Studies on the Small Body Size Mouse Developed by Mutagen N-Ethyl-N-nitrosourea

QianKun Zhang1,2, Kyu-Hyuk Cho1, Jae-Woo Cho1, Dal-Sun Cha1, Han-Jin Park1, Seokjoo Yoon1, ShouFa Zhang1 and Chang-Woo Song1

1Department of Research & Development, Korea Institute of Toxicology, Korea Research Institute of Chemical Technology P.O BOX 123, Daejeon 305-343, Korea
2Department of Veterinary Medicine, YanBian University, Longjing, China

Received January 24, 2008; Accepted February 18, 2008

Mutant mouse which show dwarfism has been developed by N-ethyl-N-nitrosourea (ENU) mutagenesis using BALB/c mice. The mutant mouse was inherited as autosomal recessive trait and named Small Body Size (SBS) mouse. The phenotype of SBS mouse was not apparent at birth, but it was possible to distinguish mutant phenotype from normal mice 1 week after birth. In this study, we examined body weight changes and bone mineral density (BMD), and we also carried out genetic linkage analysis to map the causative gene(s) of SBS mouse. Body weight changes were observed from birth to 14 weeks of age in both affected (n = 30) and normal mice (n = 24). BMD was examined in each five SBS and normal mice between 3 and 6 weeks of age, respectively. For the linkage analysis, we produced backcross progeny [(SBS x C57BL/6J) F1 x SBS] N2 mice (n = 142), and seventy-four microsatellite markers were used for primary linkage analysis. Body weight of affected mice was consistently lower than that of the normal mice, and was 43.7% less than that of normal mice at 3 weeks of age (P < 0.001). As compared with normal mice at 3 and 6 weeks of age, BMD of the SBS mice was significantly low. The results showed 15.5% and 14.1% lower in total body BMD, 15.3% and 8.7% lower in forearm BMD, and 29.7% and 20.1% lower in femur BMD, respectively. The causative gene was mapped on chromosome 10. The map order and the distance between markers were D10Mit248 - 2.1 cM - D10Mit51 - 4.2 cM - sbs - 0.7 cM - D10Mit283 - 1.4 cM - D10Mit106 - 11.2 cM - D10Mit170.

Key words: Small body size mice, Linkage analysis, Body weight, Bone mineral density.

INTRODUCTION

With the completion of human genome project, mouse and rat genome sequence, systematic determination and analysis of gene function in mammalian genome are mainstream of the major scientific challenges for the 21st century (Lander et al., 2001; Waterston et al., 2002; Gibbs et al., 2004). Mouse has been considered as a primary model for human disease research due to the similarity of its genome, developmental and biochemical pathways, and physiology to human (Soewarto et al., 2000; Brown and Hardisty, 2003). Mouse mutagenesis is a powerful tool for study of mammalian gene function (Justice et al., 1999). Currently mouse mutations have been generated in many laboratories by chemical mutagenesis (Davis et al., 1999).

N-Ethyl-N-nitrosourea (ENU) is a potent chemical mutagen, which acts through random alkylation of nucleic acids, and it can randomly cause single base pair mutations in a wide variety of organisms (Russel et al., 1979, 1982a, b; Hitotsumachi et al., 1985; Justice and Bode 1986). The risk assessment of carcinogens and teratogens are well studied but relatively mutagens like ENU are not (Purchase, 2001; Kwack and Cho, 2005; Kim et al., 2006). In the mouse testis, the action of ENU is the most potent in spermatogonial stem cells, and it produces the highest mutation rate of any germ-line mutagen tested in the mouse with its optimal dose producing about one mutation per gene in every 175 to 655 gametes (Hitotsumachi et al., 1985; Shelovsky et
al., 1993; Justice et al., 2000; Noverske et al., 2000). However low mutation frequencies of ENU-treated female mice have been reported (Ressell and Russell, 1992). Effective ENU doses and specific mutagenesis protocols have been optimized for various strains of mice to affect single gene mutations in the progeny of males exposed to the chemical. The mutant mice that are being created from ongoing ENU mutagenesis projects are an invaluable resource for the biomedical community. Further it may allow phenotype-driven approaches to isolate mutations in any gene of interest for short time to analyze gene function (Noverske et al., 2000).

Growth is an inherent property of life. Normal somatic growth requires the integrated function of many of the hormonal, metabolic, and other growth factors. The genetic etiology of growth insufficiency is very complicated. Dwarfism can be associated with a variety of complication and genetic heterogeneity, such as achondroplasia (Wang et al., 1999; Argentin and Cicchetti, 2000), osteoporosis (Eason et al., 1995), growth hormone, and many different factors (Lira et al., 1993; Shibayama et al., 1993; Zhou et al., 1995). Mutations of genes involved in the process cause several types of dwarfism in human and mouse. Many mouse models associated with dwarfism have been known, such as Snell dwarf mouse, Ames dwarf mouse, SMA-1 mouse and achondroplastic mouse, etc. Four naturally occurring murine models of congenital and autosomal recessive GH deficiency exist: the Snell (dw), the Ames (df), the Spontaneous Dwarf Rat (SDR), and the little (lt) mouse (Jansson et al., 1986; Godfrey et al., 1993; Lin et al., 1993; Li et al., 1990; Sornson et al., 1996; Takeuchi et al., 1990). The Snell mouse results from a point mutation in the gene encoding for the pituitary transcription factor Pit-1, which is required for the development of all three pituitary cell lines (Li et al., 1990). The Ames mouse results from a mutation of another transcription factor (Prop-1), which acts as activating factor of the Pit-1 gene (Sornson et al., 1996). The SDR rat has a point mutation in the GH gene (Takeuchi et al., 1990). The little mouse has a missense mutation in the extracellular domain of the GHRHR gene, which does not allow proper binding of GHRH (Godfrey et al., 1993; Lin et al., 1993). The SMA-1 mouse is a novel ENU-induced mutant mouse, which is characterized by dwarfism (Meyer et al., 2004). The achondroplastic mouse is a spontaneous mutant with an autosomal recessive gene (cn) which is characterized by disproportionate dwarfism due to disturb chondrogenesis during endochondral ossification (Tsujii and Kunieda, 2005).

