CB1 receptor affects cortical plasticity and response to physiotherapy in multiple sclerosis

ABSTRACT

Objectives: Therapeutic effects of physical therapy in neurologic disorders mostly rely on the promotion of use-dependent synaptic plasticity in damaged neuronal circuits. Genetic differences affecting the efficiency of synaptic plasticity mechanisms could explain why some patients do not respond adequately to the treatment. It is known that physical exercise activates the endocannabinoid system and that stimulation of cannabinoid CB1 receptors (CB1Rs) promotes synaptic plasticity in both rodents and humans. We thus tested whether CB1R genetic variants affect responsiveness to exercise therapy.

Methods: We evaluated the effect of a genetic variant of the CB1R associated with reduced receptor expression (patients with long AAT trinucleotide short tandem repeats in the CNR1 gene) on long-term potentiation (LTP)-like cortical plasticity induced by transcranial magnetic theta burst stimulation (TBS) of the motor cortex and, in parallel, on clinical response to exercise therapy in patients with multiple sclerosis.

Results: We found that patients with long AAT CNR1 repeats do not express TBS-induced LTP-like cortical plasticity and show poor clinical benefit after exercise therapy.

Conclusions: Our results provide the first evidence that genetic differences within the CB1R may influence clinical responses to exercise therapy, and they strengthen the hypothesis that CB1Rs are involved in the regulation of synaptic plasticity and in the control of spasticity in humans. This information might be of great relevance for patient stratification and personalized rehabilitation treatment programs. Neurology.org/nn © 2014 American Academy of Neurology

GLOSSARY

AATn — AAT trinucleotide short tandem repeat; AMT — active motor threshold; ANOVA — analysis of variance; CB1R — cannabinoid CB1 receptor; CS — conditioning stimulus; DMD — disease-modifying drug; EAE — experimental autoimmune encephalomyelitis; EDSS — Expanded Disability Status Scale; FDI — first dorsal interosseus muscle; FLAIR — fluid-attenuated inversion recovery; ICF — intracortical facilitation; ISI — interstimulus interval; ITBS — intermittent TBS; LICI — long-interval intracortical inhibition; LTP — long-term synaptic potentiation; MEP — motor evoked potential; MS — multiple sclerosis; NRS — Numerical Rating Scale; pp — paired-pulse; PT — physical therapy; RMT — resting motor threshold; RRMS — relapsing-remitting MS; SICI — short-interval intracortical facilitation; SICI — short-interval intracortical inhibition; TBS — theta burst stimulation; TMS — transcranial magnetic stimulation; TS — test stimulus; TSE — turbo spin echo.

Physical activity increases the efficiency of synaptic transmission1 and consistently causes long-term effects on neuronal function and brain structure.2 Use-dependent long-term synaptic potentiation (LTP) is considered the molecular substrate for neurorehabilitation, as enhancing synaptic strength in surviving neurons can mitigate the effects of tissue damage by restoring the excitability of neurons that have lost part of their synaptic inputs.3 Physical therapy (PT) can reduce motor deficits in disabled patients with multiple sclerosis (MS) with large interindividual differences.4

Cannabinoid CB1 receptors (CB1Rs) regulate LTP in animals,5 and phytocannabinoids favor transcranial magnetic theta burst stimulation (TBS)—induced LTP in the primary motor cortex from the Clinica Neurologica (F.M., C.G.N., H.K., F.B., S.R., D.C.), Dipartimento di Medicina dei Sistemi, and Medicina Fisica e Riabilitativa (C.L., L.G., F.F.), Dipartimento di Sanità Pubblica e Biologia Cellulare, Università Tor Vergata, Rome, Italy; and Centro Europeo per la Ricerca sul Cervello (CERC)/Fondazione Santa Lucia (F.M., C.G.N., H.K., F.B., S.R., D.C.), Rome, Italy.

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of patients with MS. Notably, both TBS-induced LTP\(^7,8\) and cannabinoids\(^9\) had some beneficial effects on spasticity and motor disability in patients with MS, further substantiating the involvement of CB1Rs in the neuroplastic adaptations mediating recovery of function after PT. Accordingly, exercise causes a dramatic upregulation of central CB1R sensitivity,\(^10\) which contributes to the beneficial clinical effects of exercise in mice with experimental autoimmune encephalomyelitis (EAE), the animal model of MS.\(^11\)

We recently demonstrated that a microsatellite polymorphism of the CB1R-encoding gene (CNR1), an AAT trinucleotide short tandem repeat (AATn) downstream of the translation site,\(^12\) affects transcription efficacy, as individuals with long-AATn (\(\geq 12\) in both alleles) express lower levels of CB1R.\(^13\) Based on the notion that CB1Rs are involved in the central effects of exercise, we explored whether clinical response variability to PT in MS depends on CB1R function secondary to different genetic background. Our results show that individuals with long-AAT repeats in the CNR1 gene have both defective cortical plasticity in response to intermittent TBS (iTBS) and poor clinical benefits in response to PT.

