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Atrophy of the left dorsolateral prefrontal cortex is associated with poor performance in verbal fluency in elderly poststroke women☆

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Abstract

This study aimed to investigate the association between atrophy in the prefrontal cortex with executive function and verbal fluency in elderly male and female patients poststroke. Thirty elderly female patients with non-aphasic ischemic stroke aged ≥ 60 years and 30 age-matched non-aphasic male patients with ischemic stroke were recruited. Automatic magnetic resonance imaging segmentation was used to assess the volume of the whole prefrontal cortex, along with its subdivisions: anterior cingulate cortex, orbitofrontal cortex and dorsolateral prefrontal cortex. The Semantic Verbal Fluency Test was administered at 3 and 15 months poststroke. At 3 months poststroke, left dorsolateral prefrontal cortex volume was significantly correlated with Verbal Fluency Test score in female patients only (partial coefficient = 0.453, P = 0.045), after controlling for age, education, diabetes, neurological deficit, white matter lesions volume, as well as the location and volume of infarcts. At 15 months poststroke, there remained a significant association between the left dorsolateral prefrontal cortex volume and Verbal Fluency Test (partial coefficient = 0.661, P = 0.001) and between the left prefrontal cortex volume and Verbal Fluency Test (partial coefficient = 0.573, P = 0.004) in female patients after the same adjustments. These findings indicate that atrophy of the left dorsolateral prefrontal cortex contributes to the impairment of verbal fluency in elderly female patients with stroke. Sex differences may be present in the neuropsychological mechanisms of verbal fluency impairment in patients with stroke.

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Key Words

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Research Highlights

(1) Automatic MRI volumetry is an accurate method to quantify regional brain atrophy in stroke patients.
(2) The standardized volume of the left dorsolateral prefrontal cortex correlated positively with the performance of semantic verbal fluency test at 3 and 15 months poststroke in both elderly male and female patients.
(3) In elderly patients with ischemic stroke, performance on executive function and semantic verbal fluency tests did not correlate with location of infarcts, regional brain atrophy or white matter lesions.
(4) These results suggest that sex differences may be present in the neuropsychological mechanisms of verbal fluency impairment in elderly patients with stroke.

INTRODUCTION

Executive function is one of the commonly impaired cognitive domains in poststroke survivors, especially in those with severe subcortical ischemic vascular diseases[1]. Generally, executive functions are those involved in complex cognitive processes, such as solving novel problems, modifying behavior in the light of new information, generating strategies or sequencing complex actions[2]. Verbal fluency, the ability to generate word lists under certain stimulus constraints, is thought to reflect executive functioning[3].

There is evidence for a close link between executive functions (including verbal fluency) and the prefrontal cortex[2, 4]. The prefrontal cortex is the largest component of the frontal lobe, comprising two major portions: the dorsolateral prefrontal cortex and the orbitofrontal cortex[4]. The dorsolateral prefrontal cortex is largely responsible for attention, executive function and working memory, whereas the orbitofrontal cortex is associated with decision-making and affect control[4]. The anterior cingulate cortex plays a role in a wide variety of autonomic functions as well as rational cognitive functions, such as reward anticipation, decision-making, empathy and emotion[4]. Patients with damage to the prefrontal cortex show impaired judgment, as well as behavioral organization, planning and decision-making. In general, “executive function” and “frontal lobe function” have become almost interchangeable terms[2].

Verbal fluency is also thought to be mediated by the frontal lobes; particularly the prefrontal cortex, that has been found to be involved in the verbal fluency network described in healthy individuals[5]. Functional MRI activating studies have demonstrated that poor word-list retrieval is associated with the dominant prefrontal region[6-8], and decreased verbal fluency has been observed in patients with left frontal lobe lesions[9]. In patients with frontotemporal dementia, poor verbal fluency is associated with atrophy of the left dorsolateral prefrontal cortex[10].

Sex differences in cognitive function have been reported in a number of domains, including verbal fluency, visual-spatial skills, motor function[11], and verbal working memory[12]. Most studies suggest that women perform better than men on language tasks[13-15]. Besides sex differences in cognitive function, structural and functional differences in the brain have been identified using MRI[16-19]. For example, men tend to have greater brain volume and higher white matter volume[17], whereas women tend to have more gray matter tissue and greater cortical complexity in the frontal and parietal regions[18], as well as a larger volume of orbitofrontal cortex[19].