In previous studies from our laboratory, we have reported generation of ENU-induced mutant mice that showed dwarfism (Cho et al., 2003). The mutation was generated and identified in a large-scale ENU-mouse-mutagenesis program (Soewarto et al., 2000; Hrabe de Angelis et al., 2000). This program represents a powerful phenotype-driven approach to gene-function analysis and increases existing mouse mutant resources.

In this study, we examine body weight changes for further elucidating phenotypic character, and examine the bone mineral density (BMD) for exploring correlation between BMD and dwarfism phenotype, and map the causative gene for cloning of causative gene in the SBS mouse.

**MATERIALS AND METHODS**

**Animals & environmental conditions.** For breeding, one male mouse was usually maintained with two female mice. Male mice were separated from female mice as soon as pregnancy was assessed from weight gain in female mice or 2 weeks after breeding pairs. Breeding cages were observed for newborns every day, and gestation duration was calculated from the time interval between first day of breeding pair and resulting offspring day at birth. Offspring were generally weaned at 21 days, and individually marked, and housed in groups of individuals of the same sex. All mice have been maintained in the barrier system under the Specific-Pathogen Free (SPF) condition with regulated light (07:00–19:00 hour), temperature (23 ± 3°C), relative humidity (50 ± 10%), and air ventilation (10–12 times per hour). Pelleted food for experimental animals was purchased from Jeil Food (Daejeon, Korea), which was irradiated by gamma-ray at 2.0 Mrad (Greenpia, Korea) and given to mice ad libitum. Tap water was given to mice ad libitum, following the UV-irradiation and filtration. All animal experiments were carried out in accordance with Guidelines for Animal Experimentation and Institutional Animal Care and Use.

**Phenotype characterization and body weight changes.** Phenotype characterization was examined from birth to 6 weeks of age (Fig. 1). Body weight was measured once a day from birth to 6 days of age and once a week from 1 to 14 weeks of age.

**BMD measurement.** Bone mineral density (BMD) was examined at 3- and 6-week old SBS and normal mice for area for forearm, femur and total body. All mice to be examined were anaesthetized by intraperitoneal injection with Ketamine and Xylazine (0.05 mg of Ketamine and 0.015 mg of Xylazine per 1 g of body weight),
Fig. 1. Photographs of phenotype in the SBS mouse (A, B and C, left: normal mouse, right: SBS mouse, A; at 1 week of age, B; at 3 weeks of age, C; at 6 weeks of age).

Measurement were determined by the PIXlmus small animal dual-energy X-ray absorptionmetry (DEXA) system (Lunar Corporation, USA). BMD is a two-dimensional measurement comprised of mineral with the area determined to be bone by the present thresholds in the PIXlmus densitometer. Calibrations were performed with a phantom of known density, and quality assurance measurements were performed prior to BMD measurement.

Genetic mapping.

Reagents and chemicals: In this study, chemicals were purchased from Sigma-Aldrich® (CA, USA) and Merck. Some products of Qiagen® (CA, USA) and Bioneer® (Cheong-won, Korea) were used in the molecular biology research.

Microsatellite markers: Information of microsatellite markers had typed polymorphism between BALB/c and C57BL/6J was obtained form the MGI (www.informatics.jax.org). Microsatellite markers were purchased from Bioneer Inc. (Cheong-won, Korea).

Mating experiment: SBS male mice were mated with female C57BL/6J mice according to 1 : 2 to produce (SBS × C57BL/6J) F₁ hybrids. Further, female mice of F₁ progeny were mated with parental SBS male mice by backcross to produce [(SBS × C57BL/6J) F₁ × SBS] N₂ progeny for linkage analysis (Fig. 2).

Genotyping of simple sequence length polymorphisms (SSLPs).

DNA extraction: Genomic DNA was extracted from the mice tails by DNNeasy tissue Kit (Qiagen, CA, USA) according to the manufacturer’s protocols. Concentration and quality of genomic DNA were measured by Nanodrop 1000 (Nanodrop, DE, USA).

