FULL-LENGTH ORIGINAL RESEARCH

Rationale for an adjunctive therapy with fenofibrate in pharmacoresistant nocturnal frontal lobe epilepsy

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Summary

Objective: **Nocturnal frontal lobe epilepsy (NFLE) is an idiopathic partial epilepsy with a family history in about 25% of cases, with autosomal dominant inheritance (autosomal dominant NFLE [ADNFLE]). Traditional antiepileptic drugs are effective in about 55% of patients, whereas the rest remains refractory. One of the key pathogenetic mechanisms is a gain of function of neuronal nicotinic acetylcholine receptors (nAChRs) containing the mutated α4 or β2 subunits. Fenofibrate, a common lipid-regulating drug, is an agonist at peroxisome proliferator-activated receptor alpha (PPARα) that is a ligand-activated transcription factor, which negatively modulates the function of β2-containing nAChR. To test clinical efficacy of adjunctive therapy with fenofibrate in pharmacoresistant ADNFLE\NFLE patients, we first demonstrated the effectiveness of fenofibrate in a mutated mouse model displaying both disease genotype and phenotype.**

Methods: **We first tested the efficacy of fenofibrate in transgenic mice carrying the mutation in the α4-nAChR subunit (Chrna4S252F) homologous to that found in humans. Subsequently, an add-on protocol was implemented in a clinical setting and fenofibrate was administered to pharmacoresistant NFLE patients.**

Results: **Here, we show that a chronic fenofibrate diet markedly reduced the frequency of large inhibitory postsynaptic currents (IPSCs) recorded from cortical pyramidal neurons in Chrna4S252F mice, and prevented nicotine-induced increase of IPSC frequency. Moreover, fenofibrate abolished differences between genotypes in the frequency of sleep-related movements observed under basal conditions. Patients affected by NFLE, nonresponders to traditional therapy, by means of adjunctive therapy with fenofibrate displayed a reduction of seizure frequency. Furthermore, digital video-polysomnographic recordings acquired in NFLE subjects after 6 months of adjunctive fenofibrate substantiated the significant effects on control of motor–behavioral seizures.**

Significance: **Our preclinical and clinical studies suggest PPARα as a novel disease-modifying target for antiepileptic drugs due to its ability to regulate dysfunctional nAChRs.**

KEY WORDS: **Peroxisome proliferator-activated receptor alpha, Fenofibrate, Neuronal nicotinic acetylcholine receptors, Nocturnal frontal lobe epilepsy.**

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Key Points

- Fenofibrate, a clinically used PPARα agonist, is effective in the mouse model of NFLE
- Fenofibrate is effective as adjunctive therapy in pharmacoresistant NFLE patients
- PPARα is a novel disease-modifying target for NFLE

Epilepsy is a common neurological disorder affecting 1% of the population worldwide.[1] Over the past decade, several idiopathic epilepsies have shown single-gene inheritance, such as nocturnal frontal lobe epilepsy (NFLE), which has a family history in about 25% of cases, with autosomal dominant inheritance (autosomal dominant NFLE [ADNFLE]).[2] Initially described as a rather benign and clinically homogeneous epilepsy, psychiatric symptoms and cognitive impairment also appear to take part in the broad NFLE\ADNFLE phenotype,[3] with intra- and interfamilial variability.[4] Traditional antiepileptic drugs are effective (mostly carbamazepine) in about two-thirds of patients, but only 20% are seizure-free; the rest remain refractory.[5,6] Nonetheless, due to the almost exclusive recurrence of seizures during sleep, NFLE patients often are scarcely aware of the presence, complexity, and frequency of attacks. As a result, residual epileptic events together with high levels of unstable non–rapid eye movement (NREM) sleep produce an objective resistance to the therapeutic purpose of antiepileptic drugs in NFLE.[7] Clinical features of NFLE are clusters of stereotypic episodes of arousal from sleep associated with dystonic neck, limb, and trunk movements that occur during stages 2–4 of NREM sleep,[5,7] and are accompanied by severe sleep instability.[8] One of the key pathogenetic mechanisms underlying this inherited form of epilepsy is the gain of function of the neuronal nicotinic acetylcholine receptors (nAChRs) containing mutated subunits.[9] After the discovery of the first mutation in α4 subunit gene,[10] mutations affecting two more genes encoding the α2[11] and β2[10] subunits of the nAChR were found to be implicated. However, phenotypes are generally indistinguishable and these mutations could be identified only in a minority of cases, with mean penetrance ranges from 60 to 80%.[3,6] On these bases, negative modulation of nAChRs might theoretically provide a therapeutic approach to treat NFLE, especially given that currently used antiepileptic drugs suppress neuronal hyperexcitability without necessarily addressing pathogenic mechanisms involved in epileptogenesis. Hence, disease-modifying drugs represent an alternative and more efficacious treatment option.

