Exp. Anim. 52(5), 433–436, 2003

—Note—

Chromosomal Mapping of the Peroneal Muscular Atrophy (pma) Gene in the Mouse

Hideki KATOH[1, 2], Yuka WATANABE[2], Michi EBUKURO[2], Kaori MUGURUMA[2], Shuji TAKABAYASHI[1], and Toshihiko SHIROISHI[3]

[1]*Institute for Experimental Animals, Hamamatsu University School of Medicine, 1–20–1 Handayama Hamamatsu Shizuoka 431-3192,* [2]*Department of Genetics, Central Institute for Experimental Animals, 1430 Nogawa, Miyamae, Kawasaki 216-0001,* [3]*Genetic Strains Research Center, National Institute of Genetics, 1–111 Yata, Mishima, Shizuoka 411-8540, Japan*

Abstract: *We conducted chromosomal mapping of the* pma *gene that is a causative gene in the peroneal muscular atrophy mouse, which shows a club foot at birth and unusual gait due to a dropped foot in the adult. Linkage analyses using backcross progeny revealed a significant linkage between the* pma *gene and three microsatellite markers,* D5Mit263 *at 73 cM,* D5Mit141 *and* D5Mit97 *at 74 cM on Chr 5. The gene order was determined as follows: centromere-*D5Mit263*-[2.65 cM]-*D5Mit141*-[2.56 cM]-*pma*-[5.13 cM]-*D5Mit97*-telomere.*

Key words: *Chr 5, mouse, peroneal muscular atrophy*

Esaki *et al.* [3] reported an autosomal recessive mutant showing an anomaly of the hind limb found in the process of inbreeding of a line derived from CF1 outbred stock. The major phenotype of this mutant is a club foot at birth and unusual gait due to a dropped foot in the adult as shown in Fig. 1A and 1B. Anatomical and pathological studies have revealed the absence of common peroneal nerves, which leads to atrophy of the posterior crural muscles (Fig. 1D) compared with normal muscles (Fig. 1C). This strain was tentatively named, PMA, which means a phenotype of peroneal muscular atrophy and the mutation was named *pma* [3]. Unfortunately, detailed genetic studies of this anomaly have not been performed to date. We report chromosomal mapping of the *pma* gene in this paper.

The MSM/Ms strain used for mating experiments was introduced from the National Institute of Genetics, Mishima, Japan. F_1 and backcross progeny were obtained by mating between PMA-*pma*/*pma* and MSM/Ms-+/+. Genotypes of the *pma* gene were determined by observation of morphology of the hind foot and the common peroneal nerve. Linkage between the *pma* gene and markers on autosomes, except for sex chromosomes, was studied using backcross progeny. Fifty-six markers on Chr 1 to Chr 19 showing genetic polymorphisms between PMA and MSM were selected for the linkage study of the *pma* gene as follows: *D1Mit1* (8.7), *D1Mit7* (41) and *Pep3** (71) on Chr 1, *D2Mit12* (50.3) on Chr 2, *Car2** (10.5), *D3Mit6* (23.3), *D3Mit17* (71.8) and *D3Mit19* (87.6) on Chr 3, *D4Mit81* (38), *D4Mit76* (55.7), *D4Mit54* (66) and *D4Mit13* (71) on Chr 4, *D5Mit13* (20), *D5Mit10* (54), *D5Mit68* (65),

(Received 14 April 2003 / Accepted 13 June 2003)

Address corresponding: H. Katoh, Institute for Experimental Animals, Hamamatsu University School of Medicine, 1–20–1 Handayama Hamamatsu Shizuoka 431-3192, Japan

