Brain Research Bulletin 97 (2013) 63–68

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

Molecular layer heterotopia of the cerebellar vermis in mutant and transgenic mouse models on a C57BL/6 background

Raddy L. Ramos[a,*], Sarah E. Van Dine[a], Elizabeth George[b], Dhruv Patel[b], Blair J. Hoplight[c], Joerg R. Leheste[a], Eric K. Richfield[d], German Torres[a]

[a] Department of Biomedical Sciences, New York Institute of Technology, College of Osteopathic Medicine, Old Westbury, NY 11568, United States
[b] Department of Life Sciences, New York Institute of Technology, Old Westbury, NY 11568, United States
[c] Department of Behavioral Sciences, New York Institute of Technology, Old Westbury, NY 11568, United States
[d] Department of Pathology and Lab Medicine, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, United States

ARTICLE INFO

Article history:
Received 15 February 2013
Received in revised form 1 May 2013
Accepted 6 May 2013
Available online 2 June 2013

Keywords:
Cerebellar dysplasia
Heterotopia
Malformation
Lamination
Foliation
Mouse models

ABSTRACT

C57BL/6 mice exhibit spontaneous cerebellar malformations consisting of heterotopic neurons and glia in the molecular layer of the vermis (Tanaka and Marunouchi, 2005; Mangaru et al., 2013). Malformations are only found between folia VIII and IX and are indicative of deficits of neuronal migration during cerebellar development. In the present report we test the prediction that mutant and transgenic mouse models on a C57BL/6 background will also exhibit these same cerebellar malformations. Consistent with our hypothesis, we found that 2 spontaneous mutant models of Parkinson's disease on a C57BL/6 background had cerebellar malformations. In addition, we found that numerous transgenic mouse lines on a full or partial C57BL/6 background including eGFP-, YFP- and Cre-transgenic mice also exhibited heterotopia. These data suggest that histological analyses be performed in studies of cerebellar function or development when using C57BL/6 or other mice on this background in order for correct interpretation of research results.

1. Introduction

C57BL/6 mice are among the most widely used in neuroscience research. Because of the widespread use of this strain, numerous transgenic and knockout (KO) models of human diseases have been engineered on a C57BL/6 background. Surprisingly, an often overlooked study indicated the presence of spontaneous cerebellar malformations affecting the vermis in C57BL/6 mice which were characterized by the presence of heterotopic granule cells in the molecular layer between folia VIII and IX (Tanaka and Marunouchi, 2005). Laminar and morphological defect of Purkinje cells, Golgi cells, and molecular layer interneurons is also observed in cerebellar heterotopia in these mice (Tanaka and Marunouchi, 2005; Mangaru et al., 2013). These data raise the possibility that malformations in C57BL/6 mice could affect results for studies assessing cerebellar anatomy, physiology, and function.

Cerebellar heterotopia in affected C57BL/6 mice are indicative of deficits in neuronal migration of granule neurons (Tanaka and Marunouchi, 2005; Mangaru et al., 2013). Consistent with a model of migration deficits, malformations are evident as early as postnatal day 4 (Tanaka and Marunouchi, 2005; Mangaru et al., 2013) when granule neurons are actively migrating to the internal granule cell layer. Moreover, the spatial arrangement and morphology of Bergman glia is altered in mice with heterotopia along with apparent defect of the glial limitans (Mangaru et al., 2013). Thus, alteration in glial limitans formation likely affects the morphology of Bergmann glia via deficits in stable end foot attachment of radial fibers to the pia. Reduced or abnormally-organized radial fibers ultimately leads to the migration defects of granule cells (Mangaru et al., 2013).

In a recent report, we demonstrated the presence of cerebellar molecular layer heterotopia (MLH) in substrains of C57BL/6 mice sold by several commercial vendors (Mangaru et al., 2013) suggesting a genetic origin of MLH formation. Consistent with this hypothesis, heterotopia were also observed in the closely-related C57BL/10 mouse strain. These data also suggest that preclinical mouse models on a C57BL/6 background might also display heterotopia, including mutant mice and transgenic mice. In the present report, we examine mutant and transgenic mouse models on a

* Corresponding author at: Department of Biomedical Sciences, New York Institute of Technology College of Osteopathic Medicine, Northern Boulevard, PO Box 8000, Old Westbury, NY 11568-8000, United States. Tel.: +1 5166861318; fax: +1 5166863750.
E-mail address: rramos02@nyit.edu (R.L. Ramos).