Peroxisome proliferator-activated receptor alpha (PPARα) activation leads to phosphorylation of β2-containing (β2*)-nAChRs with a consequent blockade of nicotine-induced activation of discrete neuronal populations in rodents.[12–14] Synthetic PPARα agonists such as fibrates are already well-established therapeutic options for the treatment of hyperlipidemia. Notably, fibrates exhibit similar antiepileptic properties[14] to traditional ketogenic diet in an experimental model of epilepsy.[15] We observed that PPARα agonists are protective against nicotine-induced seizures in behavioral and electroencephalographic (EEG) experiments in vivo, and in vitro abolish nicotine-induced spontaneous inhibitory postsynaptic current (sIPSC) increased frequency in rodents.[14]

Altogether, these observations prompted us to test both in vitro and in vivo the efficacy of a chronic diet with fenofibrate in a mutated mouse model displaying both disease genotype and phenotype. To optimize the results, experiments were carried out in a previously described mouse model of NFLE (*Chrna4*^{S252F}).[16] Next, we conducted a clinical study based on the viability of fenofibrate in both ADNFLE and NFLE drug-resistant patients. To this end, an add-on protocol was implemented in a clinical setting. Based on our working hypothesis, we expected that fenofibrate via PPARα activation might be beneficial in the treatment of NFLE.

Methods

Preclinical studies: neurophysiology and behavior of NFLE mouse model (*Chrna4*^{S252F})

Animals

Animal experiments were performed in strict accordance with the European Economic Community Council Directive of November 24, 1986 (86/609). All efforts were made to minimize pain and suffering and to reduce the number of animals used. The experimental protocols were also approved by the Animal Ethics Committee of the University of Cagliari and by the Italian Ministry of Health.

For all the experiments, we used a male, age-matched (60–80 postnatal days [PND]), wild-type (*wt*) and heterozygous mouse (*mut*) model of NFLE (*Chrna4*^{S252F}).[16] The colony founders (B6.129X1-Chrna4tm1Jbou/Mmucd, identification number 030293-UCD) were obtained from the Mutant Mouse Regional Resource Center (MMRRC), a strain repository funded by the National Center for Research Resources–National Institutes of Health, and were donated to the MMRRC by Dr. Jim Boulter of University of California, Los Angeles. Before the experiments, mice were housed six per cage under a 12-h light/dark cycle (light on at 7:00 AM), under conditions of constant temperature (21 ± 2°C) and humidity (60%), with food and water ad libitum. Animals were randomly assigned to the different experimental protocols: in vitro electrophysiology or EEG recordings coupled with behavioral observations (Fig. S3).

Chronic diet

Both *wt* and heterozygous *Chrna4*^{S252F} (*mut*) mice were randomly divided into diet treatment groups: (1) a standard

diet (control group, Harlan Teklad Global) and (2) a 0.2% fenofibrate diet (fenofibrate, Sigma-Aldrich and Harlan Teklad Global).[14,15] Mice were fed diets for 14 days. For EEG and observational studies, diets started 1 week after electrode implantation (see below; Fig. S3).

In vitro electrophysiology

The preparation of frontal cortex (FCx) slices and whole-cell patch clamp recordings from layer II/III pyramidal neurons were as described previously (see Data S1).[14,16] Briefly, male mice (PND 60–70) were anesthetized with isoflurane and killed. Coronal slices (two per animal, 300-μm thickness) containing the FCx were obtained, placed in the recording chamber, and superfused with artificial cerebrospinal fluid (32–34°C). Neurons were identified visually and voltage-clamped at a membrane potential of 0 mV. γ-Aminobutyric acid type A sIPSCs were pharmacologically isolated by blocking synaptic currents mediated by N-methyl-D-aspartate and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

Chronic EEG recordings

EEG and electromyographic (EMG) recordings were carried out in adult *wt* and *mut* mice (PND > 70; see Data S1). EEG electrodes were implanted in the right prefrontal cortex (from bregma: anteroposterior [AP], +2.8 mm; mediolateral [ML], 1.5 mm; dorsoventral [DV], 1.5 mm), and dorsal hippocampus (from bregma: AP, +2 mm; ML, 2 mm; DV, 2 mm).[17] EMG signals were recorded from two electrodes inserted in the neck musculature. Baseline recordings were performed 1 week following surgical preparation and on day 15 after diet onset. Digitally synchronized video-EEG recordings were acquired for 24 h.

Statistics

Electrophysiological experiments were sampled on- and offline with data analysis electrophysiology software by computers connected to a specific interface. Drug-induced changes in sIPSCs were calculated by averaging the effects following drug administration and normalized to the pre-drug baseline.

Data were analyzed utilizing parametric two-way analysis of variance (ANOVA) or Student t test, when they had equal variance and were normally distributed. Post hoc multiple comparisons were made using the Newman–Keuls, Tukey, or Bonferroni tests, when appropriate.

Clinical study

The clinical study (Fig. S3) was approved by the local ethical committee (N.257 03/22/2012), and informed consent, according to the Declaration of Helsinki and approved by the institutional review board, has been obtained from patients after a full explanation of the procedure.

Twelve subjects affected by drug-resistant ADNFLE and NFLE have been enrolled for the purpose of this study from outpatients attending the Epilepsy Diagnostic and Sleep Disorders Center of Cagliari and Parma (Italy).