CLINICAL REPORT

Y Yagasaki
T Yamaguchi
J Watahiki
M Konishi
H Katoh
K Maki

The role of craniofacial growth in leptin deficient (*ob/ob*) mice

Authors' affiliations:
Y. Yagasaki, Assistant, Department of Orthodontics, School of Dentistry, Showa University, Kitasenzoku, Outa-ku, Tokyo, Japan
T. Yamaguchi, Researcher, Institute of Medical Science, University of Tokyo, and Department of Orthodontics, School of Dentistry, Showa University, Kitasenzoku, Outa-ku, Tokyo, Japan
J. Watahiki, Graduate Student, Department of Orthodontics, School of Dentistry, Showa University, Kitasenzoku, Outa-ku, Tokyo, Japan
M. Konishi, Product Leader, LUNAR Group Ultrasound Sales Division GE Yokogawa Medical Systems, Hino-shi, Tokyo, Japan
H. Katoh, Associate Professor, Institute for Experimental Animals, Hamamatsu University School of Medicine, Shizuoka, Japan
K. Maki, Instructor, Department of Orthodontics, School of Dentistry, Showa University, Kitasenzoku, Outa-ku, Tokyo, Japan

Correspondence to:
Yoshimi Yagasaki
Department of Orthodontics
School of Dentistry
Showa University
2-1-1, Kitasenzoku, Outa-ku
Tokyo 145-8515
Japan
Tel.: 81-3-3787-1151, ext 262
Fax: 81-3-3784-6641
E-mail: yagasaki@senzoku.showa-u.ac.jp

Dates:
Accepted 19 June 2003

To cite this article:
Orthod Craniofacial Res **6**, 2003; 233–241
Yagasaki Y, Yamaguchi T, Watahiki J, Konishi M, Katoh H, Maki K:
The role of craniofacial growth in leptin deficient (*ob/ob*) mice

Copyright © Blackwell Munksgaard 2003

ISSN 1601-6335

Structured Abstract

Authors – Yagasaki Y, Yamaguchi T, Watahiki J, Konishi M, Katoh H, Maki K.

Objectives – To elucidate the role of leptin on maxillo-facial morphological growth using hereditary obesity model *ob/ob* mice, and to examine the presence of the leptin receptor gene expression in the mouse condylar head cartilage.

Design – Leptin was intraperitoneally administered once a day in 10 C57BL/6J (lean) and 10 C57BL/6J-*ob* (*ob/ob*) mice (leptin administration group), and phosphate-buffered saline (PBS) in 10 lean and 10 *ob/ob* mice (PBS administration group), between the fifth and 11th week after birth. The amount of fat, the body amount without fat, the rate of body fat, and the width of the condylar cervical area were measured during the 11th week, and roentgenographic cephalometric analysis was performed at the fifth, eighth, and 11th week. Furthermore, the condylar head cartilage in C57BL/6J mice was stereoscopically excised to extract total RNA, and RT-PCR method was performed regarding the leptin receptor gene.

Results – The body fat amount in *ob/ob* mice with leptin production insufficiency was greater than that in lean mice, and significant differences were noted in every measurement item regarding maxillo-facial morphology. Recovery of bone length was noted in *ob/ob* mice by administering leptin. Furthermore, the expression of the leptin receptor gene in the condylar head cartilage was confirmed.

Conclusion – Exogenous leptin administration leads to significant increases in craniofacial dimensions; and leptin receptors are expressed in mandibular condylar cartilage. These results indicate an important role for leptin in craniofacial growth and morphology. We speculate that leptin's direct peripheral effect on bone and cartilage is closely involved in this role.

Key words: craniofacial growth; leptin; obesity

Exp. Anim. 53(4), 311–320, 2004

Establishment of an Efficient BAC Transgenesis Protocol and its Application to Functional Characterization of the Mouse Brachyury Locus

Kuniya ABE[1,4], Masaaki HAZAMA[2], Hideki KATOH[3], Ken-ichi YAMAMURA[4,5], and Misao SUZUKI[5]

[1]Research & Development Team for Mammalian Cellular Dynamics, BioResource Center, RIKEN Tsukuba Institute, 3–1–1 Koyadai, Tsukuba, Ibaraki 305-0074, [2]Department of Biological Sciences, Kyoto University Faculty of Medicine, Yoshidahonmachi, Sakyo-ku, Kyoto 606-8501, [3]Experimental animals Institute, Hamamatsu University School of Medicine, 1–20–1 Handayama, Hamamatsu, Shizuoka 431-3192, [4]Institute of Molecular Embryology and Genetics, Kumamoto University, 4–24–1 Kuhonji, Kumamoto 862-0976, and [5]Center for Animal Research and Development, Kumamoto University, 1–1–1 Honjo, Kumamoto 860-0811, Japan