Polymerase chain reaction: Polymerase chain reaction (PCR) amplifications were carried out in 20 μl reaction mixture containing 20 ng template DNA, 20 pmole primers and PCR premixture (Taq DNA polymerase 1U, DNTP 250 μM, Tris-HCl 10 mM, KCI 40 mM, MgCl₂ 1.5 mM, Stabilizer and tracking dye) by Model T-Gradient (Biometra, Goettingen, Germany) and PTC-100 (MJ Research, NV, USA). PCR conditions consisted of one cycle of denaturation for 5 minutes at 94°C followed by 30 cycles with each cycle consisting of denaturation for 40 seconds at 94°C, annealing for 1 minute at 53–59°C regulated by different microstellite markers and extension for 1 minute at 72°C, and final extension 72°C for 10 minutes. The PCR products were stored at 4°C.

Electrophoresis: The PCR products were electrophoresed in denaturing 8%-10% polyacrylamide gel in 0.5 × TBE buffer for 150–300 minutes under 120–150 Volts (Hu and O’shaughnessy 2001).

Staining: The polyacrylamide gel with PCR products was stained in ethidium bromide (Et-Br) for 25–30 minutes and the DNA bands were visualized.

Image analysis: The polyacrylamide gel with PCR products was imaged by ultraviolet ray in Alpalmager™ 3400 (Alpha innotech, CA, USA) and DNA bands were analyzed by AlphaEaseFC™ system program (Alpha innotech, CA, USA).

Linkage analysis: Microsatellite markers were used for linkage analysis. We used total 79 microsatellite markers for linkage analysis; 75 markers for primary linkage screening and 4 markers for further mapping.
Table 1. Phenotype segregation of N2 progeny analyzed in this study

| Mating | Total No. mice | No. affected mice | No. normal mice | Segregation ratio | P value |
|--------|----------------|------------------|-----------------|------------------|---------|
| Backcross | 142 (74/68)* | 72 (32/40)* | 70 (42/28)* | 1 : 1 | P > 0.05 |

*(females/males).
Breeding data were analyzed by \( X^2 \)-test and considerably significant at \( P > 0.05 \).

Table 2. Secondary screen using the thirty-eight microsatellite markers in the primary linkage analysis

| Chr.* | Markers | Position (cM) | Affected | Normal | Recombination value (%) | \( X^2 \) analysis |
|-------|---------|---------------|----------|--------|-------------------------|------------------|
|       |         |               | Homo\(^b\) | Hetero\(^b\) | Homo\(^c\) | Hetero\(^c\) |
| 1     | D1Mit302 | 32.8          | 13        | 9      | 7           | 15      | 36.4 ± 7.3 | 3.273 |
| 2     | D2Mit329 | 49.2          | 12        | 10     | 11          | 11      | 47.7 ± 7.5 | 0.091 |
| 3     | D3Mit22  | 33.7          | 14        | 8      | 12          | 10      | 45.5 ± 7.5 | 0.364 |
| 4     | D3Mit19  | 61.8          | 13        | 9      | 14          | 8       | 52.3 ± 7.5 | 0.091 |
| 5     | D4Mit178 | 35.5          | 15        | 7      | 11          | 11      | 40.9 ± 7.4 | 1.455 |
| 6     | D4Mit204 | 61.9          | 12        | 10     | 7           | 15      | 38.6 ± 7.3 | 2.273 |
| 7     | D5Mit81  | 28            | 14        | 8      | 7           | 15      | 34.1 ± 7.1 | 4.455 |
| 8     | D5Mit210 | 64            | 9          | 13     | 12          | 10      | 56.8 ± 7.5 | 0.816 |
| 9     | D6Mit8   | 35.2          | 8          | 14     | 9           | 13      | 52.3 ± 7.5 | 0.091 |
| 10    | D6Mit254 | 60.55         | 10         | 12     | 14          | 8       | 59.1 ± 7.4 | 1.455 |
| 11    | D7Mit176 | 27.0          | 14         | 8      | 9           | 13      | 38.6 ± 7.3 | 2.273 |
| 12    | D7Mit220 | 52.4          | 15         | 7      | 8           | 14      | 34.1 ± 7.1 | 4.455 |
| 13    | D8Mit176 | 20            | 13         | 9      | 8           | 14      | 38.6 ± 7.3 | 2.273 |
| 14    | D8Mit249 | 49.3          | 12         | 10     | 9           | 13      | 43.2 ± 7.5 | 0.816 |
| 15    | D8Mit121 | 67            | 12         | 10     | 9           | 13      | 50.7 ± 7.5 | 0.000 |
| 16    | D9Mit191 | 26            | 10         | 12     | 10          | 12      | 50.7 ± 7.5 | 0.000 |
| 17    | D9Mit182 | 55            | 8          | 14     | 12          | 10      | 59.1 ± 7.4 | 1.455 |
| 18    | D10Mit170| 29            | 21         | 1      | 2           | 20      | 47.7 ± 3.7 | 32.818 |
| 19    | D10Mit95 | 51            | 14         | 8      | 6           | 16      | 31.8 ± 7.0 | 5.816 |
| 20    | D11Mit236| 20            | 13         | 9      | 8           | 14      | 38.6 ± 7.3 | 2.273 |
| 21    | D11Mit212| 50            | 9          | 13     | 12          | 10      | 56.8 ± 7.5 | 0.816 |
| 22    | D12Mit201| 29            | 12         | 10     | 12          | 10      | 50.7 ± 7.5 | 0.000 |
| 23    | D12Mit28 | 50            | 11         | 11     | 12          | 10      | 52.3 ± 7.5 | 0.091 |
| 24    | D13Mit221| 30            | 9          | 13     | 11          | 11      | 54.5 ± 7.5 | 0.364 |
| 25    | D13Mit213| 55            | 14         | 8      | 10          | 12      | 40.9 ± 7.4 | 1.455 |
| 26    | D14Mit203| 28.3          | 7          | 15     | 15          | 7      | 68.2 ± 7.0 | 5.818 |
| 27    | D14Mit195| 44.3          | 9          | 13     | 14          | 8      | 61.4 ± 7.3 | 2.272 |
| 28    | D15Mit63 | 29.2          | 12         | 10     | 7           | 15      | 38.6 ± 7.3 | 2.272 |
| 29    | D15Mit159| 49.6          | 10         | 12     | 4           | 18      | 36.4 ± 7.3 | 3.27 |
| 30    | D16Mit64 | 27.3          | 13         | 9      | 9           | 13      | 40.9 ± 7.4 | 1.455 |
| 31    | D16Mit114| 44.5          | 12         | 10     | 13          | 9      | 52.3 ± 7.5 | 0.091 |
| 32    | D17Mit152| 37.7          | 14         | 8      | 13          | 9      | 47.7 ± 7.5 | 0.091 |
| 33    | D17Mit221| 56.7          | 13         | 9      | 16          | 6       | 56.8 ± 7.5 | 0.818 |
| 34    | D18Mit60 | 36            | 14         | 8      | 10          | 12      | 40.9 ± 7.4 | 1.455 |
| 35    | D18Mit40 | 37            | 13         | 9      | 9           | 13      | 40.9 ± 7.4 | 1.455 |
| 36    | D19Mit61 | 9             | 11         | 11     | 10          | 12      | 47.7 ± 7.5 | 0.091 |
| 37    | D19Mit91 | 47            | 9          | 13     | 11          | 11      | 54.5 ± 7.5 | 0.364 |