Diagnoses were obtained based on reports of patients or family members witnessing the event, from serial EEG, digital video-EEG, and Holter-EEG recordings, and genetic test when available. Only primary NFLE patients, both sporadic and genetic (with genetic test when available), were considered for the study. Ascertained secondary forms were excluded by means of a high-resolution magnetic resonance imaging (MRI), customized according to the main electroclinical information employing a 1.5-T ACS-NT unit (Philips Medical Systems, Best, The Netherlands). It must be pointed out that the 1.5-T ACS-NT unit lacks the fine resolution necessary to exclude secondary forms of frontal lobe epilepsy such as MRI-negative focal cortical dysplasia. Drug resistance refers to the International League Against Epilepsy definition of drug-resistant epilepsy.[18] Recruited subjects underwent adjunctive therapy with fenofibrate. The chemical name for fenofibrate is 2-[4-(4-chlorobenzoyl) phenoxy]-2-methyl-propanoic acid, 1-methylethyl ester. Fenofibrate is usually indicated as adjunctive therapy to diet to reduce elevated low-density lipoprotein cholesterol, triglycerides, and apolipoprotein B, and to increase high-density lipoprotein cholesterol in adult patients with primary hyperlipidemia or mixed dyslipidemia. Fenofibrate was orally administered off-label at 600 mg per day, three times per day, for 6 months. Efficacy and safety adverse reactions have been documented and reported.[19] Because changes in liver function are the main cause of discontinuation of this treatment in 1.6% of patients in double-blind trials, blood examinations were carried out every 2 months during the total observation time. Furthermore, for safety, both cardiologic examination and electrocardiogram were performed at the beginning (T0) and at the end (T1-FEN) of the study. Intermediate clinical observations (i.e., nocturnal seizure and sleep diary examination) were performed every 2 months. Finally, to evaluate subjective clinical effects of fenofibrate, the Epileptic Quality of Life questionnaire (EpiQoL)[20] and a quality of life visual analog scale (VAS) were filled out by patients at T0 and T1-FEN (see Data S1 and Fig. S2).

Video-polysomnography (VPSG; see Data S1 for further details) has been visually, double-blindedly examined by two neurophysiologists expert in epilepsy and sleep disorders (G.M. and M.Pu.) and in agreement with clear-cut ictal epileptiform activity and stereotyped motor pattern. Each VPSG motor event was classified as a minor motor event (MME), whereas paroxysmal arousals, tonic–dystonic seizures, hyperkinetic seizures, and prolonged motor behaviors were classified as major events (MEs).[8] At both time points (i.e. T0 and T1-FEN), the total distribution of MEs and MMEs was analyzed throughout sleep time (NREM and REM sleep).

Statistics

Due to nonparametric distribution of data, the analysis of seizure diary and VPSG epileptic events in NFLE patients before and after add-on fenofibrate treatment was assessed by the nonparametric Wilcoxon two-tailed signed-rank test with paired comparison.

EpiQoL and VAS scores acquired at T0 and T1-FEN were analyzed by the nonparametric Wilcoxon two-tailed signed-rank test with paired comparison. Cross-correlation between the variation of seizure frequency and quality of life scores after 6 months of add-on fenofibrate treatment was calculated to evaluate an interdependency between these two conditions.

Statistical significance was set at $p < 0.05$ and determined using the calculation software GraphPad Prism.

Results

Neurophysiology and behavior of NFLE mouse model (*Chrna4*^{S252F})

The electrophysiological mechanism underlying epileptogenesis at the level of cortical circuits involves changes in synaptic activity affecting pyramidal cells as a key source of cortical EEG activity.[14] We, therefore, examined the effect of acute activation of PPARα on sIPSCs of layer II/III pyramidal cells of the FCx in a mouse model of NFLE (*Chrna4*^{S252F}).[16] Whole-cell voltage-clamp recordings of sIPSCs carried out from *wt* and *mut Chrna4*^{S252F} mouse brain slices[16] revealed that under basal conditions ($V_{holding}$ = 0 mV to isolate sIPSCs[16]), sIPSCs in *mut* mouse pyramidal cells displayed similar frequency overall (12.4 ± 1.3 Hz in *mut* vs. 12.4 ± 3.8 Hz in *wt* mice), although events > 100 pA displayed higher frequency when compared with *wt* mice (0.05 ± 0.01 Hz in *mut* vs. 0.004 ± 0.002 in *wt* mice; $p < 0.05$, unpaired t test, Fig. S1A). We next examined the effects of nicotine (5 μM, 30 s) on sIPSCs recorded from *wt* and *mut* mouse pyramidal cells. As previously described,[16] we found a larger and longer effect in *mut* when compared to *wt* mice (Fig. S1B, $p < 0.01$, $F_{30,180} = 6.62$, two-way ANOVA for repeated measures followed by Newman–Keuls post hoc test). However, irrespective of the genotype, acute nicotine-induced effects were absent following an acute bath application of the synthetic PPARα agonist WY14643 (3 μM, 5 min, Fig. S1B, $p > 0.05$, $F_{1,180}$=3.46, two-way ANOVA for repeated measures followed by Newman–Keuls post hoc test), in agreement with previous reports.[12–14] We next chronically treated the mice with a clinically available PPARα agonist, that is, fenofibrate. Both *wt* and *mut* mice were fed a diet containing 0.2% wt/wt fenofibrate[14,15] or a control diet and were randomly divided into four groups, that is, fenofibrate diet *wt* and *mut* mice, and control diet *wt* and *mut* mice. Fenofibrate diet was administered ad libitum for 14 days. Fenofibrate *wt* and *mut* mice consumed a daily average of 4.61 ± 0.22 and 4.39 ± 0.13 g of food pellets, respectively, which corresponds to approximately 300 mg/kg of fenofibrate per day.

