GRK2-Targeted Knockdown as Therapy for Multiple System Atrophy

Miguel Lopez-Cuina, MD, PhD,[1,2,3,4] Paul Guérin, PhD,[1,2] Nathalie Dutheil, PhD,[1,2] Christelle Martin, PhD,[5] Thierry Leste Lasserre, PhD,[6] Pierre-Olivier Fernagut, PhD,[7] Wassilios G. Meissner, MD, PhD,[1,2,8,9] and Erwan Bezard, PhD[1,2*]

[1]*Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France* [2]*CNRS, Institut des Maladies Neurodégénératives, UMR 5293, Bordeaux, France* [3]*Neurology Department, Hospital Universitario Reina Sofía, Córdoba, Spain* [4]*Instituto Maimónides de Investigación Biomédica de Córdoba (IMIBIC), Córdoba, Spain* [5]*Univ. de Bordeaux, Interdisciplinary Institute for Neuroscience, UMR 5297, Bordeaux, France* [6]*Univ. Bordeaux, INSERM, PUMA, Neurocentre Magendie, U1215, Bordeaux, France* [7]*Université de Poitiers, Laboratoire de Neurosciences Expérimentales et Cliniques, INSERM UMR_S 1084, Poitiers, France* [8]*CHU Bordeaux, Service de Neurologie des Maladies Neurodégénératives, IMNc, Bordeaux, France* [9]*Department of Medicine, University of Otago, Christchurch, and New Zealand Brain Research Institute, Christchurch, New Zealand*

ABSTRACT: Background: Multiple system atrophy (MSA) is a sporadic adult-onset rare neurodegenerative synucleinopathy for which counteracting central nervous system insulin resistance bears the potential of being neuroprotective. G-protein-(heterotrimeric guanine nucleotide-binding protein)-coupled receptor kinase 2 (GRK2) is emerging as a physiologically relevant inhibitor of insulin signaling.
Objectives: We tested whether lowering brain GRK2 abundance may reverse insulin-resistance.
Methods: We lowered brain GRK2 abundance through viral-mediated delivery of a GRK2-specific miRNA and quantified the reversion of a developing or an established insulin-resistant phenotype using the transgenic PLP-SYN mouse model of MSA.
Results: Viral vector delivery of a GRK2 miRNA demonstrated a neuroprotective capacity when administered (1) in utero intracerebroventricularly in developing PLP-SYN mice and (2) intrastriatally in adult PLP-SYN mice. Decreased striatal GRK2 levels correlated in both designs with neuroprotection of the substantia nigra dopamine neurons, reduction in high-molecular-weight species of α-synuclein, and reduced insulin resistance.
Conclusions: These data support GRK2 as a potential therapeutic target in MSA. © 2023 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: insulin resistance; synucleinopathy; in utero

Introduction

Multiple system atrophy (MSA) is a sporadic adult-onset rare neurodegenerative disorder clinically characterized by a variable combination of parkinsonism, cerebellar impairment, and autonomic dysfunction.[1] The cytopathological hallmark of MSA is the accumulation of α-synuclein (α-syn) aggregates in oligodendrocytes, forming glial cytoplasmic inclusions.[1,2] Currently no treatment is available to mitigate symptom severity or clinical progression. Developing neuroprotective treatments for MSA is an immediate unmet need.[3] Impaired insulin/insulin-like growth factor-1 (IGF-1) signaling and insulin resistance (ie, decreased insulin/IGF-1 signaling) are critical features of MSA.[4,5] Counteracting such insulin resistance bears the potential of being neuroprotective.[5]

G-protein-(heterotrimeric guanine nucleotide-binding protein)-coupled receptor kinase 2 (GRK2) integrates several signal transduction pathways and is emerging as a physiologically relevant inhibitor of insulin signaling.[6,7] Interestingly, GRK2 abundance is knowingly increased in humans with metabolic syndromes and in different murine models of insulin resistance.[8] Reversal of diet-induced obesity and insulin resistance by inducible genetic ablation of GRK2[8] put forward this enzyme as a potential therapeutic target for insulin resistance in general.