*Chromosome.

*Affected homo and normal hetero represent parental combination.

*Affected hetero and normal homo represent recombinant combination.

We finally selected 38 from 75 markers for secondary screening after checking polymorphism between BALB/c and C57BL/6J. Their positions of 38 markers from each 19 chromosomes are shown in Table 2. Four more markers on chromosome 10 are described in Table 3.

Statistic analysis. The BMD data was assessed using student's \( t \)-test (GraphPad Instate, V2.05a). The
Table 3. Further linkage analysis of causative gene on chromosome 10 for SBS mice

| Markers   | Position (cM) | Affected | Normal | Recombination value (%) | X² analysis |
|-----------|---------------|----------|--------|--------------------------|-------------|
|           | Homo²         | Hetero²  | Homo²  | Hetero²                  |             |
| D10Mit248 | 7             | 67       | 5      | 4                        | 66          |
| D10Mit51  | 9             | 69       | 3      | 3                        | 67          |
| sbs       | 72            | 0        | 1      | 2                        | 68          |
| D10Mit283 | 16            | 72       | 0      | 1                        | 69          |
| D10Mit106 | 17            | 71       | 1      | 2                        | 68          |
| D10Mit170 | 29            | 64       | 8      | 11                       | 59          |

²Affected homo and normal hetero represent parental combination.
²²Affected hetero and normal homo represent recombinant combination.
³P < .01, difference is very significant.

The genotypic difference of SSLPs was assessed by recombination values and X² analysis, which calculated according to recombination value = (RC/PC + RC) × 100% and the formula X² = (PC - RC)²/N, where PC = parental combinations, RC = recombinant combinations, and N = the total number of offspring scored. The standard error (SE) of the recombination value was calculated as SE = [p(1 - p)/N]¹/², where P is the recombination value.

RESULTS

Body weight changes. SBS and normal mouse are indistinguishable macroscopically in size at birth (data not shown). At 1 week of age, their difference of body size was just apparent (Fig. 1A). However, the onset of body weight changes of SBS mouse was detected from birth through body weight measured. As compared with normal mice, the body weight mean of normal mice was 1.54 ± 0.177 g, whereas the body weight mean of SBS mice was 1.37 ± 0.085 g at birth (Fig. 3A). Body weight changes were significant between SBS and normal mice from birth to 14 weeks of age. Body weight difference between them has widened from birth to 3 weeks of age. At 3 weeks of age, the body weight mean of normal mice was 11.20 ± 1.634 g, and the body weight mean of SBS mice was 4.92 ± 1.299 g. That of SBS mice was approximately 43.7% the body weight mean of normal mice and the difference was most significant (Fig. 3B). Body weight difference between them was gradually reduced from 3 to 14 weeks of age (P < 0.001 or < 0.01).

Segregation of N₂ backcross progeny. The segregation results of the phenotypes in the N₂ backcross progeny are represented in Table 1. Total 142 N₂ backcross progeny were produced resulting from mating 4 SBS males to 8 (SBS × C57BL/6J) F₁ females hybrids. All [(SBS × C57BL/6J) F₁ × SBS] N₂ hybrids were observed to be phenotypically normal. Seventy of 142 N₂ backcross mice were not affected. The incidence of dwarfism in N₂ backcross progeny was 50.7%. There were no sex differences in N₂ backcross progeny.

