Stroke

CLINICAL TRIAL

Efficacy of Intravenous Mesenchymal Stem Cells for Motor Recovery After Ischemic Stroke: A Neuroimaging Study

Jungsoo Lee, PhD*; Won Hyuk Chang, MD, PhD*; Jong-Won Chung, MD, PhD; Suk Jae Kim, MD, MSc; Soo-Kyoung Kim, MD, PhD; Jin Soo Lee, MD, PhD; Sung-Il Sohn, MD, PhD; Yun-Hee Kim, MD, PhD†; Oh Young Bang, MD, PhD†; STARTING-2 Collaborators‡

BACKGROUND AND PURPOSE: Stem cell–based therapy is a promising approach to repair brain damage after stroke. This study was conducted to investigate changes in neuroimaging measures using stem cell–based therapy in patients with ischemic stroke.

METHODS: In this prospective, open-label, randomized controlled trial with blinded outcome evaluation, patients with severe middle cerebral artery territory infarct were assigned to the autologous mesenchymal stem cell (MSC) treatment or control group. Of 54 patients who completed the intervention, 31 for the MSC and 13 for the control groups were included in this neuroimaging analysis. Motor function was assessed before the intervention and 90 days after randomization using the Fugl-Meyer assessment scale. Neuroimaging measures included fractional anisotropy values of the corticospinal tract and posterior limb of the internal capsule from diffusion tensor magnetic resonance imaging and strength of connectivity, efficiency, and density of the motor network from resting-state functional magnetic resonance imaging.

RESULTS: For motor function, the improvement ratio of the Fugl-Meyer assessment score was significantly higher in the MSC group compared with the control group. In neuroimaging, corticospinal tract and posterior limb of the internal capsule fractional anisotropy did not decrease in the MSC group but significantly decreased at 90 days after randomization in the control group. Interhemispheric connectivity and ipsilesional connectivity significantly increased in the MSC group. Change in interhemispheric connectivity showed a significant group difference.

CONCLUSIONS: Stem cell–based therapy can protect corticospinal tract against degeneration and enhance positive changes in network reorganization to facilitate motor recovery after stroke.

REGISTRATION: URL: https://www.clinicaltrials.gov; Unique identifier: NCT01716481.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: ischemic stroke ■ magnetic resonance imaging ■ stem cells ■ motor skills ■ neuroimaging

Correspondence to: Yun-Hee Kim, MD, PhD, Department of Physical and Rehabilitation Medicine, Center for Prevention and Rehabilitation, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Department of Health Sciences and Technology, Department of Medical Device Management & Research, Department of Digital Health, SAIHST, Sungkyunkwan University, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea, Email yunkim@skku.edu or Oh Young Bang, MD, PhD, Department of Neurology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, South Korea, Email ohyoung.bang@samsung.com
*J. Lee and W.H. Chang contributed equally.
†Y.-H. Kim and O.Y. Bang contributed equally.
‡A list of all STARTING-2 Collaborators is given in the Appendix.
The Data Supplement is available with this article at https://www.ahajournals.org/doi/suppl/10.1161/STROKEAHA.121.034505.
For Sources of Funding and Disclosures, see page 27.

Stroke is available at www.ahajournals.org/journal/str

Nonstandard Abbreviations and Acronyms

CST	corticospinal tract
DTI	diffusion tensor imaging
FA	functional anisotropy
FMA	Fugl-Meyer assessment
FMA-LL	lower limb score of FMA
FMA-T	total score of FMA
fMRI	functional MRI
MRI	magnetic resonance imaging
MSC	mesenchymal stem cell
PLIC	posterior limb of the internal capsule
rs-fMRI	resting-state functional MRI
STARTING-2	Stem Cell Application Researches and Trials in Neurology-2
T0	preintervention
T1	90-day follow-up

Stem cell–based therapy is a promising approach to promote neurobiological recovery and recover impaired functions after stroke.[1] According to a meta-analysis,[2] stem cell–based therapy can improve impaired functions and quality of life in patients with stroke. Although the mechanisms of stem cell–based therapies are under investigation, multiple mechanisms including cell replacement, bystander effects, neurotrophic influence, enhancement of endogenous repair process, and immune and inflammatory modulation have been suggested.[1,3] These mechanisms can contribute to neural circuit reconstruction and improve functional benefit, as reported in previous animal studies.[4–6] Particularly, induced neurogenesis, axonal sprouting, and synaptogenesis enhancement by stem cell–based therapy can cause positive changes in injured tracts and connections.[7] In this respect, neuroimaging study can be helpful in providing further evidence.

Neuroimaging approaches have been used in several animal studies.[8–10] A rat middle cerebral artery occlusion model showed an increase of white matter integrity of ischemic boundary regions under diffusion tensor imaging (DTI) and recovery of normal activation in the sensorimotor cortex on functional magnetic resonance imaging (fMRI).[8,10] In a human study, increased activation of the primary motor and premotor cortices was observed using fMRI after stem cell–based therapy in patients with ischemic stroke.[11] These studies indicate that stem cell–based therapy might have led to plastic changes at the synaptic and neuronal levels, and such neuroimaging approaches are useful to provide important implications for the effects of stem cell–based therapy. We performed the STARTING-2 trial (Stem Cell Application Researches and Trials in Neurology-2) to evaluate the efficacy of autologous mesenchymal stem cell (MSC) therapy in ischemic stroke and recently reported the results of primary and secondary outcomes.[12] In this clinical trial, neuroimaging data were acquired for DTI and resting-state fMRI (rs-fMRI). By examining changes in neuroimaging measures, we aimed to investigate the effects of stem cell–based therapy on macroscopic neural network reorganization in patients with stroke.

METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Trial Design and Participants

The STARTING-2 trial was an investigator-initiated, prospective, randomized, open-label, controlled trial with blinded outcome evaluation (PROBE [Prospective Randomized Open, Blinded End-Point] design). The trial protocol details are described elsewhere.[13] Participants were enrolled from four university medical centers and transferred to Samsung Medical Center to receive comprehensive rehabilitation and undergo serial MRI examinations. The selection of participants was based on clinical and radiological features. Blood-brain barrier manipulation using intravenous mannitol before MSC treatment and comprehensive and objective measurements using multimodal MRI and detailed functional assessments were performed.