As expected, in mice fed with a control diet, nicotine enhanced sIPSC frequency to a maximum to 171.5 ± 8.3% of baseline (22.2 ± 6.3 Hz) in *wt* and 207.1 ± 14% of baseline (25.6 ± 2.8 Hz) in *mut* mice (Fig. 1A). Statistical analysis of the effect of nicotine (0–4 min following perfusion) confirmed that the difference between genotypes is significant at 2 and 3 min postnicotine (effect of genotype $p < 0.05$, $F_{1,10} = 6.02$, two-way ANOVA for repeated measures followed by Newman–Keuls multiple comparisons post hoc test), consistent with a gain of function of α4β2*-nAChRs in *mut* mice. Experiments with mice chronically fed with fenofibrate confirmed that PPARα activation abolishes nicotine-induced increases in sIPSC frequency in FCx pyramidal neurons in both genotypes (Fig. 1A, $p < 0.0001$, $F_{3,23} = 13.88$, two-way ANOVA for repeated measures followed by Newman–Keuls multiple comparisons post hoc test). Hence, nicotine-induced increase in sIPSC frequency was 105.6 ± 10.9% of baseline in fenofibrate *wt* mice (15.1 ± 2.0 Hz baseline and 16.0 ± 2.5 Hz postnicotine; n = 7, Fig. 1A) and 84.6 ± 10.9% of baseline in fenofibrate *mut* mice (15.8 ± 4.0 Hz baseline and 13.4 ± 3.4 postnicotine; n = 8, Fig. 1A). Remarkably, fenofibrate diet abolished the differences observed between genotypes in the frequency of IPSCs > 100 pA under basal conditions ($p < 0.001$, $F_{3,25} = 7.60$; one-way ANOVA and Tukey multiple comparison test; Fig. 1B).

At variance with the description of this mutated mouse strain provided by Klaassen et al.,[16] but consistent with the phenotype described by Teper et al.[21] in a homologous knockin strain and with the human disease, we did not observe overt spontaneous seizures associated with high-voltage EEG spiking activity through the 24-h recording period. Notably, also consonant with the human pathological condition and with the role of nAChRs in sleep regulation,[22,23] *mut* mice exhibited a significantly higher number of brief (2–6 s) arousals associated with stereotypic movements during NREM sleep as compared to *wt* mice (Fig. 1C). Our analysis showed that *mut* mice (n = 4) had 1.10 ± 0.18 sleep-related events per minute, whereas *wt* mice (n = 5) had 0.65 ± 0.04 events per minute (Fig. 1C; $p < 0.05$, unpaired t test). The increase in these sleep-related movements observed in *mut* mice was equally distributed across the 24-h recording period.

When fenofibrate was chronically administered with the diet for 14 days, it reduced by approximately 50% the number of brief movement episodes during NREM sleep only in *mut* mice (effect of diet: $p < 0.05$, $F_{1,7} = 8.46$; genotype: $p > 0.05$, $F_{1,7} = 0.97$; interaction between genotype and treatment: $p < 0.01$, $F_{1,7} = 15.40$, two-way ANOVA for repeated measures followed by Bonferroni test), thus nullifying differences between genotypes (Fig. 1C). No effect of chronic fenofibrate was detected in *wt* mice (Fig. 1C).