BMD changes. Difference was found in mean fore-arm BMD, femur BMD, and total body BMD at 3 and 6 weeks of age between the SBS and normal mice. As compared with the normal mice at 3 weeks of age, the
Fig. 4. Comparison of BMD at forearm, femur, and total body between SBS mice (n = 5) and normal mice (n = 5) at 3 (A) and 6 weeks of age (B), respectively. The BMD (forearm, femur, and total body) levels in SBS mice was shown to be significantly lower than in normal mice [*p < 0.05, **p < 0.01 (student's t-test) VS normal mice].

**Genetic mapping.** In this study, N2 mice were used for linkage analysis. Affected 22 N2 mice and 22 normal mice were initially genotyped with 37 microsatellite markers on all 19 autosomal chromosomes, with an average interval of approximately 30 centimorgen (cM). However, the result of genotyping was not visible (data not shown). 38 new microsatellite markers were collected once again for secondary screen on all 19 autosomal chromosomes (Table 2). By linkage analysis of the causative gene in N2 backcross mice, a strong linkage was expected on mouse chromosome 10 around D10Mit170. Further linkage analysis was narrowed to chromosome 10 with additional 4 microsatellite markers, D10Mit248, D10Mit51, D10Mit283, and D10Mit106. A total of 142 N2 backcross mice consisted of 72 affected mice and 70 normal mice were genotyped for linkage analysis. Table 3 represents the genotypes of all N2 backcross mice for 5 microsatellite markers on chromosome 10. All affected N2 backcross mice showed dwarfism had the BALB/c homozygote genotypes at D10Mit283 on chromosome 10, and all normal N2 backcross mice had the BALB/c x C57BL/6J heterozygote genotypes at D10Mit283 on chromosome 10 except for only one normal N2 backcross mouse. The one normal N2 backcross mouse had the BALB/c homozygote genotype at D10Mit283. Fig. 5 shows the distribution of haplotypes.

**DISCUSSION**

Syndromes of heritable dwarfism in humans and animals can be caused by states of a variety of complication and genetic heterogeneity, with either dominant or recessive inheritance, some of which are related to point mutations. Using random ENU-mutagenesis, we generated novel mouse model for dwarfism which was named SBS mouse. The SBS mouse exhibit heritable dwarfism in a recessive Mendelian pattern (Cho et al., 2003). In this study, we examined body weight changes and bone mineral density (BMD), and we also carried out genetic linkage analysis to map the causative gene of SBS mouse developed by ENU mutagenesis.

The phenotypic differences in body size manifest from 1 to 14 weeks of age during puberty except birth, but body weight changes appear to be already present at birth (Fig. 3). Measurements of newborn SBS mice yielded body weights of 1.3 ± 0.08 g, comparable with
normal mice 1.5 ± 0.18 g from similar litter sizes (data not shown).

In results of bone density analysis, BMD of the forearm, femur, and total body in SBS mice were significantly low compared with normal mice (Fig. 4). BMD has been employed most commonly as the index for defining and studying osteoporosis (Deng and Recker, 2002; Wynne et al., 2002). Comparative maps of genetic, Jackson Laboratory and sequence indicate chromosomal region in which synteny is conserved (Fig. 5, 6). In this regions, possible candidate genes for dwarfism phenotypes, including postnatal body weight growth 9 (Pbwg9) and peroxisome biogenesis factor 7 (Pex7) have been mapped on mouse chromosome 10 (Brites et al., 2003; Ishikawa and Namikawa, 2004).

Postnatal body weight growth 9 (Pbwg9) was located by QTL mapping whose location is 14 cM from centromere on chromosome 10 which mainly regulated the function in the middle growth phase of entire growth process (Ishikawa and Namikawa 2004; Cheverud et al., 1996). Therefore, The Pbwg9 gene could be a candidate gene for sbs gene. Mutations in the peroxisome biogenesis factor (Pex7) encoding the receptor for a class of peroxisomal matrix enzymes have been found in patients of rhizomelic chondrodysplasia punctata (RCDP), who show many abnormal phenotype such as dwarfism, abnormal lipid chemistry, abnormal cartilage development, reduced bone density and cataracts (Brites et al., 2003; Braverman et al., 1997; Purdue et al., 1997). The Pex7 gene is considered as a strong candidate gene for sbs gene. The mapping of these candidate genes in the region on the chromosome 10 and the identification of markers loci tightly linked to the sbs locus will reveal correspondence between these genes and the dwarfism. For further studies, it may require identifying the gene mutations that occurred and demonstrate the features of the mutations by sequence

Fig. 6. A partial genetic and sequence map of chromosome 10 compared with distance of microsatellite markers from Jackson Laboratory, and the location of Pbwg9 and Pex7 gene: microsatellite markers (A), distance between markers (B), distance from causative gene (C, sbs), distance between markers from MGI (D), distance from centromere from MGI(E) and microsatellite markers sequence size (F) were presented in the map.  