Eligible participants were adults age 30 to 75 years who had moderate-to-severe persistent neurological deficits (National Institutes of Health Stroke Scale score of 6 to 21 points), stroke observed within 90 days of the onset of symptoms, and nonlacunar infarcts within the middle cerebral artery territory but sparing more than half of the ipsilateral subventricular zone. Inclusion and exclusion criteria are shown in the trial protocol.[13]

Participants were randomly assigned at a ratio of 2:1 to the MSC group or control group using computer-generated random-permuted blocks, each with 6 subjects. A total of 54 participants were completed for this study (39 patients in the MSC group and 15 patients in the control group). Of these, MRI scans could not be performed for 5 participants due to the application of a preliminary study protocol without MRI (n=2), refusal by participants (n=2), and metal implant in the brain (n=1). Two patients showed bilateral brain lesions without clinical symptoms or signs from one hemisphere. In addition, there was a failure of spatial normalization of MRI preprocessing procedures in 3 participants.

Finally, data analyses were performed on 44 patients with ischemic stroke (31 participants in the MSC and 13 in the control groups). The flowchart of the study is shown in Figure 1. Demographic and clinical information for all patients in the 2 groups is summarized in Table 1.

The clinical trial protocol and consent form were approved by the Korean Food and Drug Administration (No. 12218) and the Institutional Review Board of Samsung Medical Center, Seoul, Republic of Korea (IRB-2011-10-047). The trial was registered and reported according to the CONSORT (Consolidated Standards of Reporting Trials) statement.[14] Written informed consent was obtained from all patients and/or their first-degree relatives.

Treatments

All participants received conventional rehabilitation therapy (physical, occupational, speech/language, or cognitive rehabilitation therapy as needed) during the inpatient rehabilitation

CLINICAL TRIAL

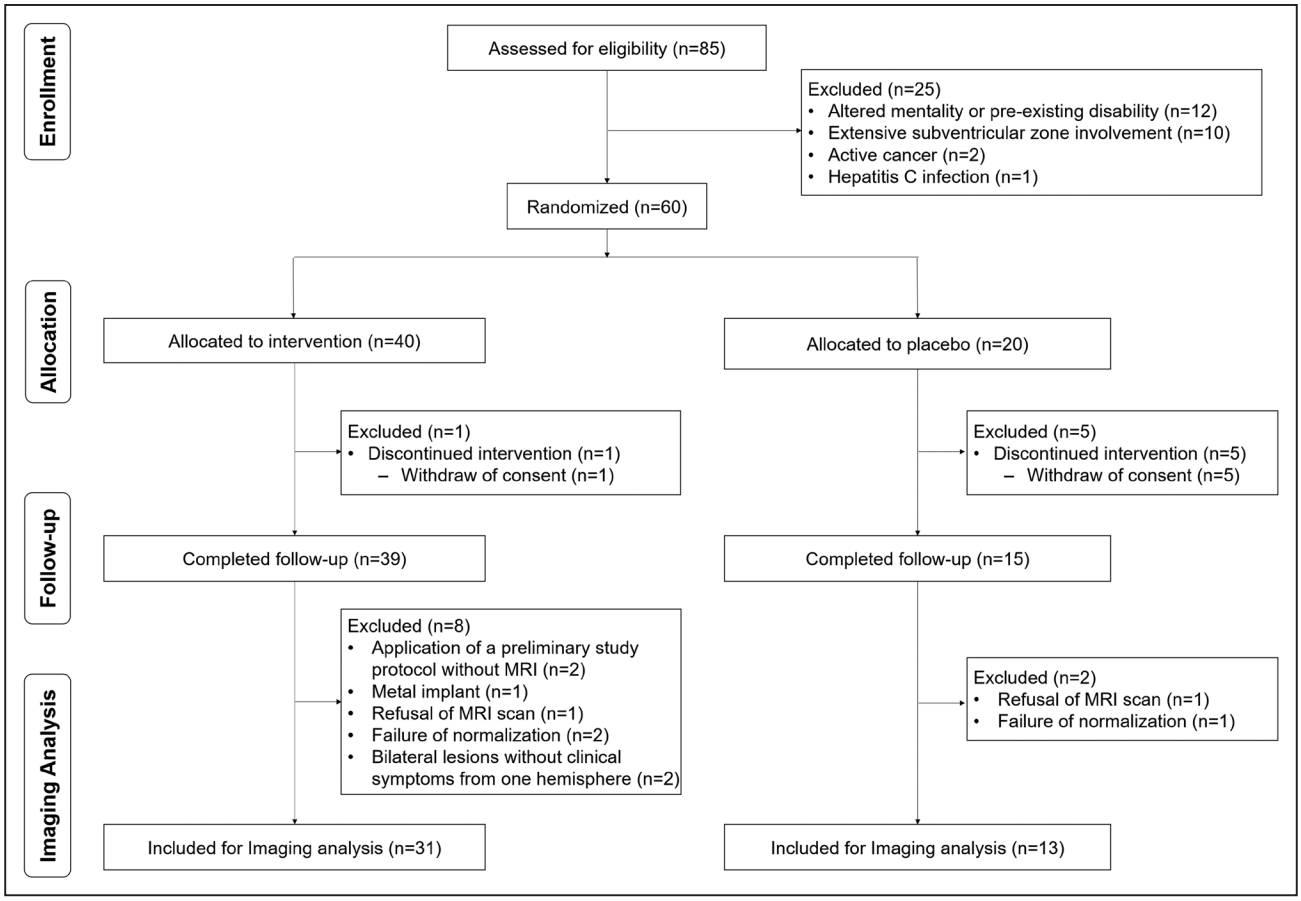

Figure 1. Flowchart of the neuroimaging study.
MRI indicates magnetic resonance imaging.

period. Participants in the MSC group additionally underwent MSC treatment. Methods for bone marrow aspiration, MSC isolation, cell preparation, and intravenous infusion were as previously described, except for the amount of aspirated bone marrow and the use of autologous serum for ex vivo cultivation of MSCs.[12,13,15,16] The control group received the same amount of rehabilitation therapy without injection of MSC.

Motor Function Assessments

Motor function of all participants was evaluated with the Fugl-Meyer Assessment (FMA)[17] by a licensed occupational therapist blinded to group allocations. The therapist completed standardized training programs including instructional lectures, test patient videos, and standardized scoring sheets before the assessment. The total score of FMA (FMA-T), upper limb score of FMA (FMA-UL), and lower limb score of FMA (FMA-LL) were recorded separately. FMA has well-established reliability and validity as an indicator of motor impairment severity across stroke recovery time points.[17] Motor function assessments were performed at baseline (preintervention [T0]) and 90 days after randomization (90-day follow-up [T1]). The improvement ratio of each score was calculated to measure functional improvement considering the baseline FMA score, which is the most influential factor in improvement of FMA score. The formula is a relative change method[18] and is defined as follows:

$$\textit{Improvement ratio} = \frac{\textit{FMA (T1)} - \textit{FMA (T0)}}{\textit{FMA (T0)}}$$

Determination of Motor-Evoked Potential Response

Determination of motor-evoked potential response was performed by single-pulse transcranial magnetic stimulation. A transcranial magnetic stimulation system (Magstim Rapid2 stimulator; Magstim, Ltd, Carmarthenshire, United Kingdom) and 70-mm figure-8 coil were used. Electromyography electrodes were attached to the contralateral first dorsal interosseous muscle. Using the international 10 to 20 system, the vertex (Cz) point was marked, and the initial scalp location was identified at 5 cm lateral to the intersection line from the vertex to the preauricular point. The presence or absence of the motor-evoked potential response was analyzed by moving the coil in 1-cm steps in each direction for an interval of 5 s. The motor-evoked potential response was determined as present if the stimuli produced motor-evoked potentials ≥50 μV peak-to-peak amplitude in 5 of 10 subsequent trials.