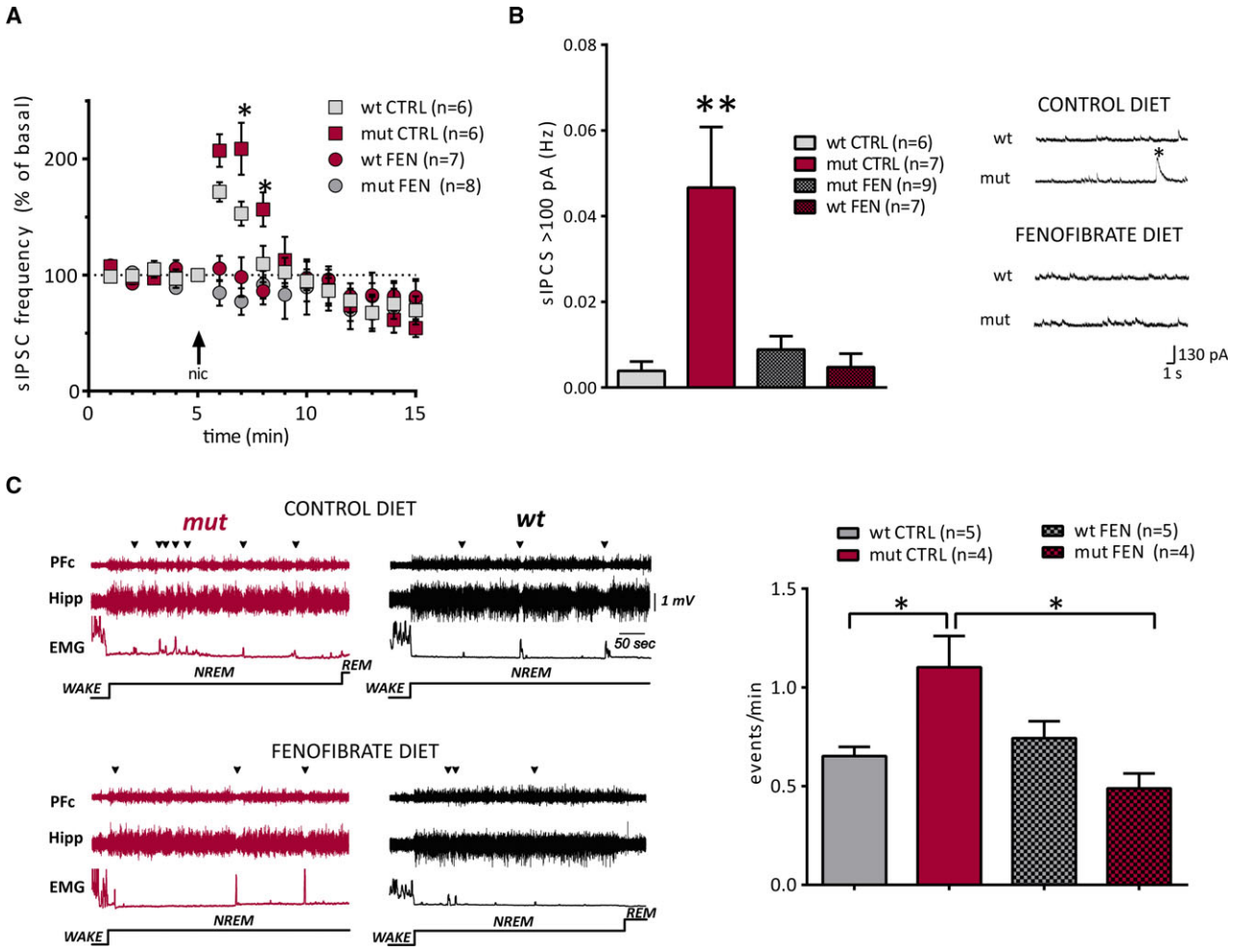

Figure 1.

Effect of chronic administration of the peroxisome proliferator-activated receptor alpha (PPARα) agonist fenofibrate in a mouse model of nocturnal frontal lobe epilepsy. (**A**) Whole-cell patch clamp recordings from wild-type (*wt*) and mutant *Chrna4*^{S252F} (*mut*) mouse cortical layer II/III pyramidal cells show that chronic fenofibrate (0.2% wt/wt for 14 days in food, FEN) fully suppressed nicotine (nic)-induced (5 μM perfused at arrow for 30 s) increase in spontaneous inhibitory postsynaptic current (sIPSC) frequency in both *wt* (red circles, n = 8) and *mut* (gray circles, n = 7) mice. Notably, in frontal cortex (FCx) slices from mice fed a control diet (CTRL), nicotine increases sIPSC frequency in pyramidal neurons irrespective of the genotype (*wt*: gray squares, n = 6; *mut*: red squares, n = 6), although the effect in *mut* mice is significantly larger and lasts longer. **(B)** The bar graph reveals that in *mut* mice (n = 7) the frequency of sIPSC > 100 pA recorded from FCx pyramidal neurons is significantly higher than in *wt* mice (n = 6), and that chronic fenofibrate abolished the differences between genotypes (*mut* FEN, n = 9; *wt* FEN, n = 7). Representative recordings of the effect of chronic fenofibrate diet on sIPSCs from pyramidal cells at $V_{holding}$ = 0 mV are depicted on the right in panel B. (**C**) Examples of recordings and scoring of brief movements during non–rapid eye movement (NREM) sleep in a *mut* (left) and a *wt* mouse (right) before and after chronic fenofibrate diet. The vigilance state of each animal was determined by an algorithm that analyzed hippocampal and electromyographic (EMG) activity changes over time. The right graph shows the number of brief sleep-related movement events per minute in *wt* and *mut* mice before and after 14 days of fenofibrate diet. Chronic administration of the PPARα agonist with food reduced the frequency of events in *mut* but not in *wt* mice. Symbols represent the average ± standard error of the mean. Two-way analysis of variance for repeated measures, *p < 0.05, **p < 0.01. Hipp, hippocampus; PFc, prefrontal cortex.

Clinical protocol for drug-resistant ADNFLE\NFLE patients

To study the effect of an adjunctive therapy with fenofibrate, 12 patients affected by ADNFLE\NFLE, who were nonresponders to traditional therapy, were recruited. Clinical data, brain MRI, and routine wake-EEG findings of NFLE subjects under traditional antiepileptic therapy are summarized in Table S1. Among subjects, eight presented nocturnal motor events since childhood, whereas the mean duration of paroxysmal motor events during sleep was approximately 3 years before diagnosis in the remaining subjects. Five subjects reported a family history of epilepsy

(ADNFLE) with confirmed mutation at genetic test (PZ 6 CHRNA4 *mut* and PZ 7-10 CHRNA2). Due to the well-established genetic heterogeneity[9,24] despite a relatively homogeneous clinical picture, genetic testing in our sporadic cases was not regarded as relevant, especially after a cost–benefit analysis**.** Seven subjects described parasomnias or nocturnal motor events in first-grade relatives. None of the subjects displayed either perinatal hypoxia, childhood febrile convulsions, or mild head injury. Diurnal seizures were rarely reported. Neurological examinations were unremarkable, and customized MRI was normal for all subjects. Regarding the nature of seizures and/or motor events observed in our patients, Terzaghi et al.[25,26] have demonstrated that NFLE patients exhibit both MEs, considered clear epileptiform manifestations, and a large quantity of highly stereotyped MMEs (involving the limbs, the axial musculature, and/or the head) that could occur in either the presence or absence of an epileptiform discharge.