To support GRK2 as a potential therapeutic target in MSA, we investigated whether lowering brain GRK2 abundance through viral-mediated delivery of a

***Correspondence to:** Dr. Erwan Bezard, Univ. de Bordeaux, Institut des Maladies Neurodégénératives, UMR 5293, F-33000 Bordeaux, France; E-mail: erwan.bezard@u-bordeaux.fr

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GRK2-specific miRNA may reverse a developing and an established insulin-resistant phenotype using the transgenic proteolipid protein human α-syn (PLP-SYN) mouse model of MSA.[5,9]

Materials and Methods

Animals. All experiments involving mice were performed following French guidelines (87-848, Ministere de l'Agriculture et de la Foret) and the European Community Council Directive (2010/63/EU) for the care of laboratory animals. Animal experiments were approved by the Institutional Animal Care and Use Committee of Bordeaux (CE50, license no.: 50120100-A). Mice were maintained in a temperature- and humidity-controlled room on a 12-hour light–dark cycle with food and water ad libitum. Mice expressing human α-syn in oligodendrocytes under the control of the proteolipid promoter (PLP-SYN) were previously produced on a C57BL/6J background. Overexpression of α-syn in oligodendrocytes promotes the accumulation of α-syn and neurodegeneration resembling MSA.[5,9,10]

All experimental details are provided in the Supporting Information.

Results

We designed a specific GRK2 miRNA for conducting in vivo experiments. After careful in vitro screening, we selected an miRNA sequence that decreases GRK2 levels by 76% compared to a scramble miRNA in NIH3T3 mouse cells (not shown). The GRK2 miRNA and scramble sequences were incorporated into an AAV2/9 cassette, co-expressing the green fluorescent protein (GFP) under a cytomegalovirus (CMV) promoter to produce 2.5×10^{13} vg titers of the AAV-CMV-emGFP-GRK2 miRNA and the AAV-CMV-emGFP-scramble miRNA.

Degeneration and symptoms of MSA begin in adulthood, but the transgenic PLP-SYN mouse model[10] involves constitutive oligodendroglial human α-syn overexpression. We sought to determine whether the intrauterine (E14) intracerebroventricular delivery[11] of GRK2 miRNA could offer adequate protection for the late-onset development of behavioral and pathological abnormalities in this mouse model (Fig. 1). The AAV-CMV-emGFP-GRK2 miRNA group (PLP GRK2 KD) showed reduced motor impairment compared to the AAV-CMV-emGFP-scramble miRNA group (PLP scramble) (Fig. 1B). The PLP GRK2 KD group was not different from the wild-type sham (WT sham) group (Fig. 1B). According to the working hypothesis, such behavioral improvement was concomitant with reduced striatal GRK2 levels in the PLP GRK2 KD compared to PLP scramble (Fig. 1C). Such GRK2-level reduction led to reduced striatal insulin resistance evidenced by decreased striatal IRS1 phospho-S307 levels in the PLP GRK2 KD compared to PLP scramble, although still higher than that in the WT sham (Fig. 1D). The number of substantia nigra pars compacta (SNc) neurons mirrored this improved behavior, with a preserved number of neurons in the PLP GRK2 KD compared to PLP scramble, not different from the WT sham (Fig. 1E). Comparably, striatal high-molecular-weight (HMW) α-syn species, ie, α-syn oligomers, were reduced in the PLP GRK2 KD compared to PLP scramble, although still higher than that in the WT sham (Fig. 1F). In utero knockdown of striatal GRK2 levels, therefore, resulted in diminished insulin resistance over time and conferred SNc neuroprotection and decreased HMW α-syn species in 6-month-old PLP-SYN mice.