MRI Data Acquisition

The DTI, rs-fMRI, and T1-weighted structural data were acquired using a 3T Philips ACHIEVA MR scanner (Philips Medical Systems, Best, the Netherlands). During the MRI scan, participants were instructed to keep their eyes closed without thinking about anything in particular and to remain motionless. Total scan time was around 18 minutes. The MRI data were acquired with the following settings: DTI data, b=1000 s/mm^2,

Table 1. Demographic and Clinical Characteristics of Participants

Group	MSC (n=31)	Control (n=13)
Age, y		
Mean±SD	63.4±14.0	61.5±13.0
Sex (*n*)		
Male	15	9
Female	16	4
Lesion side (n)		
Right	15	6
Left	16	7
Lesion volume, cc		
Mean±SD	125.0±115.7	127.3±122.7
Duration after stroke onset, d		
Mean±SD	24.6±21.0	20.9±11.9
MEP response at T0		
Presence	5	2
Absence	24	11
N/A	2	0

MEP indicates motor-evoked potential; MSC, mesenchymal stem cell; N/A, not available; and T0, preintervention.

45 noncolinear gradient directions, 60 axial slices, slice thickness=2.25 mm, no gap, matrix size=112 × 112, repetition time=8770 ms, echo time=60 ms, and field of view=220×220 mm; rs-fMRI data, 100 volumes, 35 axial slices, slice thickness=4 mm, no gap, matrix size=128×128, repetition time=3000 ms, echo time=35 ms, and field of view=220×220 mm; T1-weighted structural data, 124 axial slices, slice thickness=1.6 mm, no gap, matrix size=512×512, repetition time=13.9 ms, echo time=6.89 ms, flip angle=8°, and field of view=240×240 mm.

MRI Data Processing and Measurements

Lesion Volume

The lesions were drawn manually on the diffusion-weighted image using FSLview 4.0.1 (part of FSL software package 5.0.9). Lesion volumes were warped to Montreal Neurological Institute standard space using the transformation matrix obtained from spatial normalization of DTI data. Lesion volumes were flipped for participants with lesions in the left hemisphere, and all lesion volumes were overlaid on the right hemisphere. The lesion distribution of each group was visualized using MRIcroGL (McCausland Center for Brain Imaging, University of South Carolina, http://www.cabiatl.com/mricrogl; Figure 2). Lesion volume was calculated by counting the number of lesioned voxels on the spatially normalized lesion map and multiplying by voxel volume.

Extraction of Fractional Anisotropy Values From DTI Data

To extract functional anisotropy (FA) values of the corticospinal tract (CST) and posterior limb of the internal capsule (PLIC), individual DTI data points were preprocessed using the FDT (FMRIB's [Functional Magnetic Resonance Imaging of the Brain] Diffusion Toolbox) implemented in the FSL software package 5.0.9 (FMRIB Software Library, FMRIB, Oxford, United Kingdom, http://www.fmrib.ox.ac.uk/fsl). Corrections for eddy currents and head motion were performed, and the brain was skull-stripped using the brain extraction tool algorithm. The *DTIfit* algorithm was used to fit a tensor model and reconstruct FA maps, which were registered to Montreal Neurological Institute standard space (FMRIB58_FA standard space image) using the nonlinear registration algorithms of the tract-based spatial statistics technique. Lesioned voxels were masked out, and stroke lesion was not considered during spatial registration. The spatially normalized FA maps were assessed visually. To obtain the FA value of the CST, the CST template descending from the primary motor cortex (M1) obtained from probabilistic tractography in the nine healthy DTI data was used.[19] To obtain the FA value of the PLIC, the Johns Hopkins University white matter atlas (JHU ICBM-DTI-81)[20] was used. The CST and PLIC were binarized and masked on spatially normalized FA maps. The FA values of the CST and PLIC were obtained by averaging within each region. The proportional FA values (affected/unaffected hemisphere) were used as the integrity measure for each region.

Functional Network From rs-fMRI Data

Preprocessing of rs-fMRI data included slice timing and head motion correction, outlier detection for scrubbing using ART-based identification, registration to structural images, segmentation, lesion-masked spatial normalization, and spatial smoothing with a 6-mm full-width half-maximum gaussian kernel. All processes were performed with the SPM12 package (Welcome Trust Centre for Neuroimaging, University College London, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm). Nuisance signals were removed with a linear regression of several parameters, including head motion parameters, temporal parameters for each of the white matter, ventricle, and global signals, and scrubbing parameters for outlier volumes. Band-pass filtering between 0.009 and 0.08 Hz and linear detrending was performed to remove constant offsets and linear trends. These processes were performed with MATLAB R2016a (Mathworks, Natick, MA).

The functional network was constructed with 24 predefined (motor-related) regions (Table I in the Data Supplement) obtained from a previous meta-analysis[21] of 36 neuroimaging studies for upper extremities of patients with stroke. Lesioned voxels were masked out. The network was constructed by calculating the Pearson correlation for the mean time course of each region, defined as the 10-mm diameter sphere around the predefined Montreal Neurological Institute coordinates.

Measures of the Motor Functional Network

Strength of Connectivity

The strength of the ipsilesional, contralesional, and interhemispheric connectivity in the motor network was obtained by averaging the strength of connections within the ipsilesional hemisphere and contralesional hemisphere and between homotopic regions in bilateral hemispheres.