The same authors demonstrated that both MMEs and epileptiform discharges are mutually related with sleep instability.[25,26] In particular, hyperarousability induced by epileptiform discharges facilitates the occurrence of MME.[7] Because MMEs result from a release or disinhibition pattern, directly or indirectly related to a paroxysmal activity, we considered the effect of fenofibrate add-on on both MEs and MMEs.

Fenofibrate add-on therapy consisted of 600 mg per day for 6 months. Two ADNFLE subjects refused (signed declaration) traditional antiepileptic therapy, and fenofibrate was administered as a monotherapy. A twelfth patient dropped out because of increased levels of hepatic enzymes during the first month of therapy, although the values immediately returned to normal ranges after interruption. At baseline (T0) and after 6 months of fenofibrate add-on therapy (T1-FEN), quality of life was assessed and daily seizures diaries were collected. According to subjective seizure diaries, which were based upon subject and bed-partner reports, 8 of 11 subjects were seizure-free, and the remaining three reported a reduction > 75% of MEs (Fig. 2A, $p = 0.002$). Table 1 shows seizure rate change calculated according to the Labar rule.[27] Fig. 2B,C displays the Wilcoxon test results with regard to EpiQoL and VAS. Although none of the subjects had reported a severe discomfort in relation to their pathological condition, the scores of both quality of life tests revealed a significant improvement after 6 months of add-on therapy ($p = 0.0186$ and $p = 0.022$, respectively;

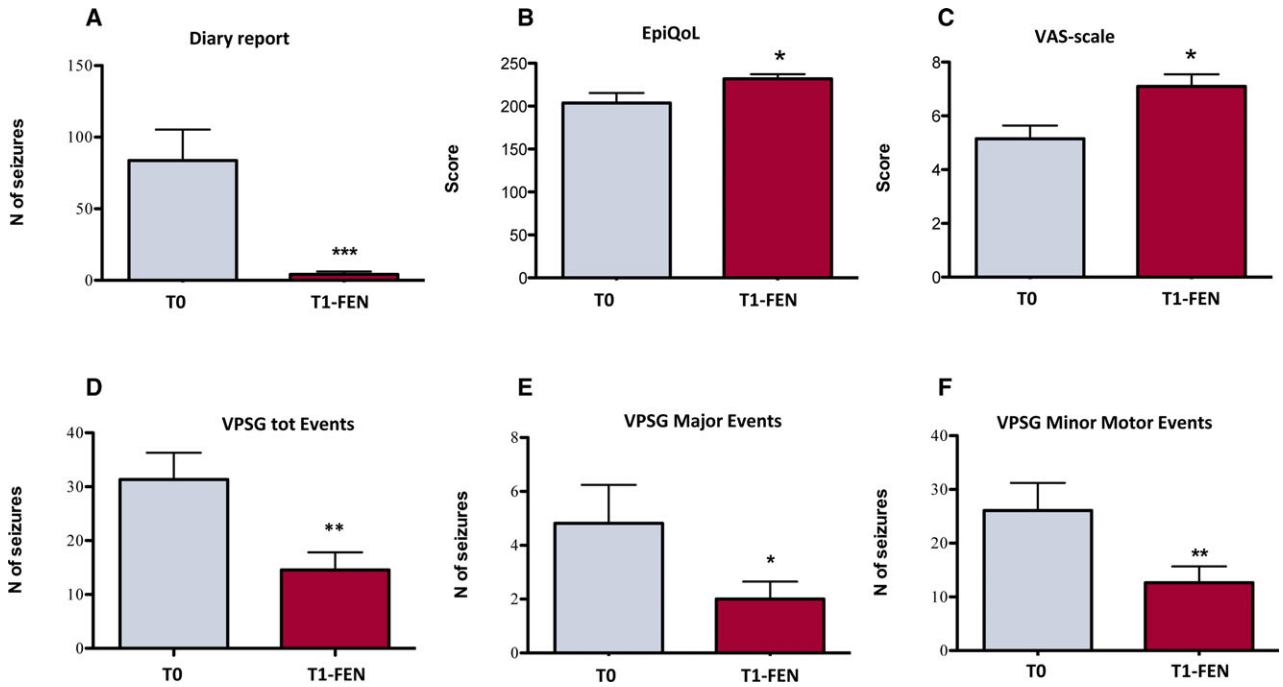

Figure 2.

