Available online at www.sciencedirect.com

SCIENCE DIRECT

Developmental Brain Research 155 (2005) 99–106

DEVELOPMENTAL BRAIN RESEARCH

www.elsevier.com/locate/devbrainres

Research report

Mutation at the *Lmx1a* locus provokes aberrant brain development in the rat

Mitsuru Kuwamura[a,*], Takehiko Muraguchi[b], Tamiko Matsui[a], Mayumi Ueno[a], Shigeo Takenaka[c], Jyoji Yamate[a], Takao Kotani[a], Takashi Kuramoto[b], Jean-Louis Guénet[d], Kazuhiro Kitada[b,e], Tadao Serikawa[b]

[a]*Laboratory of Veterinary Pathology, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan*
[b]*Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8501, Japan*
[c]*Laboratory of Cellular and Molecular Biology, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan*
[d]*Unité de Génétique des Mammifères, Institut Pasteur, 25 Rue du Docteur Roux, F-75724 Paris Cedex 15, France*
[e]*Laboratory of Mammalian Genetics, Center for Advanced Science and Technology, Hokkaido University, 060-0810 Sapporo, Japan*

Accepted 20 December 2004
Available online 24 February 2005

Abstract

A rat short-tail mutation with neurological defects (named *queue courte*, *qc*) was discovered. Histopathology in adult *qc*/*qc* rats revealed hypoplasia of the cerebellum and hippocampus, maldevelopment of the choroid plexus and corpus callosum. These abnormalities are strongly reminiscent of the phenotypic abnormalities found in the shaker short-tail or dreher (*dr*) mouse mutation at the LIM homeobox transcription factor 1 alpha locus (*Lmx1a*). The *qc* mutation is an autosomal recessive and has been mapped to the *dr* homologous region on rat chromosome 13, and Northern blot analysis demonstrated no expression of *Lmx1a* in *qc*/*qc* rats. Narrowing and distortion of the ventricles were observed from embryonic day 17 (E17) in *qc*/*qc* rats. From E17, fusion of the opposing neuroepithelium and formation of neuroepithelial rosettes were also found. Arrangements of neuroepithelial cells were disturbed and processes of radial glia were disoriented in the fused lesions. Neuronal migration analysis using BrdU immunohistochemistry revealed defective migration from the neuroepithelium toward the neocortex and mesencephalon in *qc*/*qc* rats. These findings suggest that the *qc* mutation is involved in development of the ventricular system and dorsal migration of neurons.
© 2005 Elsevier B.V. All rights reserved.

Theme: Disorders of the nervous system
Topic: Genetic models

Keywords: Dreher; *Lmx1a*; Neural migration; Neuroepithelial rosette; Ventricular system

1. Introduction

Because of its larger size, the laboratory rat (*Rattus norvegicus*), rather than the mouse, has been frequently used for investigation in basic neuroscience and neurological disorders. Using animals of this species for the development and assessment of new therapies, including gene therapy, is also attractive even if the knockout technology is not possible in the rats as it is in the mouse [21,22]. For these reasons, spontaneous rat mutations exhibiting neurological disorders must be maintained and characterized as carefully and as extensively as possible and considered as potential models for human diseases.

The mouse mutation *dreher* (*dr*-, chromosome 1) is an autosomal recessive allele that exhibits a wide range of phenotype, including behavioral, neurological, and skeletal abnormalities; hypoplasia of Müllerian duct derivatives and pigmentation anomalies [2,20]. Pathology of the brain of the *dr*/*dr* mouse reveals disruption of the neocortex and

* Corresponding author. Fax: +81 72 250 7208.
E-mail address: kuwamura@vet.osakafu-u.ac.jp (M. Kuwamura).

0165-3806/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.devbrainres.2004.12.009

hippocampus, cerebellar hypoplasia, cerebellar foliation, and lamination defects [2,16]. The *dr* mutation has been identified in the gene encoding a member of the LIM homeobox gene family: the LIM-1A homeodomain transcription factor [13]. Since this observation, the mutant allele has been renamed *Lmx1a*^{dr}.

The LIM homeobox gene family encoding the LIM homeodomain transcription factors consists of two paralogues: *Lmx1a* and *Lmx1b*. *Lmx1a* plays an important role in regional and cell fate specification during development [1,9] and the gene is expressed in roof plate of the developing neural tube, suggesting a role for roof plate formation [4,11–14,19]. *Lmx1b* contributes an essential role for limb and kidney development in mouse [3].