Network Efficiency and Network Density

The network efficiency and network density are graph theoretical measures. Network efficiency is inversely related

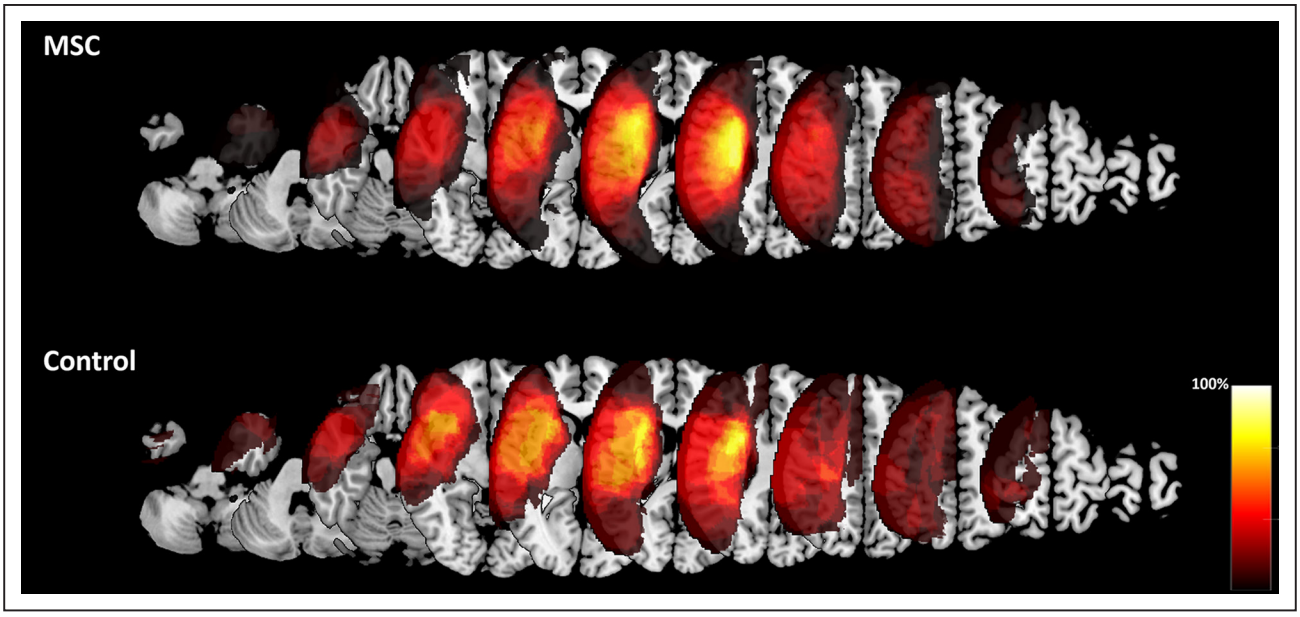

Figure 2. Lesion maps.
Stroke lesions were flipped for patients with lesions on the **left** side. All lesions were overlaid on the **right** side. MSC indicates mesenchymal stem cell.

to path length, which is the minimum number of connections required to travel from one network node to another. Therefore, the measure indicates how efficiently information is exchanged in the network structure.[22–24] Network density is the proportion of the number of existing connections to the number of possible connections in the network.[25] These measures are defined as follows:

$$Network\ efficiency = \frac{1}{n}\sum_{i\in N}\frac{\sum_{j\in N, j\neq i}(d_{ij}^{w})^{-1}}{n-1}$$

$$Network\ density = \frac{2c}{n(n-1)}$$

where n is the number of regions, d_{ij}^{w} is the shortest path length between region i and region j, and c is the number of existing connections.

Statistical Analysis

Normality testing was performed using the Shapiro-Wilk test. The independent t test and the χ^2 test were performed to evaluate significant differences in clinical characteristics. To examine the efficacy of motor recovery after MSC therapy by adjusting for baseline, group differences in change in FMA scores and imaging measures were investigated by ANCOVA. The paired t test was performed to analyze significant changes in FMA scores and imaging measures within a group. In addition, Hedges g was used to evaluate the effect size of the change in FMA scores within a group. Hedges g=0.2, 0.5, and 0.8 are interpreted as small, medium, and large effects, respectively.[26] The independent t test was performed to evaluate significant group differences in the improvement ratio of FMA scores. Statistical significance was assumed at $P<0.05$. All data were analyzed using SPSS version 24.0 (SPSS, Inc, Chicago, IL) and MATLAB R2016a (Mathworks, Natick, MA).

RESULTS

Demographic and clinical characteristics at baseline (preintervention) showed no significant difference between the MSC and control groups. In the lesion map (Figure 2), lesion distributions were similar between the MSC and control groups. There was no difference in lesion volume or any FMA score or functional imaging measure between groups at preintervention.

Changes in Motor Function

The FMA-T, FMA-UL, and FMA-LL values showed significant improvements in the MSC and control groups. However, effect sizes were different between groups. FMA-T and FMA-LL in the MSC group were interpreted as a medium effect size contrary to the control group, which showed a small effect size (Table 2). There were no significant group differences in change in FMA scores (ANCOVA; FMA-T, F=1.56, P=0.219; FMA-UL, F=0.86, P=0.359; FMA-LL, F=1.95, P=0.170).

Improvement ratios of FMA-T, FMA-UL and FMA-LL were 89.9±138.5%, 123.1±243.8%, and 75.9±104.8%, respectively, in the MSC group and 32.8±35.3%, 33.4±35.4%, and 39.8±56.0% in the control group. The improvement ratio of FMA-T was significantly higher in the MSC group compared with the control group (P=0.043). In addition, the improvement ratio of FMA-UL and FMA-LL tended to be higher in the MSC group than in the control group, although the differences were not significant (P=0.058 and 0.158, Figure 3).

Table 2. Changes of Motor Functions in Mesenchymal Stem Cell Therapy and Control Groups

	Group	T0	T1	*P* value	Hedges g	ES
FMA-T	MSC	18.7±16.1	30.8±23.0	<0.001	0.546	Medium
	Control	24.3±23.5	29.2±25.2	0.0063	0.227	Small
FMA-UL	MSC	10.1±11.3	17.9±16.6	0.0015	0.481	Small
	Control	14.9±17.3	17.8±19.0	0.0089	0.208	Small
FMA-LL	MSC	8.6±6.2	12.9±7.2	<0.001	0.592	Medium
	Control	9.4±7.5	11.4±7.2	0.0324	0.243	Small

ES indicates effect size; FMA-LL, lower limb score of Fugl-Meyer assessment; FMA-T, total score of Fugl-Meyer assessment; FMA-UL, upper limb score of Fugl-Meyer assessment; MSC, mesenchymal stem cell; T0, preintervention; and T1, 90-d follow-up.

Changes in Neuroimaging Measures

In DTI data analysis (Figure 4A), the CST and PLIC FA values at T1 significantly decreased in the control group compared with those at preintervention (CST FA, *P*=0.030; PLIC FA, *P*=0.027). In contrast, the FA values at T1 did not show a significant decrease from preintervention in the MSC group.