Fenofibrate add-on treatment improves seizure control in subjects affected by drug-resistant nocturnal frontal lobe epilepsy . Bar graphs show the clinical efficacy of add-on fenofibrate. (**A**–**C**) There was a significant reduction in reported seizures in the diaries (**A**), a significant improvement on Epileptic Quality of Life questionnaire (EpiQoL) scores (**B**), and a significant improvement on visual analogic scale (VAS) scores (**C**) after 6-month fenofibrate treatment (T1-FEN). (**D**–**F**) Bar graphs show the efficacy of add-on fenofibrate treatment in control of total (tot) video-polysomnography (VPSG)-recorded seizures (**D**), major events (**E**), and minor motor events (**F**) before (VPSG T0) and after a 6-month treatment (VPSG T1-FEN). Bars represent the average ± standard error of the mean ($n = 11$). Wilcoxon two-tailed signed-rank test with paired comparison, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 1. Effect of add-on fenofibrate treatment (percentage change after 6 months of add-on fenofibrate) on reported seizures and quality of life tests (EpiQoL, VAS)

Patient no./diagnosis	Gender	Age, yr	A No. of seizures reported in diary	B EpiQoL	C VAS
1/NFLE	M	35	92 (−97.83)	184 (19.02)	5 (28.6)
2/NFLE	F	32	25 (−96)	124 (62.10)	5 (0)
3/NFLE	F	47	36 (−100)	193 (25.90)	5 (37.5)
4/NFLE	M	29	104 (−98.07)	216 (12.03)	5 (33.3)
5/NFLE	M	29	38 (−89.47)	216 (15.74)	5 (44.4)
6/ADNFLE	M	41	93 (−100)	189 (20.63)	2.5 (58.3)
7/ADNFLE	F	24	252 (−90.48)	196 (20.41)	5 (33.3)
8/ADNFLE	F	52	24 (−87.5)	236 (3.28)	5 (0)
9/ADNFLE	F	23	168 (−98.21)	240 (−5.91)	9 (0)
10/ADNFLE	F	52	24 (−100)	220 (−3.48)	5 (28.6)
11/NFLE	M	35	NA	240 (0.5)	5 (0)
Wilcoxon test			$^{a}p = 0.001$	$^{b}p = 0.0195$	$^{b}p = 0.022$

Column A shows the number of seizures collected from a single subject daily diary at baseline and the seizure rate change calculated according to Labar rule 27 after 6 months of add-on fenofibrate treatment. Columns B and C show the scores of administered EpiQoL and VAS with relative score change after treatment with 6 months of fenofibrate.

ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; EpiQoL, Epileptic Quality of Life questionnaire; F, female; M, male; NA, data not available; NFLE, nocturnal frontal lobe epilepsy; VAS, visual analog scale.

$^{a}p < 0.001$, $^{b}p < 0.05$ by Wilcoxon two-tailed signed-rank test with paired comparison.

Table 1 and Fig. 2B,C). No significant correlation between EpiQoL improvement and the reduction in seizure frequency was unveiled (data not shown). Remarkably, about 73% of the patients voluntarily requested to continue add-on therapy even after the completion of the study.

VPSG recordings at T1-FEN

A total of 340 epileptic events were counted during VPSG recordings of NFLE subjects at T0. The prevalence of seizures (approximately 96%) was recorded during NREM sleep.[28] According to polysomnographic and video criteria, 53 attacks were classified as MEs and 287 epileptic events were classified as MMEs, and both occurred during stage 2 of NREM sleep (approximately 62%). After 6 months of fenofibrate add-on therapy (T1-FEN), VPSG recordings displayed a significant decrease in the mean total number of sleep seizures (Fig. 2D, $p = 0.001$). Particularly, a reduction of MEs (Fig. 2E, $p = 0.0131$) and MMEs (Fig. 2F $p = 0.0087$) was evident. Table 2 shows the hourly indexes of all VPSG epileptic events and of the ME and MME subgroups at T0 and T1-FEN.

Discussion

Nowadays, a beneficial therapy for NFLE still represents a significant unmet clinical need, as carbamazepine, the most efficacious drug for NFLE, completely abolishes the seizures only in ~20% of patients, and reduces seizures in other 48%.[29] This leaves approximately one-third of patients without a satisfactory therapeutic response. Gene mutations identified in the *CHRNA4*, *CHRNB2*, and *CHRNA2* genes in ADNFLE families strongly establish the role of the cholinergic system in this type of epilepsy, where functional characterization of known mutations suggests that increased gain of the receptor function is at the origin of seizures.[30] Furthermore, in vitro and in vivo studies have demonstrated a high density of nAChRs in the thalamus,[31,32] an overactivated cholinergic pathway, reinforcing the hypothesis that corticosubcortical networks, regulating arousal from sleep, play a central role in the epileptogenesis of NFLE. The major finding of the present study is the clinical efficacy of PPARα agonist fenofibrate add-on therapy in NFLE patients, which exhibited remarkable seizure reduction with good control of the disease. We also extend the evidence for the action of PPARα as a negative modulator of nAChRs in a mutated mouse model of NFLE, which displays both the genotype and the phenotype of the human disease. Several observations support our conclusions. First, the patients and their bed-partners reported in their daily seizure diary a significant reduction of events together with a marked improvement in their quality of life, as indexed by the EpiQoL questionnaire and VAS. Second, the instrumental VPSG nocturnal recordings demonstrated that the efficacy of add-on therapy is manifest not only for MEs, but also for MMEs. Notably, MMEs seriously affect the development of the disease and disrupt sleep architecture, although they are totally unperceived by the subjects.[25,28] The observation that a "single" instrumental analysis, although compared with a single night under basal conditions, substantiated the 6 months of daily reports in the diary of seizures filled out by the patients and their bed-partners, strongly underscores the efficacy of this add-on therapy. In addition, although traditional antiepileptic treatment for this type of epilepsy determines a partial (about 25%)

