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Research Paper

Mice overexpressing lamin B1 in oligodendrocytes recapitulate the age-dependent motor signs, but not the early autonomic cardiovascular dysfunction of autosomal-dominant leukodystrophy (ADLD)

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ABSTRACT

Autosomal dominant leukodystrophy (ADLD) is a rare adult-onset demyelinating disease caused by overexpression of lamin B1, a nuclear lamina filament. Early autonomic dysfunction involving the cardiovascular system before progressive somatic motor dysfunction is a striking feature of most cases of ADLD. In the *Plp-FLAG-LMNB1* transgenic mouse model, lamin B1 overexpression in oligodendrocytes elicits somatic motor dysfunction and neuropathology akin to ADLD. Here, we investigate whether *Plp-FLAG-LMNB1* mice also develop autonomic cardiovascular dysfunction before or after somatic motor dysfunction. We find that *Plp-FLAG-LMNB1* mice have preserved cardiovascular responses to changes in wake-sleep state and ambient temperature and normal indexes of autonomic modulation at 37–42 weeks of age despite a progressive somatic motor dysfunction, which includes impairments of walking ability (the ability to walk on a narrow path was impaired in 80% of mice at 34–38 weeks of age) and subtle breathing derangements. Only late in the development of the disease phenotype did *Plp-FLAG-LMNB1* mice develop a structural deficit of sympathetic noradrenergic fibers, with a 38% decrease in fiber profiles in the kidneys at 44–47 weeks of age. We demonstrate that while the *Plp-FLAG-LMNB1* mouse model recapitulates the age-dependent motor dysfunction of ADLD, it does not show signs of early autonomic cardiovascular dysfunction, raising the possibility that oligodendrocyte dysfunction may not be sufficient to cause the full spectrum of clinical features present in ADLD.

1. Introduction

Autosomal dominant leukodystrophy (ADLD; OMIM #169500) is a rare adult-onset demyelinating disease, which shows some clinical overlap with multiple sclerosis (Eldridge et al., 1984; Nahhas et al., 2016). ADLD starts clinically in the 4th–5th decades of life with 100% penetrance and no gender variation, and is fatal after a generally slow progression (Nahhas et al., 2016). Autonomic derangements, which may include constipation, bladder symptoms, erectile dysfunction, and orthostatic hypotension (Guaraldi et al., 2011; Terlizzi et al., 2016), are the first symptoms in most patients with ADLD. Orthostatic hypotension is particularly disabling, as it contributes to impair walking ability

Abbreviations: ADLD, adult-onset autosomal dominant leukodystrophy; BRS, index of cardiac baroreflex sensitivity; EEG, electroencephalogram; EMG, electromyogram; HP, heart period; *LMNB1*, lamin B1 gene; N, non-rapid-eye-movement sleep; PBS, phosphate buffered saline; *Plp1*, proteolipid protein 1; pNN8, index of parasympathetic modulation of heart period computed in the time domain; R, rapid-eye-movement sleep; RSA, index of parasympathetic modulation of heart period computed in the frequency domain; SAP, systolic arterial pressure; SD_1 and SD_2, indexes of short-term and long-term variability of total breath duration, respectively; SYM, index of sympathetic modulation of arterial pressure computed in the frequency domain; TG, mice hemizygote for the *Plp-FLAG-LMNB1* transgene; T_{TOT}, total breath duration; V_E, minute volume; V_T, tidal volume

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(Finnsson et al., 2015; Guaraldi et al., 2011; Terlizzi et al., 2016). These symptoms are accompanied by a loss of noradrenergic sympathetic innervation (Guaraldi et al., 2011; Terlizzi et al., 2016), and followed by progressive motor dysfunction, with impaired walking ability because of leg spasticity, weakness, and ataxia, and with pseudobulbar palsy in the terminal stages (Finnsson et al., 2015). Nonetheless, exceptions to this disease progression pattern have been documented: in some atypical cases, significant autonomic dysfunction either is detected after somatic motor dysfunction, or is not detected at all (Finnsson et al., 2015; Potic et al., 2013; Quattrocolo et al., 1997).

ADLD is caused by genomic mutations, i.e., duplications involving the lamin B1 (*LMNB1*) gene or deletions upstream of the gene, both leading to overexpression of lamin B1 (Giorgio et al., 2015; Padiath et al., 2006). A key role of oligodendrocytes was supported by the demonstration that *Plp-FLAG-LMNB1* transgenic mice (TG) overexpressing lamin B1 selectively in oligodendrocytes develop walking deficits at 40 weeks of age, and progress rapidly (in two months) to a terminal disease stage, when they become moribund (Heng et al., 2013). These mice also showed seizures, brainstem demyelination, and axonal degeneration (Heng et al., 2013). A later study on an independently-derived TG mouse strain established a molecular link between lamin B1 overexpression and lipid synthesis in oligodendrocytes (Rolyan et al., 2015). Autonomic cardiovascular derangements were not investigated by either of these studies.