**METHODS** Standard protocol approvals, registrations, and patient consents. The study was approved by the institutional review board. Written informed consent was signed by all study participants.

**Patients with MS.** Thirty Central-Southern Italian patients with relapsing-remitting MS (RRMS)\(^14\) were enrolled (table) by referral from the MS Center, Neurology Unit, Policlinico Tor Vergata, Rome. Data collection was performed from 2010 to 2012. Sample size was determined on the basis of a previous study.\(^8\)

Patient eligibility was based on the following criteria: (1) clinically definite RRMS,\(^14\) (2) remitting phase of disease, (3) Expanded Disability Status Scale (EDSS) score between 2 and 7, and (4) presence of spasticity in the lower limbs.

Disease onset was defined as the first episode of focal neurologic dysfunction indicative of MS. All patients were in a remitting phase in the 3 months preceding recruitment and during the study. Remission was defined as the absence of new or recurrent neurologic symptoms not associated with fever or infection lasting for at least 24 hours.

All patients started disease-modifying drugs (DMDs) at the time of diagnosis. First-line treatment prescribed was glatiramer acetate (20 mg SC daily), interferon \(\beta-1a\) (44 mg SC 3 times weekly), interferon \(\beta-1a\) (30 mg IM), or interferon \(\beta-1b\) (250 mg SC every other day). Mitoxantrone (12 mg/m\(^2\) IV every 3 months with a lifetime maximum of 140 mg/m\(^2\)) or natalizumab (300 mg IV every 4 weeks) was prescribed as second-line treatment.

**Determination of AAT repeats in the CNR1 gene.** Samples of peripheral blood from recruited patients were collected in BD Vacutainer tubes with EDTA (Beckton Dickinson, Franklin Lakes, NJ). Genomic DNA was isolated and extracted from human whole blood (200 \(\mu\)L) using a MagNA Pure LC DNA Isolation Kit and automated extractor (Roche Diagnostics GmbH, Mannheim, Germany) following manufacturer’s instructions. One hundred fifty ng of genomic DNA was used to amplify the CNR1 region, including the AAT repeats through PCR. PCR was carried out in a 25 \(\mu\)L volume containing polymerase buffer, 1 mM MgCl\(_2\), 0.2 mM of each dNTP, 0.5 pmoles of each primer (sense: 5’-CACCCTGGGCTGTAAAATA-3’; antisense: 5’-GTTGCCAGTGACCAAAGATCA-3’), and 1.5 U Taq DNA polymerase (Invitrogen, Madison, WI). Denaturation was achieved through a first step of 5 minutes at 94°C followed by 35 cycles of 45 seconds at 95°C, annealing for 1.5 minutes at 58°C and elongation for 1 minute at 72°C, and final elongation for 7 minutes at 72°C. For sequencing analysis, 10 ng of PCR products Agencourt AMPure PCR Purification kit (Agencourt Bioscience Corporation, Beverly, MA), 0.5 pmoles of the sequencing primer (5’-ACCTCCACCCAATCCAAATA-3’), and the ABI PRISM BigDye Terminator v3.1 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) were used. Sequencing reactions initiated with 1 minute

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<th>Table</th>
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<td>CNR1 AATn</td>
<td>Sex</td>
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<tr>
<td>Short(^a)</td>
<td>8M, 7F</td>
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<tr>
<td>4 IFN-(\beta1-a)</td>
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<td>3 GA</td>
<td>6,956</td>
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<tr>
<td>Long(^b)</td>
<td>6M, 9F</td>
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<td>6 IFN-(\beta1-a)</td>
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Abbreviations: AATn = AAT trinucleotide short tandem repeat; DMD = disease-modifying drug; EDSS = Expanded Disability Status Scale; GA = glatiramer acetate; IFN = interferon; LL = lesion load; NRS = Numerical Rating Scale (spasticity); PFS = pyramidal functional system subscore.

Values represent median ± SD for EDSS and EDSS-PFS, and mean ± SD for age, disease duration, lesion load, and NRS.

\(^a\)Short = patients carrying at least 1 allele with CNR1 AATn ≤11.

\(^b\)Long = patients carrying both alleles with CNR1 AATn ≥12.
Immediately before iTBS and at 2 different time points (0 and 10 pulses during FDI dorsal interosseus muscle (FDI) with surface electrodes, amplified like or long-term depression amplitude lasting after the end of repetitive TMS indicates that LTP-the stimulated region. An increase or decrease of the MEP amplitude evoked potentials (MEPs) from the muscles represented within the motor cortex, TMS can induce plastic modifications of cortical excitability that can be measured by recording the motor evoked responses with TS preceding CS at 1 of 5 different ISIs (1.5, 2.1, 2.7, 3.7, and 4.5 msec).