Table 2. Effect of add-on fenofibrate treatment on VPSG-recorded seizures

			Total number of seizures		Number of major events		Number of minor motor events	
Patient no./diagnosis	Gender	Age, yr	T0	T1-FEN	T0	T1-FEN	T0	T1-FEN
1/NFLE	M	35	48	18	1	2	47	18
2/NFLE	F	32	37	5	1	0	36	5
3/NFLE	F	47	35	16	11	2	24	14
4/NFLE	M	29	36	17	0	0	36	17
5/NFLE	M	29	9	3	3	2	6	1
6/ADNFLE	M	41	61	31	2	1	59	30
7/ADNFLE	F	24	34	31	15	4	19	27
8/ADNFLE	F	52	25	14	7	5	18	9
9/ADNFLE	F	23	33	22	7	6	26	15
10/ADNFLE	F	52	14	1	4	0	10	1
11/NFLE	M	35	8	2	2	0	6	2
Wilcoxon test				$^{a}p = 0.0046$		$^{b}p = 0.0131$		$^{a}p = 0.0087$

Total number of VPSG-recorded seizures, major events, and minor motor events, before (T0) and after 6 months of fenofibrate add-on treatment (T1-FEN) per each subject.

ADNFL, autosomal dominant nocturnal frontal lobe epilepsy; F, female; M, male; NFLE, nocturnal frontal lobe epilepsy; VPSG, video-polysomnography.

$^{a}p < 0.001$, $^{b}p < 0.05$ by Wilcoxon two-tailed signed-rank test with paired comparison.

reduction of objective VPSG seizures, sleep instability remains pathologically enhanced and is associated with persistence of daytime sleepiness.[8] Third, a significant reduction of brief sleep-related movements is also clear during recordings in mutated mice chronically fed with the fenofibrate diet. Notably, MMEs are related to a loss of cortical inhibition, which is secondary to arousal triggered by internal epileptiform stimuli of innate motor patterns generated by central pattern generators,[28,33] and might occur in either the presence or absence of an epileptiform discharge.[25] The observation that fenofibrate reduces MMEs and arousal in both a disease mouse model and humans demonstrates a helpful control of seizure activity and epileptiform internal stimuli, which consequently ameliorates sleep instability by reducing the triggered arousals. Fourth, in vitro both acute and chronic PPARα activation reduce nicotine-induced enhancement of sIPSC frequency in FCx pyramidal neurons irrespective of the genotype. This increase has been hypothesized to trigger network synchrony and ictal activity.[16] Notably, the observation that *mut* mice exhibit an augmented frequency of larger sIPSCs (>100 pA), which following the fenofibrate diet becomes comparable to *wt* mice, supports the hypothesis that network synchrony and ictal activity might account for cellular and behavioral expression of the disease. Lastly, these findings support our hypothesis that PPARα activation might prevent/reduce the loss of cortical inhibition, as previously demonstrated in a pharmacological model of the disease.[14]

The conclusions that PPARα might be a key regulator of neuronal activity through the modulation of nAChR functional properties, and that these effects might be therapeutically exploited, are in agreement with the finding that nAChRs play a major role in idiopathic and/or genetically determined forms of epilepsy. Remarkably, a dysfunction of the brain cholinergic systems is hypothesized to underlie diverse disorders, including insomnia, mania, and depression.[34–38] Accordingly, we observed a significant improvement of quality of life in our group of NFLE patients. However, as a possible antidepressant effect was not among the expected outcomes of fenofibrate treatment, we did not administer any depression scales before or after treatment. Thus, caution has to be taken when interpreting these results, as the improvement in quality of life scores might be related to a placebo effect or to the subjective feeling of well-being due the amelioration of pathology and of the quality of sleep. Nevertheless, the finding that such an effect does not correlate with seizure control, and the significant mood amelioration reported on the questionnaires, in addition to the patient determination to continue the therapy even after the completion of the study, support the preclinical findings suggestive of an independent role of fenofibrate on mood regulation.[39,40]

Taken together, our results, although preliminary, call for a double-blind placebo trial and suggest that fenofibrate is an effective and well-tolerated adjunctive therapy in NFLE due to its effects as a disease-modifying drug, and point out the role of PPARα in the regulation of neuronal functions.

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Disclosure

The authors declare no conflicts of interest. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. Materials and methods.

Table S1. Clinical history of recruited subjects.

Figure S1. In vitro electrophysiology in a mouse model of nocturnal frontal lobe epilepsy: acute effects of a synthetic peroxisome proliferator-activated receptor alpha agonist.

Figure S2. Visual analog scale scoring.

Figure S3. Study design.