Considering that autonomic dysfunction is a cardinal symptom in most ADLD cases, we carried out an extensive characterization of autonomic cardiovascular deficits in TG mice in order to better understand the mechanisms underlying ADLD disease pathology. In particular, we tested the hypothesis that autonomic cardiovascular dysfunction precedes the occurrence of somatic motor dysfunction in TG mice, as it is found in most patients with ADLD.

2. Material and methods

2.1. Animal research ethics

The study protocol complied with the EU Directive 2010/63/EU for animal experiments and was approved by the Committees on the Ethics of Animal Experiments of the University of Bologna and of the Italian Ministry of Education, University, and Research. Surgery was performed under isoflurane anesthesia, and all efforts were made to minimize suffering.

2.2. Mice

Experiments were performed on TG (*Plp-FLAG-LMNB1*) mice (Rolyan et al., 2015) and on their wild-type (WT) control littermates on a FVB/N background. TG mice overexpress the lamin B1 gene under control of the murine proteolipid protein 1 (*Plp1*) promoter, which is preferentially expressed in oligodendrocytes. A mouse colony was established at the University of Bologna (Department of Veterinary Medical Sciences, Ozzano dell'Emilia, Italy and Department of Biomedical and Neuromotor Sciences, Bologna, Italy) from founder mice from the colony of Prof. Q. Padiath at the Department of Human Genetics, University of Pittsburgh, PA, USA, and from FVB/N mice purchased from Charles River, Italy (Calco, Italy). Breeding was performed with hemizygote × WT mating and TG mice were identified by genotyping with standard polymerase chain reactions (Rolyan et al., 2015). Mice were housed under a 12:12-h light–dark cycle with ambient temperature set at 23 °C and free access to water and food (4RF21diet; Mucedola, Settimo Milanese, Italy).

2.3. Overview of the experimental protocol

The experiments were performed on mice of 3 age groups. The age groups I and III corresponded to 24–27 and 44–47 (range) weeks of age, respectively. The age group II corresponded to 34–38 weeks of age for walking and water licking tests. A subset of mice of age group II was surgically instrumented after these tests to undergo respiratory phenotyping at 36–40 weeks of age, cardiovascular phenotyping at 37–42 weeks of age, and euthanasia immediately afterwards with tissue explant for immunohistochemistry. Based on the previous reports on TG mice (Heng et al., 2013; Rolyan et al., 2015), somatic motor deficits were expected to be absent for age group I, mild for age group II, and severe for age group III. It was thus expected that TG mice of age group II would be the focus of the work, aimed at investigating whether these mice had autonomic defects before somatic motor defects became severe in age group III. At present, there is no evidence indicating gender variation of ADLD (Nahhas et al., 2016). We performed all experiments on female mice. In addition, we replicated on male mice the tests of walking ability for each age group and of sympathetic innervation for age group III.

2.4. Tests of walking ability

Mice were tested for their walking ability on a narrow path (ledge test) and in an open field using a structured assessment (Guyenet et al., 2010). For the ledge test, mice were gently lifted and positioned on the 5-mm ledge of a rectangular cage. Mice were assigned the following scores: 0, walk on the cage ledge without hind paw slips, and/or descent into the cage landing on paws; 1, repeated hind-paw slips; 2, ineffective use of the hind legs, or uncoordinated descent into the cage not landing on paws; 3, falls off the ledge and/or refusal to move despite encouragement by gentle nudges. For the open field walking test, mice were left free to walk on a large flat surface and assigned the following scores: 0, coordinated walk with body weight support on all limbs, the abdomen not touching the ground, and even participation of both hind limbs; 1, mild tremor and/or limp; 2, severe tremor and/or limp, lowered pelvis, or feet pointing away from the long axis of the body; 3, difficulty moving forward, with the abdomen dragging along the ground. These evaluations were performed by trained investigators blind to the mouse genotype, and were repeated 3 times, with the final scores computed as the median values of the 3 trial scores for each test. The third trial was omitted for mice that received scores of 0 at the ledge and open field tests for the first and second trial. The sample sizes for the tests of walking ability are reported in Table S1.

2.5. Test of water licking

Water licking was recorded during the 12-h dark period in the mouse home cage by measuring the junction potential between the steel spout of a water bottle and an aluminum foil on the cage bottom every time the mouse licked the spout, thereby closing the electrical circuit between the foil and the spout with its body (Hayar et al., 2006). The junction potential voltage signals were manually reviewed to reject artefacts and identify trains of ≥ 4 licks. The inter-lick time interval (onset to onset) was computed for each lick, and its median value over the whole recording was retained for each mouse. The sample sizes for the test of water licking are reported in Table S2.

2.6. Surgery

Mice underwent surgery under isoflurane anesthesia (1.8–2.4% in O_2) with intra-operative analgesia (Carprofen 0.1 mg subcutaneously, Pfizer Italy, Latina) as previously described in detail (Silvani et al., 2009). Briefly, mice were instrumented with 2 screw electrodes for electroencephalogram (EEG) recordings (frontal-parietal differential lead) and 2 wire electrodes in the neck muscles for electromyogram (EMG) recordings. A calibrated telemetric arterial pressure transducer (TA11-PAC10, DSI, Tilburg, The Netherlands) was implanted subcutaneously, with the catheter tip advanced via the femoral artery until below the renal arteries.