Abstract: *Transgenesis using large DNA such as YAC or BAC has extended the range of applications in functional genomics. Here we describe an efficient BAC transgenesis protocol using a simple BAC DNA preparation method adopted from YAC DNA purification methods. This method allowed us to isolate BAC DNA from small scale culture of BAC-containing cells in sufficient quantity and purity for microinjection. More than 40 founders have been produced with linearized BAC DNA prepared by this method, and 85% of them contained intact BAC transgenes. In contrast, when circular BAC DNA was injected, an approximately three-fold reduction of transgene integration rate was observed and fewer intact transgene integrations were obtained. A line of transgenic mice carrying a 170-kb BAC clone generated in this way successfully rescued tail and embryonic lethality phenotypes of the mouse* Brachyury (T) *mutants, further demonstrating the utility of this method in functional analysis of the mouse genome.*

Key words: *BAC, development, T-locus, transgenesis*

Introduction

Transgenic technology has been extremely useful and successful for studying functions of genes of interest at an organism level. However, there are several technical limitations in conventional transgenic techniques. Since introns and essential regulatory elements required for correct *in vivo* expression tend to be omitted in the constructs, transgene expression often fails to follow the expression patterns of the corresponding endogenous gene. The chromosome 'position-effect' affects foreign gene expression, depending on the chromosomal integration site, compromising the transgene expression. In addition, the maximum size of the transgene is only

(Received 10 November 2003 / Accepted 25 February 2004)

Address corresponding: K. Abe, Research & Development Team for Mammalian Cellular Dynamics, BioResource Center, RIKEN Tsukuba Institute, 3–1–1 Koyadai, Tsukuba, Ibaraki 305-0074, Japan

実験動物と環境 10 (1), 24-27, 2002

MＨＶ汚染コロニーからの動物の受け入れ

胚移植による清浄化における汚染除去確認法

（追加発言：N蛋白コード遺伝子解析による流行株の同定）

山田　靖子
国立感染症研究所　動物管理室

１．はじめに

マウス肝炎ウイルス（MHV）感染の病態はMHVの株，マウスの系統・週令・免疫状態等によって様々である。MHVには多くの株が存在するが，肝炎・脳炎等を起こすpolytropicな株と，腸炎を主病変とするenterotropicな株に大別される。自然感染ではenterotropicな株の流行が多く，幼仔では下痢の症状を呈し死亡する例もあるが，成熟マウスでは不顕性である場合が多い。ヌードマウスやscidマウスなどの免疫不全動物では慢性感染を引き起こす。最近，国内実験動物施設でMHVの汚染が多発している。汚染が確認された場合，汚染施設及び飼育動物の清浄化には多大な労力と資金を要する。飼育動物の清浄化は従来の子宮切断術あるいは帝王切開法に加え，近年では胚移植による事例も増えている。いずれの方法においても，作出動物の汚染除去が確実に行われているかを確認することが必要である。MHV汚染検出には大きくふたつの方法が挙げられる。血清中の抗MHV抗体の検出とウイルスあるいはウイルス遺伝子の検出である。２つの方法にはそれぞれ長所と短所があり，検査にあたっては両検査法の特徴を把握しておくことが不可欠である。