We wondered whether striatal GRK2 knockdown in 2-month-old adult PLP-SYN mice, ie, before degeneration of SNc neurons that occurs between 3 and 6 months of age in this model, could achieve similar protection (Fig. 2), adopting a clinically relevant design. Reduced striatal GRK2 levels were completed in the PLP GRK2 KD group compared to the PLP scramble group (Fig. 2B), as was reduced insulin resistance measured through striatal IRS1 phospho-S307 levels (Fig. 2C). Such reductions were concomitant with a decrease in motor abnormalities (Fig. 2D), protected SNc neurons (Fig. 2E), and reduced striatal HMW α-syn levels (Fig. 2F) without affecting overall α-syn levels (Fig. 2G) in the GRK2 KD group compared to the PLP scramble group. Adulthood striatal knockdown of GRK2 levels resulted in diminished insulin resistance over time, conferred SNc neuroprotection, and decreased HMW α-syn species in 8-month-old PLP-SYN mice, without affecting murine or human α-syn mRNA levels in this transgenic model (Fig. S1).

Discussion

Growing evidence suggests that insulin resistance contributes to the progressive loss of neurons in MSA,[5] similar to what has been reported for the most frequent neurodegenerative disorders, Alzheimer's disease, and Parkinson's disease (PD).[4] These findings have led to numerous studies in preclinical models and clinical trials in neurodegenerative disorders (including a small ongoing open-label trial in MSA, NCT04431713) targeting insulin/IGF-1 and GLP-1 signaling with currently available anti-diabetics. The present results demonstrate that reducing insulin resistance through direct signaling interference and restoring proper signaling through the insulin receptors have positive effects on