In rs-fMRI data analysis (Figure 4B), the strength of ipsilesional connectivity in the motor network significantly increased in the MSC group (*P*=0.042) but not in the control group. The strength of contralesional connectivity in the MSC and control groups did not change at T1 compared to preintervention. However, the strength of interhemispheric connectivity significantly decreased in the control group (*P*=0.030) but not in the MSC group. There was a significant group difference in change in the strength of interhemispheric connectivity (ANCOVA, *F*=4.87, *P*=0.033). The network efficiency and network density did not show significant changes in either group. However, nonsignificant trends were demonstrated in network efficiency and density, which tended to increase in the MSC group and decrease in the control group.

Alterations of local connectivity in the motor network were investigated in each group (Figure 4C). In the MSC group, most connections with ipsilesional regions, including sensorimotor cortices, were increased at T1. In contrast, most connections were decreased at T1 in the control group.

DISCUSSION

In this subanalysis of the STARTING-2 trial, MSC therapy might be effective to facilitate motor recovery and positive changes in motor network reorganization by investigating changes in the CST and PLIC FA values of DTI and the interhemispheric and ipsilesional functional connectivity of rs-fMRI.

Our main study recently reported no significant difference between groups in modified Rankin Scale as a primary outcome.[12] However, secondary and exploratory end point analyses suggested a clinically significant improvement in motor function of the MSC group. In participants of this neuroimaging study, effect sizes of FMA-T and FMA-LL in the MSC group were larger than those in the control group. Additionally, the improvement ratio of FMA-T was significantly greater in the MSC group than in the control group. Specifically, the control group showed a small standard deviation of motor improvement ratio, which might indicate that motor improvement is similar among patients with severe stroke. In contrast, a large standard deviation of motor improvement ratio

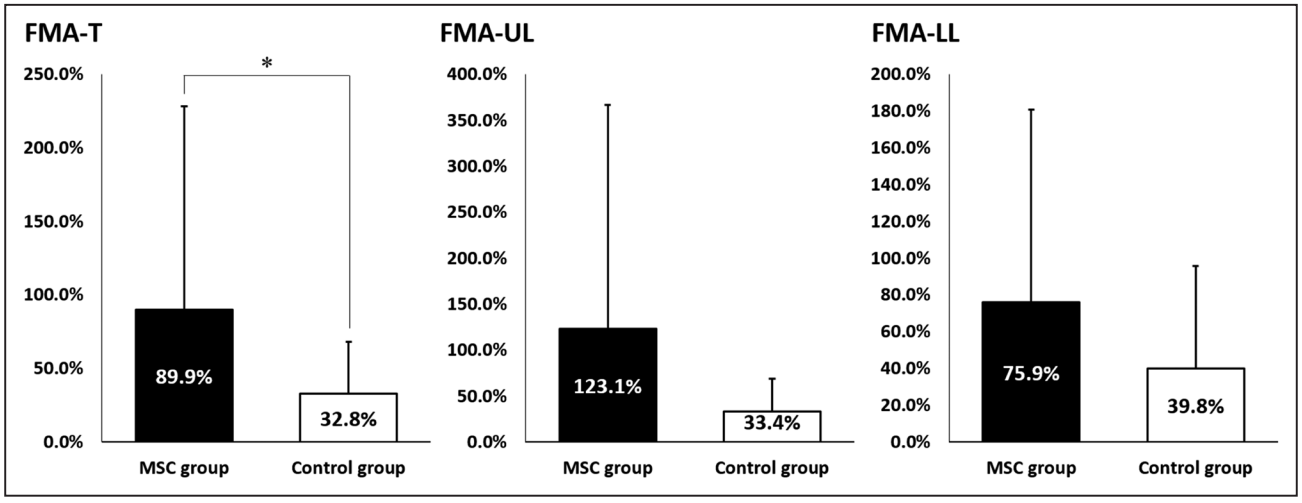

Figure 3. Improvement ratio of motor function in both groups.
FMA-LL indicates lower limb score of Fugl-Meyer assessment; FMA-T, total score of Fugl-Meyer assessment; FMA-UL, upper limb score of Fugl-Meyer assessment; and MSC, mesenchymal stem cell.