In this report, we describe the observation of a new spontaneous rat mutation with abnormal behavior and tail anomalies. Breeding records indicated that the mutation is inherited as an autosomal recessive trait with complete penetrance but variable expressivity. The phenotypic resemblance to the short-tail mutant of the mouse *dr* locus arose our interest to analyze in genetically and pathologically from the point of view of comparative study between rat and mouse. Here, we describe the identification of the causative gene by a positional candidate cloning approach using the current rat genome sequence database, and pathological findings of *qc*/*qc* rats, especially in the brain chronologically analyzed.

2. Materials and methods

2.1. Animals

The short-tail mutation, "queue courte" in French (with *qc* as a symbol), occurred spontaneously in 1994, in the ACI/Pas inbred strain of rat maintained at the Institute Pasteur (Paris, France), and was kept segregating in this stock. Since importation into the Institute of Laboratory Animals at Kyoto University, it has been maintained as a congenic strain F344.ACI-*qc* using F344/NSlc as an inbred partner.

Affected (*qc*/*qc*) and control (+/*qc* or +/+) rats used in pathological analysis were obtained from N2F2. F344.ACI-*qc*/*qc* and F344/NSlc rats used for Northern blot analyses were obtained from the Institute of Laboratory Animals, Kyoto University. Rats were handled according to the Guidelines for Animal Experimentation, Osaka Prefecture University and the Guideline for Animal Experiments of Kyoto University. BN/Orl inbred rats were purchased from the Centre de Techniques Avancées of CNRS (CDTA, Orléans-la-Source, France).

2.2. Genetic markers and linkage analysis

Primers for microsatellite markers used in this experiment were purchased from Research Genetics (Huntsville, Ala.). A total of 80 markers, evenly distributed over the whole rat genetic map and revealing polymorphism between ACI/Pas and BN/Orl, were selected and used for the systematic analysis of haplotype segregation in *qc*/*qc* rats produced from the N2 cross. Another set of 11 polymorphic markers was added to refine the *qc* locus. Genomic DNA preparation, PCR amplifications, and gel-electrophoresis were performed as described previously [17].

2.3. RNA isolation and Northern blotting

Total RNA was prepared from the cerebellum of 7-day-old *qc*/*qc* rat and control F344/NSlc rats using ISOGEN (NIPPON GENE, Tokyo, Japan). Poly-A RNA was purified using Oligotex-dT30 Super (Roche Diagnostics, Mannheim, Germany). Two micrograms of poly-A RNA was electrophoresed on formaldehyde-denaturing agarose gel and blotted onto Hybond N+ Nylon membrane (Amersham Bioscience Corp., NJ, USA). A 584-bp partial cDNA fragment was amplified by RT-PCR from the total RNA of F344/NSlc cerebellum and used as a probe for hybridization. The oligonucleotide primers were designed from the rat *Lmx1a* gene (XM_222902) and their nucleotide sequences were 5′-ATTGCGCCCAATGAGTTTGT-3′ and 5′-GTGGGCAACGTTGTATAGGG-3′.

2.4. Histopathology

To characterize the mutant phenotype, *qc*/*qc* rats aged 2 (n = 6), 3 (n = 3), 4 (n = 2), 5 (n = 2), >16 (n = 8) weeks were sacrificed under anesthesia, the brain and spinal cord were immediately removed and fixed in 10% neutral buffered formalin. Brains were cut coronally at various levels of the cerebrum, cerebellum, and brain stem, or sagitally at the midline. The samples were embedded in paraffin and sections were stained with hematoxylin and eosin (HE) and Nissl's stain. For chronological investigation of brain development in *qc*/*qc* rat, we examined embryonic day 17 (E17), E19, E21 (the day with the first sperm-positive vaginal smear being counted as embryonic day 1), postnatal day 0 (P0), and 21-week-old rats. At least four affected mutant and control rats were examined at each point. Genotypes of fetus and neonates were assessed by PCR analysis with microsatellite markers closely flanking the *qc* locus. The brains were removed and fixed with methacarn solution (methanol 6:chloroform 3:acetic acid 1) or Bouin fixative solution (picric acid 15:formalin 5:acetic acid 1) and embedded in paraffin wax. Sections were then cut and stained with HE for histological evaluation. Representative sections were also stained for myelin with Luxol fast blue staining.

2.5. BrdU labeling and immunohistochemistry

Pregnant females and neonate rats were injected intraperitoneally with 0.5 mg bromodeoxyuridine (BrdU, Sigma, CA, USA), which was dissolved in physiological saline, on E17, E19, and E21. To confirm the distribution of BrdU-

Download English Version:

https://daneshyari.com/en/article/9414687

Download Persian Version:

https://daneshyari.com/article/9414687

Daneshyari.com