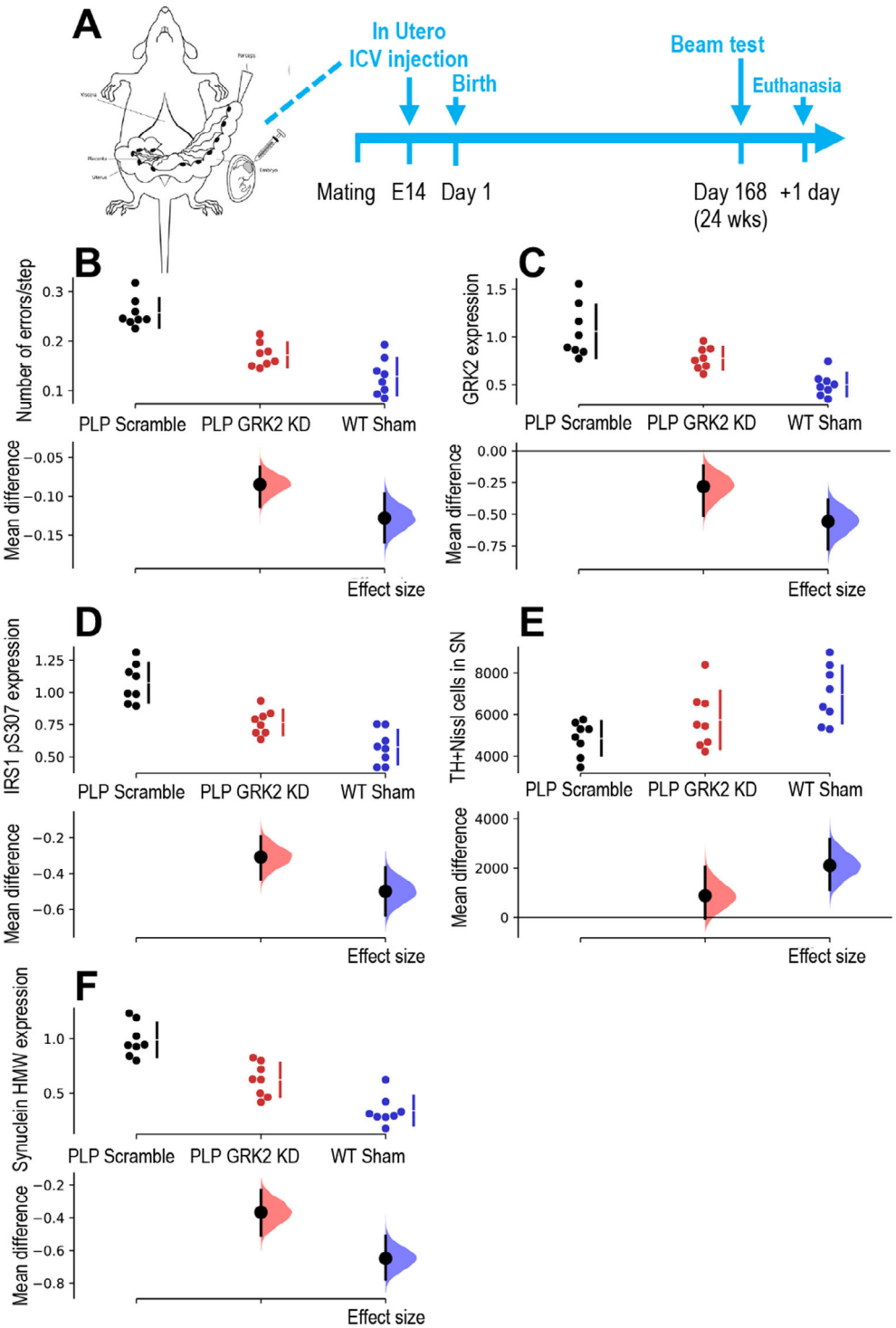

FIG. 1. In utero intracerebroventricular delivery of GRK2 miRNA protects against late-onset development of behavioral and pathological abnormalities in the PLP-SYN (proteolipid protein human α-syn) mouse model of MSA (multiple system atrophy). (**A**) Experimental procedure and design, (**B**) Beam test, (**C**) striatal GRK2 levels measured using Western blotting, (**D**) striatal IRS1 phospho-S307 levels measured using Western blotting, (**E**) stereological counting of substantia nigra pars compacta number of tyrosine-hydroxylase- (TH) and Nissl-positive neurons, and (**F**) striatal high-molecular-weight (HMW) α-synuclein levels measured using Western blotting in PLP scramble, PLP GRK2 KD, and WT (wild-type) sham groups. Data are presented as estimation graphics called "Gardner–Altman plot" (see Supporting Information). [Color figure can be viewed at wileyonlinelibrary.com]

the surrogate markers of neurodegeneration and behavioral outcome in a preclinical model. That GRK2 levels are increased in the MSA frontal cortex (n = 7 MSA patients versus n = 6 control subjects; GRK2 Western blotting, $P < 0.05$; Fig. S2) strengthens its therapeutic profile.

We should also consider the possibility of a direct role of GRK2 knockdown on synucleinopathy. GRK2