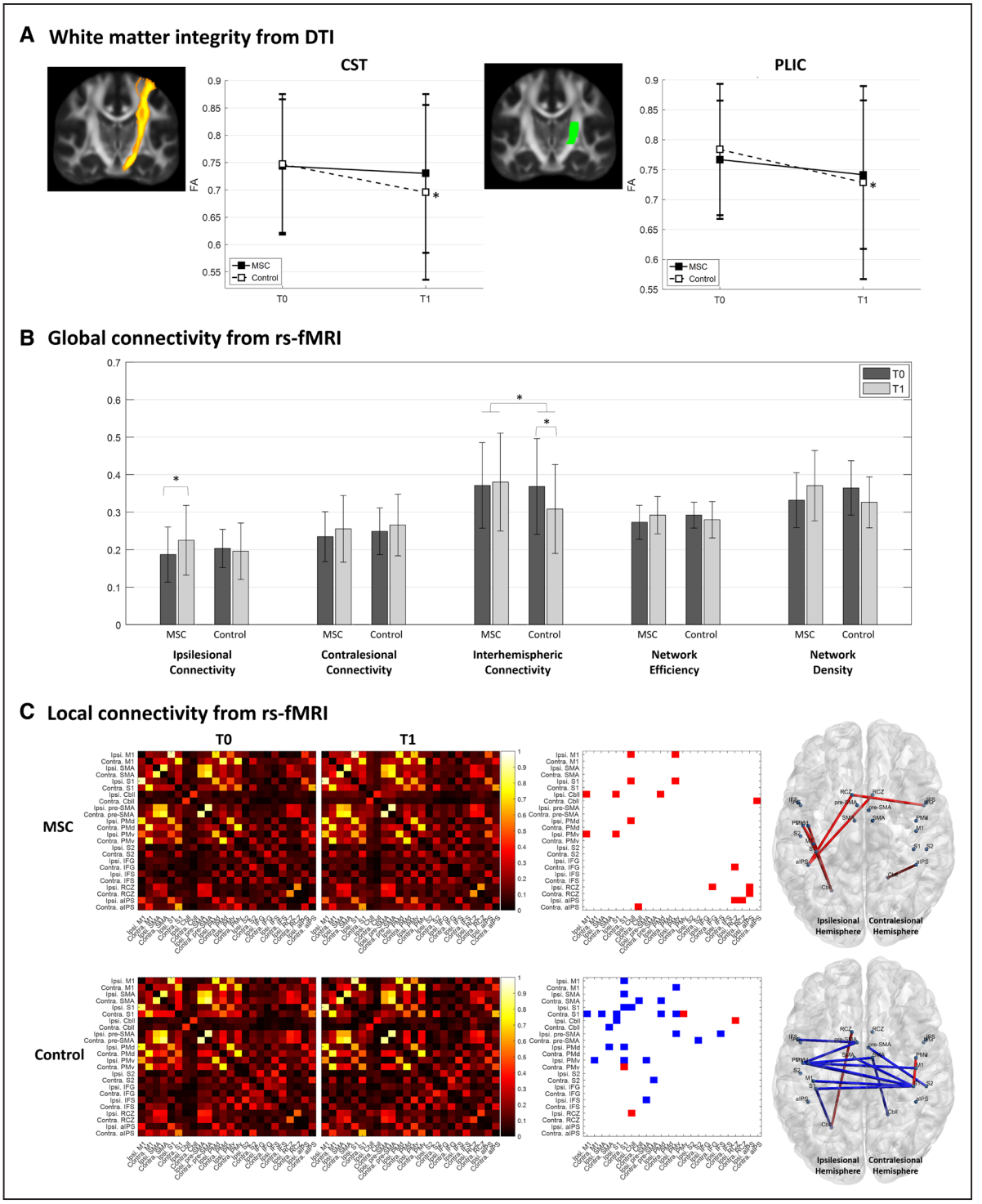

Figure 4. Changes in neuroimaging measures on diffusion tensor imaging (DTI) and resting-state functional magnetic resonance imaging (rs-fMRI).

A, Altered white matter integrity on diffusion tensor imaging. Changes in fractional anisotropy (FA) values of the corticospinal tract (CST) and posterior limb of the internal capsule (PLIC) on diffusion tensor imaging. FA values of both white matter regions did not change in the mesenchymal stem cell (MSC) group, but the values significantly decreased in the control group (*P<0.05). **B**, Altered global connectivity on rs-fMRI. Global connectivity of the ipsilesional connectivity, interhemispheric connectivity, network efficiency, and network density tended to increase in the mesenchymal stem cell therapy group and decrease in the control group. The strength of ipsilesional connectivity significantly increased in the MSC group. The strength of interhemispheric connectivity significantly decreased in the control group, and there was a significant difference between changes in the strength of interhemispheric connectivity in both groups (*P<0.05). **C**, Altered local connectivity on rs-fMRI. **Left** and **center** matrices represent the strength of local connectivity in the motor network at preintervention and follow-up, respectively. **Right** matrices and glass-brain views represent significantly altered connectivity. Red indicates an increased connection after the intervention, and blue indicates a decreased connection. aIPS indicates anterior intraparietal sulcus; Cbll, cerebellum; contra., contralesional; IFG, inferior frontal gyrus; IFS, inferior frontal sulcus; ipsi., ipsilesional; M1, precentral gyrus; PMd, dorso-lateral precentral gyrus/sulcus; PMv, ventro-lateral precentral gyrus/sulcus; RCZ, rostral cingulate zone; S1, postcentral gyrus; S2, parietal operculum; SMA, medial superior frontal gyrus; T0, preintervention; and T1, 90-d follow-up.

after MSC therapy might infer that MSC therapy induces greater changes in some patients with stroke.

Alterations of white matter integrity and functional connectivity were investigated using DTI and rs-fMRI data. The FA value obtained from DTI represents an index for the amount of diffusion asymmetry within a voxel. The FA value is used frequently to examine the degree of damage of white matter tracts and their recovery after stroke because it is highly sensitive to microstructural changes.[27,28] The FA value changes according to ischemia, myelination, axonal damage, and maturation.[27] In general, the FA value of a damaged tract decreases from the acute to the chronic stage after stroke due to Wallerian degeneration.[29,30] In our study, CST and PLIC FA values significantly decreased only in the control group. However, these values did not decrease in the MSC group. This phenomenon suggests that stem cell–based therapy might modulate degeneration of the damaged tract by stroke.

Network measures obtained from rs-fMRI in patients with stroke tend to be lower than those of healthy subjects.[31–33] In alterations of intrahemispheric connectivity, the strength of ipsilesional connectivity was increased only in the MSC group. Contralesional connectivity in both groups and ipsilesional connectivity in the control group did not show significant change. These results imply that stem cell–based therapy might directly involve a damaged hemisphere. Furthermore, changes in interhemispheric connectivity differed between 2 groups. The strength of connectivity significantly decreased in the control group but not in the MSC group. Interhemispheric connectivity is an important measure to reflect damage to CST and is related to functional outcome and recovery.[34,35] In particular, the value is significantly lower than that of healthy subjects due to the interhemispheric imbalances caused by stroke.[31,32] Network efficiency and density also are important network measures in the damaged brain.[36–38] These measures did not show significant changes between or within the 2 groups. However, alterations of interhemispheric connectivity were demonstrated to increase in the MSC group and decrease in the control group. In alterations of local connectivity, significantly decreased connectivity was dominant in the control group, but significantly increased connectivity was dominant in the MSC group. According to a previous imaging study,[39] it might be difficult to expect positive changes in network measures by rs-fMRI from subacute to chronic phases of patients with severe stroke. Nevertheless, network measures demonstrated relatively positive changes in the severely impaired patients with stroke of the MSC group of this study.

This study has some limitations. There was relatively weak statistical significance to demonstrate the effect of MSC therapy on the improvement of motor function in patients with stroke. In neuroimaging analysis, a significant difference between groups was noticed only in alteration of interhemispheric connectivity, although some trends were observed in other measures. The number of participants in the control group was not sufficient compared to that in the MSC group and might have decreased the statistical power to investigate differences in changes of functional and imaging values between 2 groups. A future study is recommended with a larger sample size.

In conclusion, intravenous application of preconditioned autologous MSC might improve motor function in patients with major stroke. In addition, the neuroimaging approach was useful to measure the effectiveness of stem cell–based therapy on plastic changes of neural networks. This neuroimaging study purports tentative conclusions as follows. Stem cell–based therapy might be effective in motor network reorganization after stroke, as shown by changes in FA value of motor-specific white matter tracts and strength of interhemispheric and ipsilesional connectivity in rs-fMRI. Also, MSC therapy might be engaged directly in the damaged area, as seen through a change in the strength of ipsilesional connectivity. Stem cell–based therapy may help to facilitate motor recovery from stroke by reducing degeneration and leading to positive network reorganization. To provide additional evidence and to support the clinical application, further studies are needed to determine the characteristics of responders and nonresponders to stem cell–based therapy after stroke.