| Chr 10 | Chr 10 | Chr 10 |
|-------|-------|-------|
| A | B | C |
| D | E | F |
| G | H | I |
| J | K | L |
| M | N | O |
| P | Q | R |
| S | T | U |
| V | W | X |
| Y | Z | |

| Genetic map | Jackson Laboratory |
|-------------|---------------------|
| A | B | C |
| D | E | F |
| G | H | I |
| J | K | L |
| M | N | O |
| P | Q | R |
| S | T | U |
| V | W | X |
| Y | Z | |

| Sequence map | |
|--------------|---|
| 6.4 Mb | 6.3 Mb | 6.2 Mb |
| 6.1 Mb | 6.0 Mb | 5.9 Mb |
| 5.8 Mb | 5.7 Mb | 5.6 Mb |
| 5.5 Mb | 5.4 Mb | 5.3 Mb |
| 5.2 Mb | 5.1 Mb | 5.0 Mb |
| 4.9 Mb | 4.8 Mb | 4.7 Mb |
| 4.6 Mb | 4.5 Mb | 4.4 Mb |
| 4.3 Mb | 4.2 Mb | 4.1 Mb |
| 4.0 Mb | 3.9 Mb | 3.8 Mb |
| 3.7 Mb | 3.6 Mb | 3.5 Mb |
| 3.4 Mb | 3.3 Mb | 3.2 Mb |
| 3.1 Mb | 3.0 Mb | 2.9 Mb |
| 2.8 Mb | 2.7 Mb | 2.6 Mb |
| 2.5 Mb | 2.4 Mb | 2.3 Mb |
| 2.2 Mb | 2.1 Mb | 2.0 Mb |
| 1.9 Mb | 1.8 Mb | 1.7 Mb |
| 1.6 Mb | 1.5 Mb | 1.4 Mb |
| 1.3 Mb | 1.2 Mb | 1.1 Mb |
| 1.0 Mb | 0.9 Mb | 0.8 Mb |
| 0.7 Mb | 0.6 Mb | 0.5 Mb |
| 0.4 Mb | 0.3 Mb | 0.2 Mb |
| 0.1 Mb | 0.0 Mb | |
was no difference in the number between male and female mice at intervals of one week. The difference in body weight, which was very significant, was verified.

Inheritance mode of the mouse was further confirmed through mating experiment in this study. There was no difference in the number of normal and affected animals from birth to 6 days of age and another is from 1 to 14 weeks of age at intervals of one week. The difference of body weight, which was very significant, was verified.

Difference of body weight changes were elucidated in detail between affected mice and normal mice, which were examined by two time phases; one is once one day from birth to 6 days of age and another is from 1 to 14 weeks of age at intervals of one week. The difference of body weight, which was very significant, was verified.

Inheritance mode of the mouse was further confirmed through mating experiment in this study. There was no difference in the number of normal and affected animals showed segregation ratio of 1 : 1 in backcross progeny. It was determined that mutant gene feature is autosomal single recessive inheritance based on Mendelian law.

Difference of bone mineral density (BMD) between normal and affected mice at 3 and 6 weeks of age during puberty is very notable. Thus, we expect SBS mice might be a good model for the study of osteoporosis related with BMD for studies in the future.

For genetic study, we narrowed down the genetic map position between D10Mit51 and D10Mit283, and demonstrated the possibility of postnatal body weight growth (Pbwg9) and the peroxisome biogenesis factor (Pex7) as candidate gene for sbs.

This study has not been able to entirely elucidate every phenotype of SBS mouse, but the biochemical and physiological analysis and fine mapping for positional cloning of the mutant gene will be in progress.

ACKNOWLEDGEMENT

This study was supported by National Research Laboratory Program (2000-N-NL-01-c-207) from the Ministry of Science and Technology of Korea.

REFERENCES

Argentin, G. and Cicchetti, R. (2000). In vitro proliferation of acondroplastic and normal mouse chondrocytes, before and after basic fibroblast growth factor stimulation. Cell Prolif., 33, 397-405.

Beamer, W.G., Shultz, K.L., Churchill, G.A. and Donahue, L.R. (1999). Quantitative trait loci for bone density in C57BL/6J and CAST/EiJ inbred mice. Mamm. Genome., 10, 1043-1049.

Beamer, W.G., Shultz, K.L., Donahue, L.R. and Rosen, C.J. (2001). Quantitative trait loci for femoral and lumbar vertebral bone mineral density in C57BL/6J and C3H/HeJ inbred strains of mice. J. Bone Miner. Res., 16, 1195-1206.

Benes, H., Weinstein, R.S., Zheng, W., Thaden, J.I. and Shmookler, R.R.J. (2000). Chromosomal mapping of osteopenia-associated quantitative trait loci using closely related mouse strains. J. Bone Miner. Res., 15, 626-633.

Braga, V., Sangalli, A., Malerba, G., Mottes, M., Mirandola, S., Gatti, D., Rossini, M., Zamboni, M. and Adami, S. (2002). Relationship among VDR (Bsm and FokI), COLIA1, and CTR polymorphisms with bone mass, bone turnover markers, and sex hormones in men. Calcif. Tissue Int., 70, 457-462.

Braverman, N., Steel, G., Obic, C., Moser, A., Moser, H., Gould, S.J. and Valle, D. (1997). Human PEX7 encodes the peroxisomal PTS2 receptor and is responsible for rhizomelic chondrodysplasia punctata. Nat. Genet., 15, 369-376.

Brites, P., Motley, A.M., Gressens, P., Mooyer, P.A., Plou‐gaert, I., Everts, V., Evrard, P., Carmeliet, P., Dewerchin, M., Schoonjans, L., Duran, M., Waterham, H.R., Wandes, R.J. and Baes, M. (2003). Impaired neuronal migration and endochondral ossification in Pex7 knockout mice: a model for rhizomelic chondrodysplasia punctata. Hum. Mol. Genet., 12, 2255-2267.

