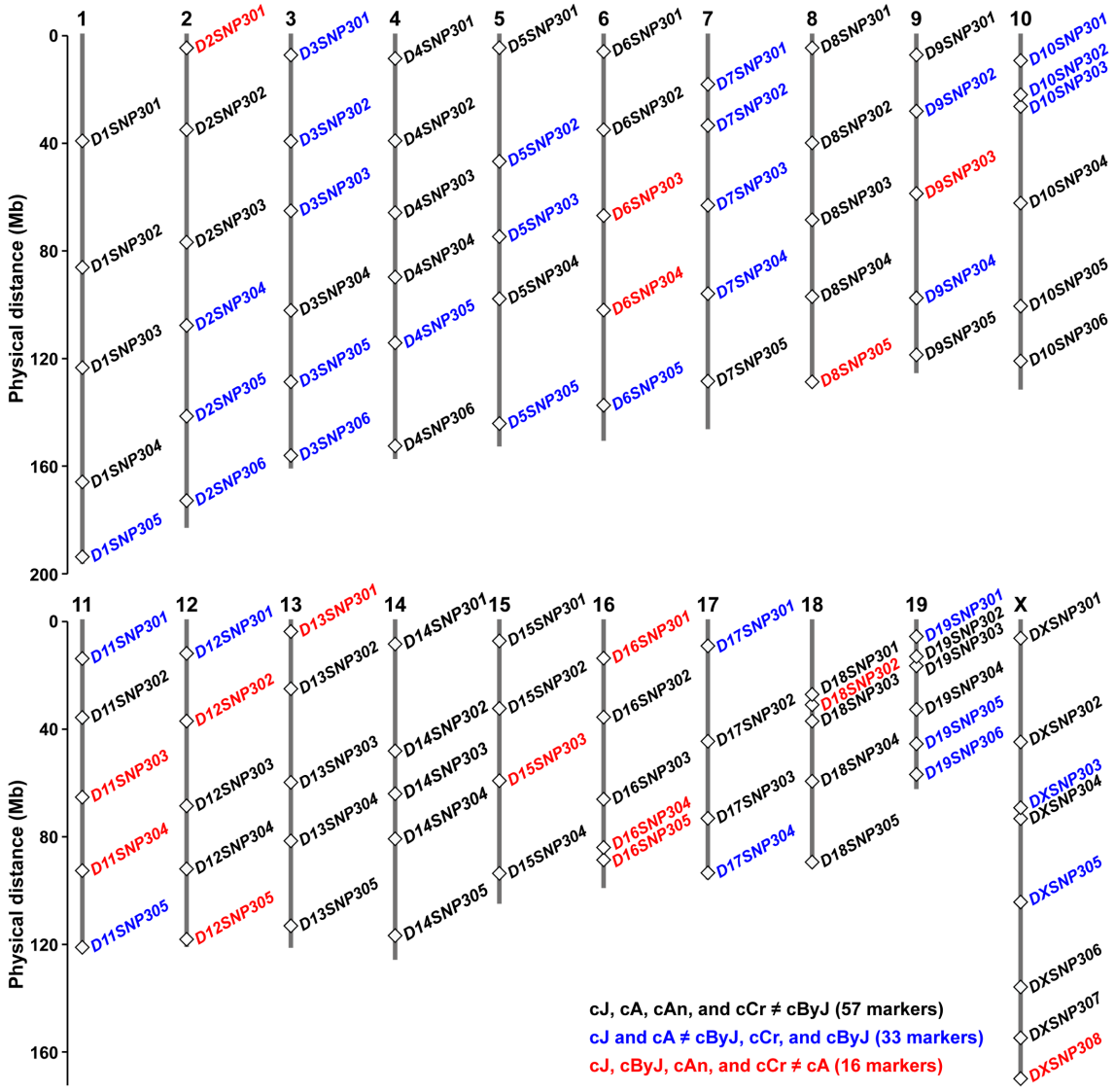

Fig. 2. Chromosomal map of the 106 SNP markers for genotyping of genetic crosses between the BALB/c (BALB/cJ, BALB/cA, BALB/cByJ, BALB/cAn and BALB/cCr) substrains. The vertical gray lines and white diamonds indicate chromosomes and locations of SNP markers, respectively. The genotypes of the SNP markers are shown in different colors (black, red, and blue) for each marker name.