0361-9230/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.brainresbull.2013.05.001

C57BL/6 background and indeed document the presence of cerebellar MLH.

2. Materials and methods

Pitx3^{ak}/2J mice (*aphakia*; The Jackson Laboratory) harbor a spontaneous mutation of the *Paired-like homeodomain transcription factor 3* (*Pitx3*) locus resulting in a null allele which spontaneously arose in a colony of 129S1/SvSl/J mice (Varnum and Stevens, 1968; Rieger et al., 2001). This strain was then maintained on a mixed C57BLKS/J and C57BL/6J background. *Aphakia* mice were obtained from Dr. C.J. Ziess (Yale University School of Medicine). Homozygous null mice which are easily identifiable due to obvious microphthalmia (Varnum and Stevens, 1968; Rieger et al., 2001) were bred to maintain a homozygous line at the New York Institute of Technology (NYIT). C57BL/6JOlaHsd mice which harbor a chromosomal deletion of the α-synuclein locus (*Scna*) (Specht and Schoepfer, 2001, 2004) were obtained from Harlan Laboratories and bred to maintain a homozygous line as previously described (Prasad and Richfield, 2008; Prasad et al., 2011). B6.Cg-Tg(Thy1-YFP)HJrs/J mice (Thy1-YFP mice) which express yellow fluorescent protein in a number of cortical and subcortical structures were purchased from The Jackson Laboratory (stock no. 003782) and bred at NYIT. According to the initial description of these mice (Feng et al., 2000), the genotype of transgenic founders were C57BL6/J-CBA F1 hybrids. Transgenic founders were backcrossed to C57BL/6J mice for 1–4 generations (Feng et al., 2000). B6.Cg-Tg(RP23-268L19-EGFP)2Mik/J mice (ChAT-eGFP mice) which express eGFP in central and peripheral cholinergic neurons (Tallini et al., 2006) were purchased from The Jackson Laboratory and bred at NYIT. Founders were created by pronuclear injection into B6/D2 F2 fertilized mouse eggs. Transgenic founders were then backcrossed to C57BL/6J mice for at least 5 generations (jaxmice.jax.org/strain/007902.html).

Aphakia, ChAT-eGFP, and Thy1-YFP mice were bred and housed in temperature-controlled facilities, maintained between 20 and 22.5 °C, 20% humidity, with 12 hour light/dark cycles, in standard plastic cages (11.75 x 7.5 x 5; in inches) with no more than 5 mice per cage (3 mice for breeding cages), and with food and water available *ad libitum*. All *aphakia*, ChAT-eGFP, and Thy1-YFP mice used in the present study were from a breeding colony in our vivarium established over 10 generations ago. Mice were aged between 60 and 180 days since by 30 days of age, all granule cells have migrated to the internal granule layer in the normal mouse brain. Moreover, we have not observed any changes in the cytoarchitecture of heterotopia after postnatal day 21 and heterotopia are identical in male and female mice (Mangaru et al., 2013). As previously described, (Lipoff et al., 2011), breeding schemes at New York Institute of Technology were mixed and included both sibling and non-sibling matings. All measures were taken to minimize pain or discomfort in mice and experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23). All procedures were approved by the Institutional Animal Care and Use Committee at the New York Institute of Technology.

Mice were sacrificed, brains removed, and histological methods were used as previously described (Lipoff et al., 2011; Mangaru et al., 2013).