Brown, S.D.M. and Hardisty, R.E. (2003). Mutagenesis strategies for identifying novel loci associated with disease phenotypes. Semin. Cell Dev. Biol., 14, 19-24.

Cheverud, J.M., Routman, E.J., Duarte, F.A., van Swinderen, B., Cothran, K. and Perel, C. (1996). Quantiative trait loci for murine growth. Genetics, 142, 1305-1319.

Cho, J.W, You, J.K., Cho, K.H., Han, S.S. and Song, C.W. (2003). The Development of Dwarfism Mice Using ENU Mutagenesis. Kor. J. Lab. Anim. Sci., 19, 65-69.

Davis, A.P., Woychik, R.P. and Justice, M.J. (1999). Effective chemical mutagenesis in FVB/N mice requires low doses of ethynitrosourea. Mamm. Genome., 10, 308-310.

Deng, H.W., Shen, H., Xu, F.H., Deng, H.Y., Conway, T., Zhang, H.T. and Recker, R.R. (2002). Tests of linkage and/or association of genes for vitamin D receptor, osteocalcin, and parathyroid hormone with bone mineral density. J. Bone Miner. Res., 17, 678-686.

Deng, H.W. and Recker, R.R. (2004). Gene mapping and identification for osteoporosis. J. Musculoskelet. Neuronal Interact., 4, 91-100.

Dietrich, W.F., Miller, J., Steen, R., Merchant, M.A., Damron‐Boles, D., Husain, Z., Dredge, R., Daly, M.J., Ingalls, K.A. and O'Conner, T.J. (1996). A comprehensive genetic map of the mouse genome. Nature, 380, 149-152.

Drake, T.A., Schadt, E., Hannani, K. and Lewis, J. (2001). Genetic loci determining bone density in mice with diet‐induced atherosclerosis. Physiol. Genom., 5, 205-215.

Duncan, E.L., Brown, M.A., Sinhshime, J., Bell, J., Carr, A.J., Wordsworth, B.P. and Wass, J.A. (1999). Suggestive linkage of the parathyroid receptor type 1 to osteoporosis. J. Bone Miner. Res., 14, 1993-1999.

Eason, J., Hall, C.M. and Trounce, O.J. (1995). Renal tubular leakage complicating microcephalic osteodysplastic primordial dwarfism. J. Med. Genet., 32, 234-235.

Gibbs, R.A., Weinstock, G.M., Metzker, M.L., Muzny, D.M., Sodergren, E.J., Scherer, S., Scott, G. and Rat Genome Sequencing Project Consortium (2004). Genome sequence...
of the Brown Norway rat yields insights into mammalian evolution. Nature, 428, 493-521.

Godfrey, P., Rahal, J.O., Beamer, W.G., Copeland, N.G., Jenkins, N.A., and Mayo, K.E. (1993). GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. Nat. Genet., 4, 227-232.

Hitotsumachi, S., Carpenter, D.A. and Russel, W.L. (1985). Receptor-associated resistance to growth hormone shown to be associated with a missense mutation in the extracellular domain that disrupts receptor function. Nat. Genet., 25, 444-447.

Hu, C.T. and O’shaughnessy, K.M. (2001). Glycerol-enhanced mini-polyacrylamide gel electrophoresis for the separation of differentially expressed DNA fragments in cDNA repre-

Hwang, Q.Y., Recker, R.R. and Deng, H.W. (2003). Searching for osteoporosis genes in the post-genome era: progress and challenges. Osteoporos. Int., 14, 701-715.

Ishikawa, A. and Namikawa, I. (2004). Mapping major quantitative trait loci for postnatal growth in an intersubspecific backcross between C57BL/6J and Philippine wild mice by using principal component analysis. Electrophoresis, 22, 1063-1068.

Jansson, J.O., Downs, R.J., Beamer, W.G. and Frohman, L.A. (1986). Induction of new mutations in mouse spermatogonia. Proc. Natl. Acad. Sci., 82, 6619-6621.

Jin, J., Zhang, W., and Gu, K. (1999). Identification of new quantitative trait loci for osteoporosis genes in transgenic mice. Mol. Endocrinol., 7, 694-701.

Klein, R.F., Carlos, A.S., Varatnian, K.A., Chambers, V.K., and Orwell, E.S. (2001). Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. J. Bone Miner. Res., 16, 1539-1547.

Klein, R.F., Carlos, A.S., Varatnian, K.A., Chambers, V.K. and Orwell, E.S. (2001). Confirmation and fine mapping of chromosomal regions influencing peak bone mass in mice. J. Bone Miner. Res., 16, 1539-1547.

Kunc, J.R., and Cho, D.H. (2005). The recommended approaches and recent trends in reproductive and developmental toxicology. J. Toxicol. Pub. Health, 21, 271-278.

Lander, E.S., Linton, L.M., Birren, B., Nusbaum, C., Zody, M.C., Baldwin, J., Devon, K., Dewey, K. and Wyman, D. (2001). Initial sequencing and analysis of the human genome. Nature, 409, 860-921.