es and long stretches of a single nucleotide in the flanking sequence of all candidate SNPs. We also could not detect the SNPs on the proximal region (1–28.12 Mb) of chromosome 18 because there were no candidate SNPs in this region (Supplementary Table 3).

Regarding BALB/c substrains, the whole-genome sequence of BALB/cJ mice has been published by the Wellcome Trust Sanger Institute [23]. In addition, 106 SNPs were genotyped in BALB/cAnNCrlCrlj (BALB/cAn) and BALB/cCrSIc (BALB/cCr) mice. Table 3 shows comparisons of the genotypes of the 106 SNPs among the five substrains. The genotypes of BALB/cJ and BALB/cA mice were identical at 90 SNPs. In contrast, the same genotypes were detected in only 16 positions in BALB/cJ and BALB/cByJ mice. The genotypes of the 106 SNPs matched exactly in BALB/cAn and BALB/cCr mice. The genotypes at 73, 57, and 49 SNPs of BALB/cAn and BALB/cCr were identical to those of BALB/cJ, BALB/cA, and BALB/cByJ, respectively.

Discussion

This study provides information on available SNP marker sets for genotyping of C57BL/6 and BALB/c substrains. As mentioned in the Introduction, a useful application of these marker sets is linkage and QTL analyses of the phenotypes produced by ENU mutagenesis. Large-scale ENU mutagenesis studies in mice have been powerful discovery platforms for identifying novel genes or gene functions associated with disease phenotypes [1, 5]. We also identified several genes and gene functions associated with several disease phenotypes, such as hair formation [34, 35], amelogenesis [36], development of single positive thymocytes [37], and abnormal behavior due to neurological disorders [38–40]. However, we could not identify the loci associated with several phenotypes by linkage and QTL analyses. In particular, the genetic mapping of several mild morphological and behavioral phenotypes, which were detected using a modified SHIRPA (SmithKline, Harwell, Imperial College, Royal Hospital, Phenotype Assessment) protocol, failed [20]. We have previously used interstrain genetic crosses between ENU-treated C57BL/6J and DBA/2J (or C3H/HeJ) mice for linkage and QTL analyses. We predicted that these mild phenotypes might be overlooked by the genetic background effects of DBA/2J and C3H/HeJ mice. In contrast, several studies have identified causative mutations associated with disease phenotypes using intersubstrain genetic crosses. Hossain *et al.* (2016) identified obesity-related genes based on genetic mapping of the N_2 progeny between ENU-treated C57BL/6J and C57BL/6N mice [14]. Funato *et al.* (2016) and Miyoshi *et al.* (2019) identified sleep-related genes by a forward genetics approach using the same intersubstrain cross [13, 15]. As it was assumed that target phenotypes in these studies are susceptible to genetic background effects, we predict that substrain crosses will become a key strategy for the successful identification of these causative genes.

In addition, we expect that the marker sets developed in the present study will be useful tools for identifying loci and mutated genes associated with phenotypic differences between substrains of C57BL/6 and BALB/c mice. Kirkpatrick *et al.* (2017) identified a robust difference in binge eating between C57BL/6 substrains and a major genetic factor associated with the phenotype by QTL mapping using C57BL/6J × C57BL/6N-F_2 mice [41]. Kumar *et al.* (2013) confirmed that substrain differences in the locomotor response to cocaine in C57BL/6J and C57BL/6N mice were caused by an SNP [16]. Previous studies have reported several phenotypic differences among substrains of C57BL/6 and BALB/c mice [9, 11, 42, 43]. Although almost all causative genes and mutations are still unknown, they may be identified by linkage and QTL analyses using intersubstrain crosses and SNP markers in both substrains.