ARTICLE INFORMATION

Received February 1, 2021; final revision received May 24, 2021; accepted June 14, 2021.

The podcast and transcript are available at https://www.ahajournals.org/str/podcast.

Affiliations

Department of Physical and Rehabilitation Medicine, Center for Prevention and Rehabilitation, Heart Vascular Stroke Institute, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, South Korea (J.L., W.H.C., Y.-H.K.). Department of Neurology, Samsung Medical Center, Sungkyunkwan University, Seoul, South Korea (J.-W.C., S.J.K., O.Y.B.). Department of Health Sciences and Technology, Department of Medical Device Management & Research, Department of Digital Health, SAIHST, Sungkyunkwan University, Seoul, South Korea (Y.-H.K.). Translational and Stem Cell Research Laboratory on Stroke, Samsung Medical Center, Seoul, South Korea (J.-W.C., O.Y.B.). Department of Neurology, Gyeongsang National University School of Medicine, Jinju, South Korea (S.-K.K.). Departments of Neurology, Ajou University Hospital, School of Medicine, Suwon, South Korea (J.S.L.). Department of Neurology, Keimyung University Dongsan Medical Center, Keimyung University School of Medicine, Daegu, South Korea (S.-I.S.).

Acknowledgments

We thank the STARTING-2 (Stem Cell Application Researches and Trials in Neurology-2) investigators. Y.-H. Kim and Dr Bang, the cocorresponding authors, were the principal investigators, participated in the study design, obtained funding, and proofread the manuscript. J. Lee and Dr Chang, the cofirst authors, contributed to data collection, data analysis, and wrote the report. Dr Chung, S.J. Kim, S.-K. Kim, J.S. Lee, and Dr Sohn contributed to study design and data collection and gave scientific advice.

Sources of Funding

This study was supported by a grant from the Korea Health Technology R&D Project, Ministry of Health & Welfare (HI14C16240000), and by a National Research Foundation of Korea (NRF) grant funded by the Korean government (NRF-2020R1A2C3010304).

Disclosures

Dr Bang reports a patent to US10,072,247 B2 licensed. The other authors report no conflicts.

CLINICAL TRIAL

Supplemental Materials

Online Table I

APPENDIX

STARTING-2 Investigators: Oh Young Bang, MD, PhD; Jong-Won Chung, MD, PhD; Suk Jae Kim, MD, MSc; Soo-Kyoung Kim, MD, PhD; Jin Soo Lee, MD, PhD; Sung-Il Sohn, MD, PhD; Yun-Hee Kim, MD, PhD; Won Hyuk Chang, MD, PhD; Jungsoo Lee, PhD; Yeon Hee Cho, MS; Ji Hee Sung, MS; Eun Hee Kim, PhD; Jeong Pyo Son, PhD; Dong Hee Kim, PhD; Eun-Hyeok Choi, MD; Sookyung Ryoo, MD; Yoon Mi Kang, MS; Yong Man Kim, PhD; Hyun Soo Kim, MD, PhD; Jun Ho Jang, MD, PhD.