Li, S., Crenshaw III, E.B., Rawson, E.J., Simmonds, D.M., Swanson, L.V. and Rosenfeld, M.G. (1990). Dwarf locus mutants lacking three pituitary cell types result from mutations in the POU-domain gene pit-1. Nature, 347, 528-533.

Liu, X., Masinde, G., Gu, W., Wergedal, J., and Baylink, D.J. (2002). Genetic dissection of femur breaking strength in a large population (MRL/Mpj x SJL/J) of F2 mice: single QTL effects, epistasis, and pleiotropy. Genomics, 73, 734-740.

Lin, S.C., Sim, C.R., Gukovsky, I., Lusis, A.J., Sawchenco, P.E., and Rosenfeld, M.G. (1993). Molecular basis of the little mouse phenotype and implications for cell-type specific growth. Nature, 364, 209-214.

Lira, S.A., Kalla, K.A., Glass, C.K., Daurot, D.W., and Rosenfeld, M.G. (1993). Synergistic interactions between Pit1 and other elements are required for effective somatotroph rat growth hormone gene expression in transgenic mice. Mol. Endocrinol., 7, 694-701.

Masinde, G.L., Li, X., Gu, W., Wergedal, J., Mohan, S., and Baylink, D.J. (2002). Quantitative trait loci for bone density in mice: the genes determining total skeletal density and femur density show little overlap in F2 mice. Calcif. Tissue Int., 71, 421-428.

Meyer, C.W., Korthaus, D., Jagla, W., Cornali, E., Grosse, J., Fuchs, H., Klingenspor, M., Roemheld, S., Tschoch, M., Heldmaier, G., Dewar, K., and Wyman, D. (2001). Confirmation and fine mapping of quantitative trait loci affecting peak bone mass in transgenic mice. Bone Miner. Res., 16, 1953-1963.
Rosen, C.J., Beamer, W.G. and Donahue, L.R. (2001). Defining the genetics of osteoporosis: Using the mouse to understand man. Osteoporos. Int., 12, 803-810.

Shelovsky, A., McDonald, J.D., Symula, D.M. and Dove, W.F. (1993). Mouse models of human phenylketonuria. Genetics, 134, 1205-1210.

Shibayama, K., Ohyama, Y., Ono, M. and Furudate, S. (1993). Expression of mRNA coding for pituitary hormones and pituitary-specific transcription factor in the pituitary gland of the rdw rat with hereditary dwarfism. J. Endocrinol., 138, 307-313.

Shimizu, M., Higuchi, K., Bennett, B., Xia, C. and Hosokawa, M. (1999). Identification of peak bone mass QTL in spontaneously osteoporotic mouse strain. Mamm. Genome, 10, 81-87.

Shimizu, M., Higuchi, K., Kasai, S., Nakamura, T. and Hosokawa, M. (2001). Chromosome 13 locus, Pbd2, regulates bone density in mice. J. Bone Miner. Res., 12, 1972-1982.

Soewarto, D., Fella, C., Teubner, A., Rathkolb, B., Pargent, W., Heffner, S. and Angelis, M.H. (2000). The large-scale Munich ENU-mouse-mutagenesis screen. Mamm. Genome, 11, 507-510.

Sooson, M.W., Wu, W. and Dasen, J.S. (1996). Pituitary lineage determination by the Prophet of Pit-1 homeodomain factor defective in Ames dwarfism. Nature, 384, 327-333.

Tsui, T. and Kunieda, T. (2005). A loss-of-function mutation in natriuretic peptide receptor 2 (Npr2) gene is responsible for disproportionate dwarfism in cnv/cnv mouse. J. Biol. Chem., 280, 14288-14292.

Takeuchi, T., Suzuki, H., Sakurai, S., Nogami, H., Okuma, S. and Ishikawa, H. (1990). Molecular mechanism of growth hormone (GH) deficiency in the spontaneous dwarf rat: detection of abnormal splicing of GH messenger ribonucleic acid by polymerase chain reaction. Endocrinology, 126, 31-38.

Wang, Y., Spatz, M.K., Kannan, K., Hayk, H., Avivi, A., Gorivodsky, M., Pines, M., Yayon, A., Lonai, P. and Givol, D. (1999). A mouse model for achondroplasia produced by targeting fibroblast growth factor receptor 3. Proc. Natl. Acad. Sci., 96, 4455-4460.

Waterston, R.H., Lindblad-Toh, K., Birney, E., Rogers, J., Abril, J.F., Agarwal, P., Agarwala, R. and Mouse Genome sequencing Consortium (2002). Initial sequencing and comparative analysis of the mouse genome. Nature, 420, 520-562.

WHO (1994). Assessment of fracture risk and its application to screening for postmenopausal osteoporosis. World Health Organ Tech. Report Series No. 843, Geneva: WHO, 1-129.

Wynne, F., Drummond, F., O’Sullivan, K., Daly, M., Shanahan, F., Molloy, M.G. and Quane, K.A. (2002). Investigation of the genetic influence of the OPG, VDR (Fok1), and COLA1 Sp1 polymorphisms on BMD in the Irish population. Calcif. Tissue Int., 71, 26-35.

Zhou, X., Benson, K.F., Ashar, H.R. and Chada, K. (1995). Mutation responsible for the mouse pygmy phenotype in the developmentally regulated factor HMGIC. Nature, 376, 771-774.