２．検査法の長所と短所

抗体検出法は感染後抗体価が上昇した後は長期間確実に汚染が検出できる点が最大の利点である。多くのサンプルを同時に処理出来るので日常的な汚染検査に最適である。しかし，感染初期の抗体価上昇前の動物や抗体を作らない免疫不全動物では検出不能であり，これらの場合汚染を見落とす可能性がある。MHVの多くの株間で抗原性は交叉するので，抗原作製に使用した株に左右されずに抗体検査が可能である反面，抗体反応では流行株の識別はできない。抗体検査の手技のうち，ELISA法や蛍光抗体法は感度が良好であるが，CF反応はそれらより感度が劣るので注意が必要である。一方，ウイルスあるいはウイルス遺伝子の検出は抗体検査では検出不能である感染初期や免疫不全動物でも有効である点が第１の長所である。糞便からRT-PCR法によって検出が可能である[1]ので，動物からの採材が容易で，貴重な動物であっても目的とする動物個体がウイルスを排出しているかを直接知ることができる。ES細胞など動物以外の汚染検査にも有用である。しかし，検体中にウイルスあるいはウイルス遺伝子が存在する場合のみ検出可能であるので，感染後の有効期間が限定される。成熟マウスに実験的に弱毒株を経口投与した場合，糞便中のウイルス遺伝子は感染後10週まで検出されたが，採材した検体や時期によってウイルス排出にばらつきが見られ，採材を単発で行うと汚染を見落とす可能性が挙げられる。実際の汚染コロニーにおいて抗体検査とウイルス遺伝子検出（採材１回）を行ったところ，抗体検査がより高率に陽性であった[1]。RT-PCR法は感度が高いので，サンプル間のcross contaminationを起こさないよう，またウイルス分離は感染性ウイルスを取り扱うので，分離ウイルスが新たな汚染源とならないよう注意を要する。RT-PCR法によって増

Exp. Anim. 53(1), 37–41, 2004

—Note—

Detection of MHV-RNAs in Mouse Intestines and in Filter Dust in Mouse Room Ventilation Duct by a Modified RT-Nested PCR

Mitsuru OYANAGI[1)], Akiko KATO[1)], Yasuko K. YAMADA[2)], and Norimitsu L. SATO[1)]

[1)]*Animal Resources Branch, Center for Bioresource-based Research, Brain Researches Institute, Niigata University, 1-757 Asahimachi-dori, Niigatacity, Niigata 951–8585, and* [2)]*Division of Experimental Animal Research, National Institute of Infectious Diseases, 1–23–1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan*

Abstract: *We applied RT-nested PCR for the detection of MHV genomic RNA in a modified manner to obtain RNA from the intestines of mice and from filter dust in the ventilation ducts of the room in which a contaminated mouse colony was kept. Since the sequences of MHV-RNA that were extracted from the intestine of a serologically MHV-positive mouse in room No. 2 (MS2) and from the filter dust in a ventilation duct in the same room (FD2) were identical, amplified product from filter dust was demonstrated to come from the MHV contaminated room. Furthermore, sequences of FD2 and of filter dust from another contaminated mouse room, No. 7 (FD7) showed 38 nucleotide exchanges among 368-bp (10.3%), suggesting that two different MHV strains were contaminating our facilities. SSCP analysis of Dra I-digested PCR product of 393 bp also showed different patterns in FD2 and FD7 samples.*

Key words: *mouse hepatitis virus, MHV-RNA genome in filter dust, RT-nested PCR*

Mouse hepatitis virus (MHV) contamination in transgenic and knockout mouse colonies has remained prevalent despite continuing efforts to protect against it in animal facilities. Transfer of infected mice to other facilities must be inhibited to avoid the spread of MHV infection among laboratories. It is also absolutely necessary to detect MHV in a mouse colony as early as possible. Reverse transcription and nested polymerase chain reaction (RT-nested PCR) has been shown to be reliable in detecting MHV excreted in the feces during the early stages of a natural outbreak [1–3].

Many types of transgenic or knockout mice have been maintained in our animal facilities in a SPF condition. Recently, however, we noticed MHV contamination in mouse colonies with serological detection of MHV-specific IgG antibody by ELISA (ICLAS, Kanagawa Japan). We used the RT-nested PCR method to detect viral RNA in mice intestines and fresh feces. We also tried to detect MHV-RNA in filter dust in the mouse room ventilation duct by means of RT-nested PCR. The present experiments were undertaken under the Guiding Principles for Animal Experimentation of Niigata

(Received 3 April 2003 / Accepted 8 August 2003)

Address corresponding: M. Oyanagi, Animal Resources Branch, Center for Bioresource-based Researches, Brain Research Institute, Niigata University, 1–757 Asahimachi-dori, Niigatacity, Niigata 951-8585, Japan