The phenotypic differences are estimated to be associated with approximately 10,000 SNPs between the C57BL/6J and C57BL/6N substrains [11, 23, 42]. In addition, Mekada *et al.* (2015) reported genetic differences among substrains of C57BL/6J and C57BL/6N mice produced by breeders [44]. We also detected several SNPs in both the C57BL/6J and C57BL/6N substrains from different breeders. Although we investigated only Japanese breeders, the genetic differences in mice from different breeders would probably be detected in mice from different breeders in other countries. Therefore, it is essential to pay attention to the genetic background of the mutant and control mice for the production of genetic crosses to perform linkage and QTL analyses using SNP markers between the C57BL/6J and C57BL/6N substrains.

In this study, we investigated and confirmed the SNPs between the BALB/cByJ and BALB/cA substrains purchased from CLEA Japan for linkage and QTL analyses, since this approach has been successful in discovering several phenotypic differences between these substrains. However, most reports have described phenotypic differences between BALB/cByJ and BALB/cJ substrains [45–48]. Although 90 markers within our marker sets are available for the genotyping of these substrains, SNP markers were absent in several long-range genomic regions (e.g., *D6SNP302–305*, *D9SNP302–304*,

Table 3. SNPs among the BALB/c substrains within the markers developed in this study

Marker	Chr	dbSNP_ID	cJ	cA	cByJ	cAn	cCr
D1SNP301	1	rs33109340	C	C	T	C	C
D1SNP302	1	rs33057227	C	C	T	C	C
D1SNP303	1	rs33365833	A	A	T	A	A
D1SNP304	1	rs31397868	G	G	A	G	G
D1SNP305	1	rs33202907	T	T	A	A	A
D2SNP301	2	rs243288053	G	A	G	G	G
D2SNP302	2	rs27169047	G	G	T	G	G
D2SNP303	2	rs27981830	G	G	A	G	G
D2SNP304	2	rs238060283	A	A	G	G	G
D2SNP305	2	rs27250976	C	C	T	T	T
D2SNP306	2	rs27622613	T	T	C	C	C
D3SNP301	3	rs46220062	C	C	T	T	T
D3SNP302	3	rs29938481	G	G	T	T	T
D3SNP303	3	rs36544985	A	A	G	G	G
D3SNP304	3	rs36506426	G	G	A	G	G
D3SNP305	3	rs221745548	G	G	A	A	A
D3SNP306	3	rs30857183	A	A	G	G	G
D4SNP301	4	rs231525592	C	C	T	C	C
D4SNP302	4	rs27767340	C	C	T	C	C
D4SNP303	4	rs3706082	T	T	C	T	T
D4SNP304	4	rs28126314	G	G	A	G	G
D4SNP305	4	rs27512361	C	C	T	T	T
D4SNP306	4	rs32877825	G	G	A	G	G
D5SNP301	5	rs31133755	C	C	A	C	C
D5SNP302	5	rs50896597	C	C	T	T	T
D5SNP303	5	rs52019376	G	G	A	A	A
D5SNP304	5	rs31892920	T	T	C	T	T
D5SNP305	5	rs32347776	G	G	A	A	A
D6SNP301	6	rs32502983	C	C	T	C	C
D6SNP302	6	rs36885644	G	G	T	G	G
D6SNP303	6	rs216689099	C	T	C	C	C
D6SNP304	6	rs230573527	C	T	C	C	C
D6SNP305	6	rs31952322	G	G	A	A	A
D7SNP301	7	rs32408583	A	A	C	C	C
D7SNP302	7	rs32445754	G	G	A	A	A
D7SNP303	7	rs33056660	A	A	C	C	C
D7SNP304	7	rs31482312	T	T	G	G	G
D7SNP305	7	rs51219759	A	A	T	A	A
D8SNP301	8	rs47376668	G	G	A	G	G
D8SNP302	8	rs30733023	C	C	T	C	C
D8SNP303	8	rs36881999	G	G	A	G	G
D8SNP304	8	rs38305200	G	G	A	G	G
D8SNP305	8	rs262469470	G	A	G	G	G
D9SNP301	9	rs51304512	T	T	C	T	T
D9SNP302	9	rs37879512	G	G	A	A	A
D9SNP303	9	rs38922776	A	G	A	A	A
D9SNP304	9	rs33134771	G	G	A	A	A
D9SNP305	9	rs50594159	C	C	T	C	C
D10SNP301	10	rs45892832	C	C	T	T	T
D10SNP302	10	rs33688332	C	C	A	A	A
D10SNP303	10	rs50315385	C	C	A	A	A
D10SNP304	10	rs46748961	C	C	T	C	C
D10SNP305	10	rs47483530	T	T	A	T	T
D10SNP306	10	rs233492562	A	A	G	A	A
D11SNP301	11	rs26833083	G	G	A	A	A
D11SNP302	11	rs26912518	T	T	C	T	T
D11SNP303	11	rs220626677	G	A	G	G	G
D11SNP304	11	rs252587388	C	T	C	C	C
D11SNP305	11	rs27014504	C	C	T	T	T
D12SNP301	12	rs46528175	A	A	G	G	G
D12SNP302	12	rs231844185	A	G	A	A	A
D12SNP303	12	rs37803925	A	A	C	A	A
D12SNP304	12	rs51364971	C	C	T	C	C
D12SNP305	12	rs247716364	G	A	G	G	G