REFERENCES

1. Boese AC, Le QE, Pham D, Hamblin MH, Lee JP. Neural stem cell therapy for subacute and chronic ischemic stroke. *Stem Cell Res Ther.* 2018;9:154. doi: 10.1186/s13287-018-0913-2
2. Chen L, Zhang G, Khan AA, Guo X, Gu Y. Clinical efficacy and meta-analysis of stem cell therapies for patients with brain ischemia. *Stem cells Int.* 2016;2016:6129579. doi: 10.1155/2016/6129579
3. Marei HE, Hasan A, Rizzi R, Althani A, Afifi N, Cenciarelli C, Caceci T, Shuaib A. Potential of stem cell-based therapy for ischemic stroke. *Front Neurol.* 2018;9:34. doi: 10.3389/fneur.2018.00034
4. Huang W, Mo X, Qin C, Zheng J, Liang Z, Zhang C. Transplantation of differentiated bone marrow stromal cells promotes motor functional recovery in rats with stroke. *Neurol Res.* 2013;35:320–328. doi: 10.1179/1743132812Y.0000000151
5. Liu Z, Li Y, Zhang L, Xin H, Cui Y, Hanson LR, Frey WH 2nd, Chopp M. Subacute intranasal administration of tissue plasminogen activator increases functional recovery and axonal remodeling after stroke in rats. *Neurobiol Dis.* 2012;45:804–809. doi: 10.1016/j.nbd.2011.11.004
6. Tohill M, Mantovani C, Wiberg M, Terenghi G. Rat bone marrow mesenchymal stem cells express glial markers and stimulate nerve regeneration. *Neurosci Lett.* 2004;362:200–203. doi: 10.1016/j.neulet.2004.03.077
7. Li J, Zhang Q, Wang W, Lin F, Wang S, Zhao J. Mesenchymal stem cell therapy for ischemic stroke: a look into treatment mechanism and therapeutic potential. *J Neurol.* 2020;1-13. doi: 10.1007/s00415-020-10138-5
8. Ramos-Cabrer P, Justicia C, Wiedermann D, Hoehn M. Stem cell mediation of functional recovery after stroke in the rat. *PLoS One.* 2010;5:e12779. doi: 10.1371/journal.pone.0012779
9. Modo M, Mellodew K, Cash D, Fraser SE, Meade TJ, Price J, Williams SC. Mapping transplanted stem cell migration after a stroke: a serial, in vivo magnetic resonance imaging study. *Neuroimage.* 2004;21:311–317. doi: 10.1016/j.neuroimage.2003.08.030
10. Jiang Q, Zhang ZG, Ding GL, Silver B, Zhang L, Meng H, Lu M, Pourabdillah-Nejed-D S, Wang L, Savant-Bhonsale S, et al. MRI detects white matter reorganization after neural progenitor cell treatment of stroke. *Neuroimage.* 2006;32:1080–1089. doi: 10.1016/j.neuroimage.2006.05.025
11. Bhasin A, Srivastava M, Bhatia R, Mohanty S, Kumaran S, Bose S. Autologous intravenous mononuclear stem cell therapy in chronic ischemic stroke. *J Stem Cells Regen Med.* 2012;8:181–189. doi: 10.46582/jsrm.0803011
12. Chung JW, Chang WH, Bang OY, Moon GJ, Kim SJ, Kim SK, Lee JS, Sohn SI, Kim YH; STARTING-2 Collaborators. Efficacy and safety of intravenous mesenchymal stem cells for ischemic stroke. *Neurology.* 2021;96:e1012–e1023. doi: 10.1212/WNL.0000000000011440
13. Kim SJ, Moon GJ, Chang WH, Kim YH, Bang OY; STARTING-2 (STem cell Application Researches and Trials In NeuroloGy-2) collaborators. Intravenous transplantation of mesenchymal stem cells preconditioned with early phase stroke serum: current evidence and study protocol for a randomized trial. *Trials.* 2013;14:317. doi: 10.1186/1745-6215-14-317
14. Schulz KF, Altman DG, Moher D; CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332. doi: 10.1136/bmj.c332
15. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY; STARTING collaborators. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells.* 2010;28:1099–1106. doi: 10.1002/stem.430
16. Bang OY, Lee JS, Lee PH, Lee G. Autologous mesenchymal stem cell transplantation in stroke patients. *Ann Neurol.* 2005;57:874–882. doi: 10.1002/ana.20501
17. Fugl-Meyer AR, Jääskö L, Leyman I, Olsson S, Steglind S. The post-stroke hemiplegic patient. 1. a method for evaluation of physical performance. *Scand J Rehabil Med.* 1975;7:13–31.
18. Törnqvist L, Vartia P, Vartia YO. How should relative changes be measured? *Am Stat.* 1985;39:43-46. doi: 10.1080/00031305.1985.10479385
19. Schulz R, Park CH, Boudrias MH, Gerloff C, Hummel FC, Ward NS. Assessing the integrity of corticospinal pathways from primary and secondary cortical motor areas after stroke. *Stroke.* 2012;43:2248–2251. doi: 10.1161/STROKEAHA.112.662619
20. Mori S, Oishi K, Jiang H, Jiang L, Li X, Akhter K, Hua K, Faria AV, Mahmood A, Woods R, et al. Stereotaxic white matter atlas based on diffusion tensor imaging in an ICBM template. *Neuroimage.* 2008;40:570–582. doi: 10.1016/j.neuroimage.2007.12.035
21. Rehme AK, Eickhoff SB, Rottschy C, Fink GR, Grefkes C. Activation likelihood estimation meta-analysis of motor-related neural activity after stroke. *Neuroimage.* 2012;59:2771–2782. doi: 10.1016/j.neuroimage.2011.10.023
22. Achard S, Bullmore E. Efficiency and cost of economical brain functional networks. *PLoS Comput Biol.* 2007;3:e17. doi: 10.1371/journal.pcbi.0030017
23. Latora V, Marchiori M. Efficient behavior of small-world networks. *Phys Rev Lett.* 2001;87:198701. doi: 10.1103/PhysRevLett.87.198701
24. Sporns O, Zwi JD. The small world of the cerebral cortex. *Neuroinformatics.* 2004;2:145–162. doi: 10.1385/NI:2:2:145
25. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci.* 2009;10:186–198. doi: 10.1038/nrn2575
26. Durlak JA. How to select, calculate, and interpret effect sizes. *J Pediatr Psychol.* 2009;34:917–928. doi: 10.1093/jpepsy/jsp004
27. Alexander AL, Lee JE, Lazar M, Field AS. Diffusion tensor imaging of the brain. *Neurotherapeutics.* 2007;4:316–329. doi: 10.1016/j.nurt.2007.05.011
28. Feldman HM, Yeatman JD, Lee ES, Barde LH, Gaman-Bean S. Diffusion tensor imaging: a review for pediatric researchers and clinicians. *J Dev Behav Pediatr.* 2010;31:346–356. doi: 10.1097/DBP.0b013e3181dcaa8b
29. Werring DJ, Toosy AT, Clark CA, Parker GJ, Barker GJ, Miller DH, Thompson AJ. Diffusion tensor imaging can detect and quantify corticospinal tract degeneration after stroke. *J Neurol Neurosurg Psychiatry.* 2000;69:269–272. doi: 10.1136/jnnp.69.2.269
30. Thomalla G, Glauche V, Weiller C, Röther J. Time course of wallerian degeneration after ischaemic stroke revealed by diffusion tensor imaging. *J Neurol Neurosurg Psychiatry.* 2005;76:266–268. doi: 10.1136/jnnp.2004.046375
31. Siegel JS, Ramsey LE, Snyder AZ, Metcalf NV, Chacko RV, Weinberger K, Baldassarre A, Hacker CD, Shulman GL, Corbetta M. Disruptions of network connectivity predict impairment in multiple behavioral domains after stroke. *Proc Natl Acad Sci USA.* 2016;113:E4367–E4376. doi: 10.1073/pnas.1521083113
32. Lee J, Park E, Lee A, Chang WH, Kim DS, Kim YH. Alteration and role of interhemispheric and intrahemispheric connectivity in motor network after stroke. *Brain Topogr.* 2018;31:708–719. doi: 10.1007/s10548-018-0644-9
33. Zhang J, Zhang Y, Wang L, Sang L, Yang J, Yan R, Li P, Wang J, Qiu M. Disrupted structural and functional connectivity networks in ischemic stroke patients. *Neuroscience.* 2017;364:212–225. doi: 10.1016/j.neuroscience.2017.09.009
34. Carter AR, Patel KR, Astafiev SV, Snyder AZ, Rengachary J, Strube MJ, Pope A, Shimony JS, Lang CE, Shulman GL, et al. Upstream dysfunction of somatomotor functional connectivity after corticospinal damage in stroke. *Neurorehabil Neural Repair.* 2012;26:7–19. doi: 10.1177/1545968311411054
35. Carter AR, Astafiev SV, Lang CE, Connor LT, Rengachary J, Strube MJ, Pope DL, Shulman GL, Corbetta M. Resting interhemispheric functional magnetic resonance imaging connectivity predicts performance after stroke. *Ann Neurol.* 2010;67:365–375. doi: 10.1002/ana.21905
36. van den Heuvel MP, Stam CJ, Kahn RS, Hulshoff Pol HE. Efficiency of functional brain networks and intellectual performance. *J Neurosci.* 2009;29:7619–7624. doi: 10.1523/JNEUROSCI.1443-09.2009
37. Langer N, von Bastian CC, Wirz H, Oberauer K, Jäncke L. The effects of working memory training on functional brain network efficiency. *Cortex.* 2013;49:2424–2438. doi: 10.1016/j.cortex.2013.01.008
38. Philips GR, Daly JJ, Príncipe JC. Topographical measures of functional connectivity as biomarkers for post-stroke motor recovery. *J Neuroeng Rehabil.* 2017;14:67. doi: 10.1186/s12984-017-0277-3
39. Lee J, Park E, Lee A, Chang WH, Kim DS, Kim YH. Recovery-related indicators of motor network plasticity according to impairment severity after stroke. *Eur J Neurol.* 2017;24:1290–1299. doi: 10.1111/ene.13377