Long-interval intracortical inhibition (LICI), mediated by GABA
was tested through a CS at 120% RMT intensity. Two conditions were presented in a random order: TS alone and CS preceding TS at an ISI of 100 msec. For each experimental condition 10 responses were collected. MEP changes at each ISI were expressed as percentage of the mean unconditioned MEP amplitude.

Physical therapy. Two to 4 weeks after TMS evaluation, patients started the therapeutic exercise program at the Physical Rehabilitation Department of the University Hospital Policlinico Tor Vergata in Rome. Exercises were performed on a daily basis for 2 weeks and consisted of 1 hour on land followed by another hour in 28–30°C water in a 150-cm wide pool, as in a previous study. The program was devised and coordinated by a physician specializing in physical medicine and rehabilitation and consisted of both passive and active therapeutic exercises specifically aimed at restoring or maintaining muscular flexibility, range of motion, balance, coordination of movements, postural passages and transfers, and ambulation. Different types of therapeutic exercises were scheduled according to the individual disability status and administered by qualified physiotherapists. The exercises consisted of (1) repetition of different movements (i.e., tips and heels, 90° flexed hips and knees) for ambulation and stair climbing; (2) repetition of crossed patterns of movements for coordination; (3) postural reactions while standing with eyes open and closed, including the use of oscillatory boards for balance; (4) strengthening lower limb antigravity muscles (i.e., gluteus minor and major, quadriceps femoris); and (5) low-intensity and long-duration static stretching of iliofemoral, rectus femoralis, hamstring, triceps surae, and lumbar spinal muscles for muscular flexibility and range of motion.

During each session patients performed either 2 or 3 sets per exercise type, with each set consisting of about 15 repetitions. Intensity of exercise was graded on the level of disability. Compensative pauses of time were included in relation to each patient's tolerance to the exercise regimen, for proper restoring.

Data analysis. As in previous studies, patients with MS were grouped according to the number of AAT repeats of the CNR1 gene. In the short-AATn group patients had 1 or 2 alleles with ≤11 repeats of AAT triplets, and in the long-AATn group patients had 2 alleles with ≥12 repeats. Student t test, Mann–Whitney test, Fisher exact test, and repeated-measures analysis of variance (ANOVA) were used to analyze differences between groups, as appropriate. Type of DMD was entered as a covariate because of its effect as a potential confounder. Duncan post hoc correction was performed. Correlation between EDSS and TMS parameters was explored through regression analysis. Data are presented as mean ± SEM. p < 0.05 was considered significant.

RESULTS The experimental procedures were well-tolerated by all study participants and no adverse reactions were reported. Long- and short-AATn CNR1 patients showed no clinico-demographic...
After the 2-week physical therapy program, patients carrying a short number of AAT repeat (AAT ≤11 in at least 1 allele) show a better clinical response in both EDSS (A) and spasticity NRS (B). *p < 0.05; error bars indicate SD. AATn – AAT trinucleotide short tandem repeat; EDSS – Expanded Disability Status Scale; NRS – Numerical Rating Scale.
Multiple sclerosis patients with a long number of AATn repeats (AATn ≥12 on both alleles) on the CNR1 gene show defective LTP response to iTBS in the motor cortex. In contrast, patients with a short number of AATn repeats (AATn ≤11 in at least 1 allele) show a preserved LTP response after iTBS (A). No differences were found between short- and long-AATn carriers in the SICI/ICF (B), LICI (C), and SICF (D) paired-pulse TMS protocols. *p < 0.05; error bars indicate SEM. AATn carriers in the SICI/ICF (B), LICI (C), and SICF (D) paired-pulse TMS protocols. *p < 0.05; error bars indicate SEM. AATn = AAT trinucleotide short tandem repeat; ICF = long-term intracortical facilitation; ISI = interstimulus interval; iTBS = intermittent theta burst stimulation; LICI = long-interval intracortical inhibition; LTP = long-term synaptic potentiation; MEP = motor evoked potential; SICF = short-interval intracortical facilitation; SICI = short-interval intracortical inhibition; TMS = transcranial magnetic stimulation.