Table 3. (continued)

Marker	Chr	dbSNP_ID	cJ	cA	cByJ	cAn	cCr
D13SNP301	13	rs33751539	C	A	C	C	C
D13SNP302	13	rs29965851	T	T	A	T	T
D13SNP303	13	rs30245925	C	C	T	C	C
D13SNP304	13	rs47717539	C	C	T	C	C
D13SNP305	13	rs38228777	G	G	C	G	G
D14SNP301	14	rs51648802	G	G	A	G	G
D14SNP302	14	rs50148147	T	T	C	T	T
D14SNP303	14	rs46892447	C	C	T	C	C
D14SNP304	14	rs32226962	A	A	G	A	A
D14SNP305	14	rs51211980	C	C	A	C	C
D15SNP301	15	rs48122948	G	G	C	G	G
D15SNP302	15	rs212086590	C	C	T	C	C
D15SNP303	15	rs220101808	C	T	C	C	C
D15SNP304	15	rs38263969	G	G	A	G	G
D16SNP301	16	rs49608214	A	G	A	A	A
D16SNP302	16	rs46364658	C	C	T	C	C
D16SNP303	16	rs50933320	C	C	T	C	C
D16SNP304	16	rs254664546	G	A	G	G	G
D16SNP305	16	rs227119859	A	T	A	A	A
D17SNP301	17	rs49061265	G	G	A	A	A
D17SNP302	17	rs45641253	C	C	A	C	C
D17SNP303	17	rs49487329	G	G	A	G	G
D17SNP304	17	rs47460807	G	G	A	A	A
D18SNP301	18	rs51512767	A	A	T	A	A
D18SNP302	18	rs221424480	C	T	C	C	C
D18SNP303	18	rs31785839	T	T	C	T	T
D18SNP304	18	rs39281136	C	C	T	C	C
D18SNP305	18	rs37625494	T	T	G	T	T
D19SNP301	19	rs36448887	G	G	A	A	A
D19SNP302	19	rs37039649	G	G	A	G	G
D19SNP303	19	rs38082986	G	G	A	G	G
D19SNP304	19	rs50157060	T	T	C	T	T
D19SNP305	19	rs36495646	A	A	G	G	G
D19SNP306	19	rs48624001	C	C	T	T	T
DXSNP301	X	rs33287577	T	T	A	T	T
DXSNP302	X	rs30035452	A	A	C	A	A
DXSNP303	X	rs29043150	T	T	C	C	C
DXSNP304	X	rs241499720	G	G	T	G	G
DXSNP305	X	rs8268104	C	C	A	A	A
DXSNP306	X	rs3718396	C	C	T	C	C
DXSNP307	X	rs29299426	G	G	A	G	G
DXSNP308	X	rs31827558	T	C	T	T	T