We used the Allen Brain Atlas (ABA; brain-map.org) as previously described (Ramos et al., 2008; Mangaru et al., 2013). In particular, data from the Mouse Connectivity database which includes histological data from numerous Cre "driver" transgenic mouse lines (*Projection* dataset) as well as Cre-driver lines that have been crossed with lox "reporter" mouse lines (*Transgenic Characterization* dataset). A total of 1043 cases from 153 unique transgenic and Cre-lox crossed lines were examined from the *Transgenic Characterization* dataset. A total of 128 cases from 35 unique Cre-driver lines were examined from the *Projection* dataset. Approximately 6–10 photomicrographs from each case in these datasets were examined and mice with MLH were documented and digital photomicrographs were archived and annotated. Detailed information regarding the methods and mice used in the creation of the Mouse Connectivity database can be found on the ABA website.

3. Results

Heterotopia identical to those described by Tanaka and Marunouchi (Tanaka and Marunouchi, 2005) and our group (Mangaru et al., 2013) were observed in both *aphakia* and C57BL/6JOlaHsd mice. In particular, 3 of 11 (27.27%) male and 4 of 13 (30.77%) female *aphakia* mice exhibited MLH, while 3 of 9 (33.33%) female and 1 of 6 (16.67%) male C57BL/6JOlaHsd mice exhibited MLH. Representative examples are illustrated in Fig. 1. MLH in both *aphakia* and C57BL/6JOlaHsd mice were found along the midline of the vermis and exclusively affected folia VIII and IX as is observed in C57BL/6 and C57BL/10 mice (Tanaka and Marunouchi, 2005; Mangaru et al., 2013). Chi-square analysis of the prevalence of MLH in *aphakia* and C57BL/6JOlaHsd revealed no significant differences ($p > 0.05$), nor where there any sex differences observed. These data demonstrate that mutant mice on a C57BL/6 background or mutant mice backcrossed into a C57BL/6 background can exhibit MLH.

As a first-step in testing whether transgenic mice on a C57BL/6 background might also exhibit MLH, we rigorously examined digital photomicrographs found in the Mouse Connectivity database of the ABA using methods previously described (Ramos et al., 2008; Mangaru et al., 2013). This type of analysis can provide evidence that a given mouse line exhibits some prevalence of MLH when at least one case of heterotopia is identified. However, this analysis cannot exclude the possibility that MLH are found in a given line

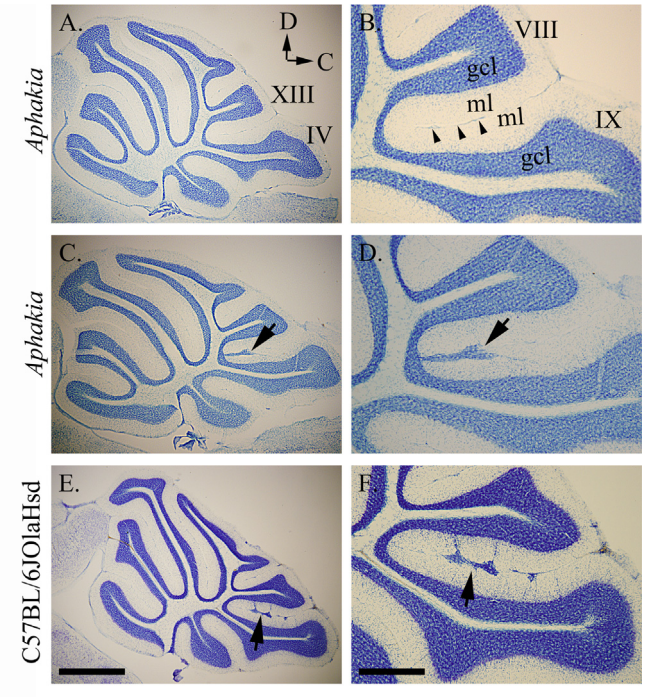

Fig. 1. *Aphakia* mice and C57BL/6JOlaHsd mice have cerebellar MLH. Representative photomicrographs of *aphakia* mice with normal cerebellar lamination (A) and (B) and with heterotopia affecting folia VIII and IX (C) and (D). Representative photomicrographs of MLH in a C57BL/6JOlaHsd mouse (E) and (F). Abbreviations: ml = molecular layer; gcl = granule cell layer. Scalebars in microns: A, C and E = 1000; B, D and F = 400.