Objective changes in spasticity. Spasticity derives from hyperactivity of the stretch reflex caused by damaged corticospinal tract function,4 and motor cortex LTP induced by iTBS5,8 alleviates this symptom by increasing the excitability of the corticospinal tract. In this respect, the CB1R has been implicated in LTP in both animals5 and humans, as treatment with cannabis in MS favors TBS-induced LTP.6 Notably, iTBS-induced effects originate cortically at impinging on pyramidal output cells,26 which are also particularly sensitive to CB1R modulation.27

It is interesting that the brain-derived neurotrophic factor, an essential player in LTP induction and maintenance, is increased by exercise,28,29 and its genetic polymorphism can impair both TBS-induced LTP30 and exercise-induced cortical reorganization in humans.31

The results of our investigation could be extended to PT effects in patients with diseases other than MS, such as stroke. However, it is also possible that the specific mechanisms of brain damage in MS, which are greatly dependent on the release of inflammatory molecules by infiltrating immune cells, could exacerbate the negative influence of the genetically determined defective CB1R function on synaptic plasticity and motor recovery, as both EAE and MS have been associated with reduced CB1R function.32

Indeed, downregulation of CB1R caused by both genetic defect or subchronic tetrahydrocannabinol exposure causes activation of the microglia in mouse cerebellum. This microglia activation causes deficits in cerebellar associative learning and motor coordination mediated by release of proinflammatory cytokines such as interleukin-1β.33

Accordingly, proinflammatory molecules (namely interleukin-1β) have been demonstrated to inhibit CB1Rs in mice.34 Moreover, other cytokines that can be altered during inflammatory activity, such as the platelet-derived growth factor, have been shown to influence TBS-induced LTP and accumulation of disability in MS.35,36

TBS aftereffects can last up to 1 hour; however, in our study the recording was limited to the first 15 minutes only. Moreover, because determination of AMT requires voluntary activation of the target muscle, this may have caused metaplastic interactions with the subsequent iTBS protocol.37 Therefore our findings may be limited to early LTP induction and may be related to either iTBS-induced LTP or its metaplastic interaction with prior voluntary activation. Finally, even though PT was administered following a detailed program, exercises differed among patients according to their level of impairment and disability. In addition, the effects of training on ambulation, balance, safety, and range of motion were not measured through specific scales. PT programs usually last several months to produce effective results.18 Because our PT program lasted only 2 weeks, we cannot exclude the possibility that long-AATn CNR1 patients may still be responsive to a longer PT program. These limitations may prevent the conclusion that exercise is more beneficial in patients with short-AATn. Further studies are needed to confirm our results and hypothesis and to test the effects of the CNR1 genetic variants both in healthy individuals and in other disease conditions and how they interact with other factors that may potentially influence TBS-induced LTP and effects of exercise.

Our results provide the first evidence that genetic differences within the CB1R may determine at least in part the variable clinical response to PT and strengthen the proposed involvement of CB1Rs in the control of spasticity. This information might be of great relevance for patient stratification and personalized PT.
Defective plasticity mechanisms in long-AATn CNR1 patients with MS could also account for worse clinical evolution, as plasticity plays a key role in spontaneous compensation of brain damage, implying that defective plasticity reserve may contribute to clinical progression. It is interesting that genome-wide association studies did not identify the genetic area containing the CNR1 gene as related to MS susceptibility; however, long-AATn CNR1 (≥13) alleles are more frequent in patients with primary progressive MS, a condition characterized by progressive clinical worsening of disability with no periods of remission.

**AUTHOR CONTRIBUTIONS**

Dr. Mori: study concept and design, acquisition of data, analysis, interpretation, and manuscript preparation. Dr. Ljoka: study concept and design, acquisition of data. Dr. Nicoletti: acquisition of data. Dr. Kopecà: acquisition of data. Dr. Buttari: acquisition of data. Ms. Goidani: acquisition of data. Dr. Rossi: study concept and design. Dr. Foti: study concept and design. Dr. Centonze: study concept and design, interpretation, and manuscript preparation.

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**DISCLOSURE**

F. Mori received travel funding from Teva Pharmaceuticals and Merck-Serono, received speaker honoraria from Merck-Serono, is an Associate Editor for BMC Neurology, and consulted for Grifols Italia. C. Ljoka, C.G. Nicoletti, and H. Kopecà report no disclosures. F. Buttari has received speaker honoraria from Novartis and Teva. L. Goidani report no disclosures. S. Rossi is on the advisory board for Biogen Idec, Teva, and Novartis; has received speaker honoraria from Novartis, Teva, and Biogen Idec; and has received travel funding from Novartis, Teva, Merck-Serono, and Sanofi-Aventis. C. Foti is on the editorial board for Bio Med Center Med Sport. D. Centonze is on the scientific advisory board for Teva Pharmaceutical Industries Ltd, Merck-Serono, Bayer Schering, and Novartis; has received speaker honoraria and travel funding from Sanofi-Aventis, Merck-Serono, Serono Symposia International Foundation, Bayer Schering Pharma, and Biogen-Dompé AG; and has received research support from Merck-Serono, Teva, Novartis, Bayer Schering, Sanofi-Aventis, Biogen Idec, Italian National Ministry of Education, and Fondazione Italiana Sclerosi Multipla (FISM). Go to Neurology.org/nm for full disclosures.

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