Locations of SNPs are according to the mouse genome reference assembly (GRCm38.p6). SNPs with the genomic sequence (Mouse Genome Project: https://www.sanger.ac.uk/sanger/Mouse_SnpViewer/rel-1505) of BALB/cJ (cJ) are highlighted in gray. cA, BALB/cAJcl; cByJ, BALB/cByJJcl; cAn, BALB/cAnNCrlCrlj; and cCr, BALB/cCrSlc.

D11SNP302–305, and proximal and distal regions on chromosome 16; Fig. 2 and Table 3). Additional markers will need to be developed at several positions to perform detailed linkage and QTL analyses using genetic crosses between BALB/cByJ and BALB/cJ substrains. In addition, we screened the genotypes of SNPs in BALB/cAn and BALB/cCr mice purchased from the Charles River Laboratories Japan and Japan SLC, respectively; however, the genotypes of these strains were the same for all SNPs. Our SNP markers might be used for linkage and QTL analyses to cross these mice (BALB/cAn and BALB/cCr) and BALB/cByJ mice. However, whole-genome genotyping is difficult for crosses between BALB/cA, BALB/cJ, BALB/cAn, and BALB/cCr mice using only our SNP markers because of the lack of polymorphisms in many chromosomal regions. Whole-genome sequencing of BALB/cAn and BALB/cCr mice can help screen the SNPs and compare them with those in other BALB/c substrains.

In the present study, we developed a genotyping system on 384-well plates using TaqMan assays. The approach can promptly distinguish the homozygous and heterozygous genotypes in progeny from the genetic crosses between C57BL/6 substrains, as shown in Supplementary Fig. 1. This genotyping system can provide researchers with the ability to identify the responsible

and modifier genes via linkage and QTL analyses using genetic crosses between the C57BL/6 substrains. We are currently developing a similar high-throughput genotyping system for BALB/c substrains using the SNPs identified in the present study.