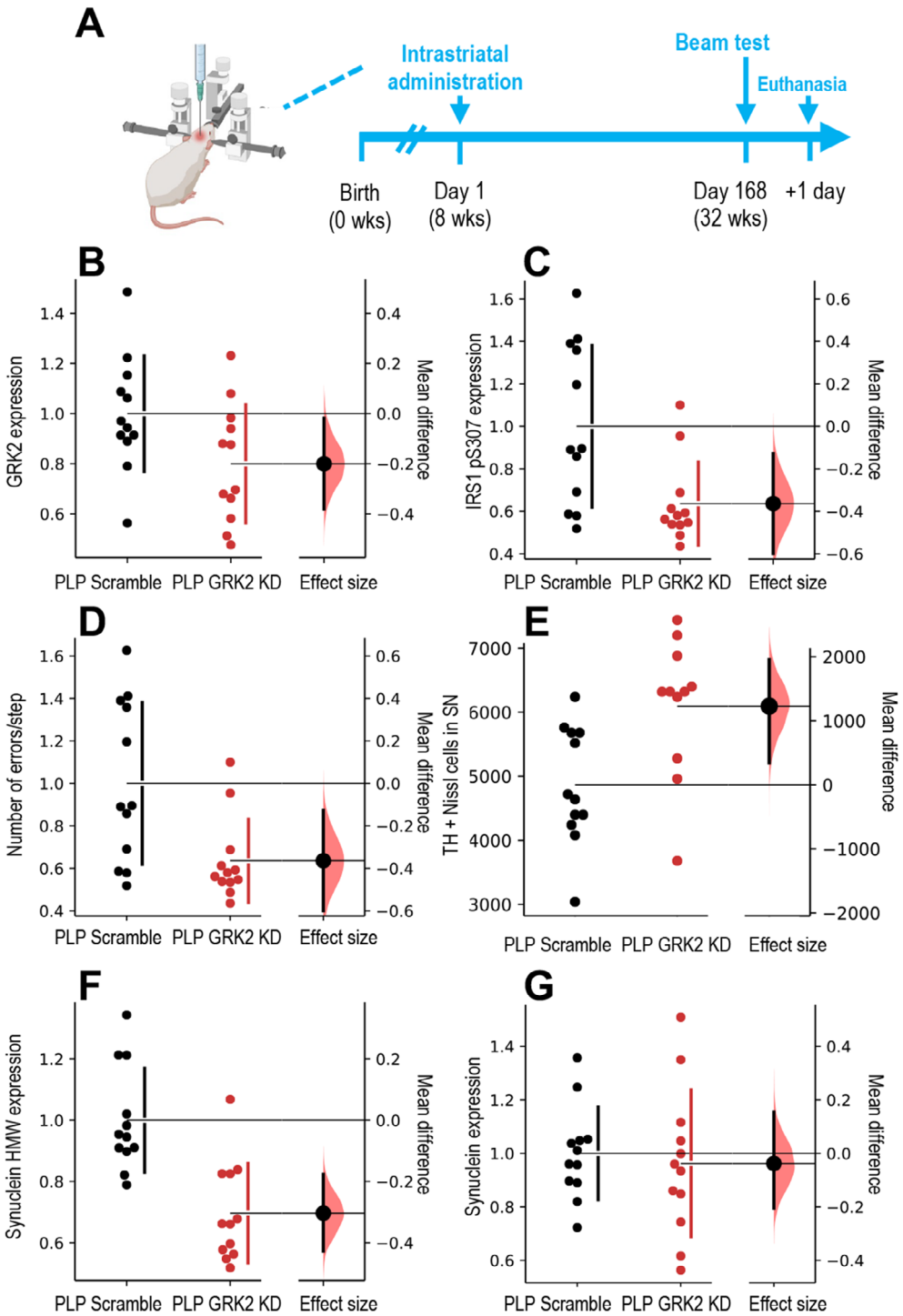

FIG. 2. Adulthood intrastriatal delivery of GRK2 miRNA protects against late-onset development of behavioral and pathological abnormalities in the PLP-SYN (proteolipid protein human α-syn) mouse model of MSA (multiple system atrophy). (**A**) Experimental procedure and design, (**B**) striatal GRK2 levels measured using Western blotting, (**C**) striatal IRS1 phospho-S307 levels measured using Western blotting, (**D**) Beam test, (**E**) stereological counting of substantia nigra pars compacta number of tyrosine-hydroxylase- (TH) and Nissl-positive neurons, and striatal (**F**) high-molecular-weight (HMW) and (**G**) total α-synuclein levels measured using Western blotting in PLP scramble and PLP GRK2 KD groups. Data are presented as estimation graphics called "Gardner–Altman plot" (see Supporting Information). [Color figure can be viewed at wileyonlinelibrary.com]

phosphorylates α-syn,[12] exacerbating its toxicity in a PD drosophila model[13] and accelerating the neurodegeneration in a PD rat model.[14] The effect of downregulating GRK2 might then be dual for achieving neuroprotection by restoring insulin signaling and decreasing α-s phosphorylation and aggregate maturation.

This proof-of-concept preliminary study is not, however, without limitations. We recently showed that

S129-phosphorylated α-syn corresponds to the accumulation of phosphorylated α-syn monomers/oligomers and not to the appearance of the distinctive fibrillar α-syn aggregates that are present in the brains of MSA or PD patients.[15] Demonstration remains, therefore, to be provided in a model of fibrillar formation, possibly using specific preformed fibrils inducing a pathology clearly reminiscent of MSA.[16]

Our results pave the way for a more comprehensive analysis of the role of GRK2 in MSA. The metabolic role of GRK2 in insulin resistance and associated conditions receives much attention. Several other approaches have been tested for peripheral indications, including small molecules, peptides, or aptamers.[17] They are worth trying for central nervous system indications, possibly through direct intracerebral infusion, to avoid peripheral side effects. ■

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Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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Supporting Data

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.