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References

1. Gondo Y, Makino S, Fukumura R. Chapter 28 − Forward and Reverse Genetics to Model Human Diseases in the Mouse. In: Conn PM, editor. Animal Models for the Study of Human Disease (Second Edition). Cambridge: Academic Press; 2017. pp. 727–752.
2. Takahashi JS, Pinto LH, Vitaterna MH. Forward and reverse genetic approaches to behavior in the mouse. Science. 1994; 264: 1724–1733. [Medline] [CrossRef]
3. Gurumurthy CB, Grati M, Ohtsuka M, Schilit SL, Quadros RM, Liu XZ. CRISPR: a versatile tool for both forward and reverse genetics research. Hum Genet. 2016; 135: 971–976. [Medline] [CrossRef]
4. Paigen K. One hundred years of mouse genetics: an intellectual history. II. The molecular revolution (1981-2002). Genetics. 2003; 163: 1227–1235. [Medline] [CrossRef]
5. Acevedo-Arozena A, Wells S, Potter P, Kelly M, Cox RD, Brown SDM. ENU mutagenesis, a way forward to understand gene function. Annu Rev Genomics Hum Genet. 2008; 9: 49–69. [Medline] [CrossRef]
6. Potter PK, Bowl MR, Jeyarajan P, Wisby L, Blease A, Goldsworthy ME, et al. Novel gene function revealed by mouse mutagenesis screens for models of age-related disease. Nat Commun. 2016; 7: 12444. [Medline] [CrossRef]
7. Bull KR, Rimmer AJ, Siggs OM, Miosge LA, Roots CM, Enders A, et al. Unlocking the bottleneck in forward genetics using whole-genome sequencing and identity by descent to isolate causative mutations. PLoS Genet. 2013; 9: e1003219. [Medline] [CrossRef]
8. Yasuda SP, Miyasaka Y, Kikkawa Y. Effects of genetic background on susceptibility and the acceleration of hearing loss in mice. In: Hatzopoulos S, Ciorba A, editors. An excursus into hearing loss. Rijeka: IntechOpen; 2018.
9. [No authors listed]. The BALB/c mouse. Genetics and immunology. Curr Top Microbiol Immunol. 1985; 122: 1–253. [Medline]
10. Glant TT, Bárdos T, Vermes C, Chandrasekaran R, Valdéz JC, Otto JM, et al. Variations in susceptibility to proteoglycan-induced arthritis and spondylitis among C3H substrains of mice: evidence of genetically acquired resistance to autoimmune disease. Arthritis Rheum. 2001; 44: 682–692. [Medline] [CrossRef]
11. Mekada K, Yoshiki A. Substrains matter in phenotyping of C57BL/6 mice. Exp Anim. 2021; 70: 145–160. [Medline] [CrossRef]
12. Simpson EM, Linder CC, Sargent EE, Davisson MT, Mobraaten LE, Sharp JJ. Genetic variation among 129 substrains and its importance for targeted mutagenesis in mice. Nat Genet. 1997; 16: 19–27. [Medline] [CrossRef]
13. Miyoshi C, Kim SJ, Ezaki T, Ikkyu A, Hotta-Hirashima N, Kanno S, et al. Methodology and theoretical basis of forward genetic screening for sleep/wakefulness in mice. Proc Natl Acad Sci USA. 2019; 116: 16062–16067. [Medline] [CrossRef]
14. Hossain MS, Asano F, Fujiyama T, Miyoshi C, Sato M, Ikkyu A, et al. Identification of mutations through dominant screening for obesity using C57BL/6 substrains. Sci Rep. 2016; 6: 32453. [Medline] [CrossRef]
15. Funato H, Miyoshi C, Fujiyama T, Kanda T, Sato M, Wang Z, et al. Forward-genetics analysis of sleep in randomly mutagenized mice. Nature. 2016; 539: 378–383. [Medline] [CrossRef]
16. Kumar V, Kim K, Joseph C, Kourrich S, Yoo SH, Huang HC, et al. C57BL/6N mutation in cytoplasmic FMRP interacting protein 2 regulates cocaine response. Science. 2013; 342: 1508–1512. [Medline] [CrossRef]
17. Ohnishi T, Miura I, Ohba H, Shimamoto C, Iwayama Y, Wakana S, et al. A spontaneous and novel *Pax3* mutant mouse that models Waardenburg syndrome and neural tube defects. Gene. 2017; 607: 16–22. [Medline] [CrossRef]
18. Nolan PM, Peters J, Strivens M, Rogers D, Hagan J, Spurr N, et al. A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. Nat Genet. 2000; 25: 440–443. [Medline] [CrossRef]
19. Clark AT, Goldowitz D, Takahashi JS, Vitaterna MH, Siepka SM, Peters LL, et al. Implementing large-scale ENU mutagenesis screens in North America. Genetica. 2004; 122: 51–64. [Medline] [CrossRef]
20. Masuya H, Inoue M, Wada Y, Shimizu A, Nagano J, Kawai A, et al. Implementation of the modified-SHIRPA protocol for screening of dominant phenotypes in a large-scale ENU mutagenesis program. Mamm Genome. 2005; 16: 829–837. [Medline] [CrossRef]
21. Cho KH, Cho JW, Song CW. Studies on *N*-ethyl-*N*-nitrosourea mutagenesis in BALB/c mice. Toxicol Res. 2008; 24: 59–68. [Medline] [CrossRef]
22. Mekada K, Abe K, Murakami A, Nakamura S, Nakata H, Moriwaki K, et al. Genetic differences among C57BL/6 substrains. Exp Anim. 2009; 58: 141–149. [Medline] [CrossRef]
23. Keane TM, Goodstadt L, Danecek P, White MA, Wong K, Yalcin B, et al. Mouse genomic variation and its effect on phenotypes and gene regulation. Nature. 2011; 477: 289–294. [Medline] [CrossRef]
24. Yang H, Ding Y, Hutchins LN, Szatkiewicz J, Bell TA, Paigen BJ, et al. A customized and versatile high-density genotyping array for the mouse. Nat Methods. 2009; 6: 663–666. [Medline] [CrossRef]
25. Li H. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data. Bioinformatics. 2011; 27: 2987–2993. [Medline] [CrossRef]
26. Cingolani P, Platts A, Wang LL, Coon M, Nguyen T, Wang L, et al. A program for annotating and predicting the effects of single nucleotide polymorphisms, SnpEff: SNPs in the genome of Drosophila melanogaster strain w1118; iso-2; iso-3. Fly (Austin). 2012; 6: 80–92. [Medline] [CrossRef]
27. Sarsani VK, Raghupathy N, Fiddes IT, Armstrong J, Thibaud-Nissen F, Zinder O, et al. The genome of C57BL/6J "Eve", the mother of the laboratory mouse genome reference strain. G3 (Bethesda). 2019; 9: 1795–1805. [Medline] [CrossRef]
28. Waterston RH, Lindblad-Toh K, Birney E, Rogers J, Abril JF, Agarwal P, et al. Initial sequencing and comparative analysis of the mouse genome. Nature. 2002; 420: 520–562. [Medline] [CrossRef]
29. Skarnes WC, Rosen B, West AP, Koutsourakis M, Bushell W,

Iyer V, et al. A conditional knockout resource for the genome-wide study of mouse gene function. Nature. 2011; 474: 337–342. [Medline] [CrossRef]
30. Dickinson ME, Flenniken AM, Ji X, Teboul L, Wong MD, White JK, et al. High-throughput discovery of novel developmental phenotypes. Nature. 2016; 537: 508–514. [Medline] [CrossRef]
31. Fontaine DA, Davis DB. Attention to background strain is essential for metabolic research: C57BL/6 and the international knockout mouse consortium. Diabetes. 2016; 65: 25–33. [Medline] [CrossRef]
32. Pettitt SJ, Liang Q, Rairdan XY, Moran JL, Prosser HM, Beier DR, et al. Agouti C57BL/6N embryonic stem cells for mouse genetic resources. Nat Methods. 2009; 6: 493–495. [Medline] [CrossRef]
33. Frazer KA, Eskin E, Kang HM, Bogue MA, Hinds DA, Beilharz EJ, et al. A sequence-based variation map of 8.27 million SNPs in inbred mouse strains. Nature. 2007; 448: 1050–1053. [Medline] [CrossRef]
34. Kikkawa Y, Oyama A, Ishii R, Miura I, Amano T, Ishii Y, et al. A small deletion hotspot in the type II keratin gene *mK6irs1*/*Krt2-6g* on mouse chromosome 15, a candidate for causing the wavy hair of the caracul (*Ca*) mutation. Genetics. 2003; 165: 721–733. [Medline] [CrossRef]
35. Tanaka S, Miura I, Yoshiki A, Kato Y, Yokoyama H, Shinogi A, et al. Mutations in the helix termination motif of mouse type I IRS keratin genes impair the assembly of keratin intermediate filament. Genomics. 2007; 90: 703–711. [Medline] [CrossRef]
36. Masuya H, Shimizu K, Sezutsu H, Sakuraba Y, Nagano J, Shimizu A, et al. Enamelin (*Enam*) is essential for amelogenesis: ENU-induced mouse mutants as models for different clinical subtypes of human amelogenesis imperfecta (AI). Hum Mol Genet. 2005; 14: 575–583. [Medline] [CrossRef]
37. Kakugawa K, Yasuda T, Miura I, Kobayashi A, Fukiage H, Satoh R, et al. A novel gene essential for the development of single positive thymocytes. Mol Cell Biol. 2009; 29: 5128–5135. [Medline] [CrossRef]
38. Clapcote SJ, Lipina TV, Millar JK, Mackie S, Christie S, Ogawa F, et al. Behavioral phenotypes of *Disc1* missense mutations in mice. Neuron. 2007; 54: 387–402. [Medline] [CrossRef]
39. Furuse T, Wada Y, Hattori K, Yamada I, Kushida T, Shibukawa Y, et al. Phenotypic characterization of a new *Grin1* mutant mouse generated by ENU mutagenesis. Eur J Neurosci. 2010; 31: 1281–1291. [Medline] [CrossRef]
40. Furuse T, Yamada I, Kushida T, Masuya H, Miura I, Kaneda H, et al. Behavioral and neuromorphological characterization of a novel *Tuba1* mutant mouse. Behav Brain Res. 2012; 227: 167–174. [Medline] [CrossRef]
41. Kirkpatrick SL, Goldberg LR, Yazdani N, Babbs RK, Wu J, Reed ER, et al. Cytoplasmic FMR1-Interacting Protein 2 Is a Major Genetic Factor Underlying Binge Eating. Biol Psychiatry. 2017; 81: 757–769. [Medline] [CrossRef]
42. Simon MM, Greenaway S, White JK, Fuchs H, Gailus-Durner V, Wells S, et al. A comparative phenotypic and genomic analysis of C57BL/6J and C57BL/6N mouse strains. Genome Biol. 2013; 14: R82. [Medline] [CrossRef]
43. Kopić A, Benamara K, Schuster M, Leidenmühler P, Bauer A, Glantschnig H, et al. Coagulation phenotype of wild-type mice on different genetic backgrounds. Lab Anim. 2019; 53: 43–52. [Medline] [CrossRef]
44. Mekada K, Hirose M, Murakami A, Yoshiki A. Development of SNP markers for C57BL/6N-derived mouse inbred strains. Exp Anim. 2015; 64: 91–100. [Medline] [CrossRef]
45. Sittig LJ, Jeong C, Tixier E, Davis J, Barrios-Camacho CM, Palmer AA. Phenotypic instability between the near isogenic substrains BALB/cJ and BALB/cByJ. Mamm Genome. 2014; 25: 564–572. [Medline] [CrossRef]
46. Dam SA, Jager A, Oomen CA, Buitelaar JK, Arias-Vasquez A, Glennon JC. Inhibitory control in BALB/c mice sub-strains during extinction learning. Eur Neuropsychopharmacol. 2019; 29: 509–518. [Medline] [CrossRef]
47. Jager A, Dam SA, Van Der Mierden S, Oomen CA, Arias-Vasquez A, Buitelaar JK, et al. Modulation of cognitive flexibility by reward and punishment in BALB/cJ and BALB/cByJ mice. Behav Brain Res. 2020; 378: 112294. [Medline] [CrossRef]
48. Gan P, Patterson M, Watanabe H, Wang K, Edmonds RA, Reinholdt LG, et al. Allelic variants between mouse substrains BALB/cJ and BALB/cByJ influence mononuclear cardiomyocyte composition and cardiomyocyte nuclear ploidy. Sci Rep. 2020; 10: 7605. [Medline] [CrossRef]