In the elderly, several reports have demonstrated more whole-brain and frontal atrophy in men than in women[20-21]. Functional MRI studies have shown that women have more robustly activated areas of the brain associated with pain, verbal fluency, and imagination than men[22-25]. Thus, in elderly poststroke patients, some cognitive functions such as executive function (including verbal fluency) may not be equally impaired in the two sexes.

In ischemic stroke patients, prefrontal cortex atrophy is common[26]. Vascular risk factors may exacerbate brain aging and account for part of the observed decline in volume, as the prefrontal cortex shows increased vulnerability to cerebral small vessel disease[26]. An MRI volumetry study showed that atrophy of the superior frontal and fronto-orbital gyri predicted decline in executive function independent of the white matter lesion volume in patients with cerebral small vessel disease[27]. Impairment of verbal fluency has been found in elderly patients with subcortical ischemic vascular disease[28], and has been linked to atrophy of the corpus callosum in otherwise healthy older adults[29]. However, to the best of our knowledge, there has been no study reporting the relationship between the size of the prefrontal cortex or dorsolateral prefrontal cortex and executive function or verbal fluency in patients with ischemic stroke (through a search of PubMed).

We postulated that atrophy of the prefrontal cortex and its subdivisions may contribute to poor performance in executive function or verbal fluency in ischemic stroke patients, and that there may be sex differences in this association. Thus, we performed an MRI volumetric study of the prefrontal cortex and its subdivisions in non-aphasic stroke patients with the aim of investigating the association between atrophy in these regions on executive function and verbal fluency in men and women.

RESULTS

Quantitative analysis of subjects

Eighty-three elderly patients with acute ischemic stroke, 50 males and 33 females, were initially included in this study. Among the 33 female patients, 30 passed the

baseline assessment and neuropsychological testing. From the initial group of 50 males, 30 age-matched male patients who passed the baseline assessment and neuropsychological testing were selected as controls. Finally, 60 patients with acute ischemic stroke were suitable for final analysis.

Baseline data and MRI variables between male and female patients with acute ischemic stroke

The demographic, clinical, and radiological features of the subjects according to sex are shown in Table 1.

Table 1 Comparisons of demographic, clinical, neuropsychological and radiological characteristics between male and female patients with acute ischemic stroke

Variable	Female (n = 30)	Male (n = 30)	$t/z/\chi^2$	P
Age (year)[a]	73.3±7.2	72.1±6.9	–0.695	0.490
Education (year)[b]	3.1±3.1	5.3±3.1	–2.644	0.010
Hypertension[c]	22(73)	18(60)	1.200	0.273
Diabetes mellitus[c]	16(53)	6(20)	7.177	0.007
Previous stroke[c]	5(17)	5(13)	0.131	0.653
NIHSS[b]	5.2±3.8	4.6±2.4	–0.516	0.485
MMSE (3 m)[b]	23.4±3.2	26.4±2.9	–3.371	0.001
FAB (3 m)[b]	11.5±2.8	13.6±2.6	–2.777	0.005
VFT (3 m)[a]	22.3±6.5	21.9±6.2	–0.242	0.809
GDS (3 m)[b]	4.4±4.3	3.7±3.2	–0.340	0.734
MMSE (15 m)[b]	24.1±3.6	26.7±3.6	–3.234	0.001
FAB (15 m)[b]	11.9±3.4	14.3±3.0	–2.907	0.004
VFT (15 m)[a]	22.8±6.2	24.0±8.3	0.627	0.533
GDS (15 m)[b]	6.6±4.8	5.5±3.9	–0.860	0.390

NIHSS: the National Institutes of Health Stroke Scale; MMSE: Mini-Mental State Examination; FAB: Frontal Assessment Battery; VFT: Verbal Fluency Test; GDS: Geriatric Depression Scale. [a]Mean ± SD, t-test; [b]mean ± SD, Mann-Whitney U test; [c]n(%), chi-square test. m: Months.

There were no significant differences in terms of demographic and clinical variables between male and female patients except that female patients had less education, more severe diabetes mellitus, lower Mini-Mental Status Examination and Frontal Assessment Battery scores. Verbal Fluency Test score did not differ significantly between male and female patients. In terms of MRI variables, female patients also had a significantly higher volume of right orbitofrontal cortex and right anterior cingulate cortex (Table 2).

Associations between Verbal Fluency Test score and MRI variables (3 months poststroke)

At 3 months poststroke, the left dorsolateral prefrontal cortex volume was significantly correlated with the Verbal Fluency Test score in female patients but not in male patients (Table 3 and Figure 1A).

Variables which differed between male and females, as well as age and the National Institutes of Health Stroke Scale scores, were adjusted by subsequent partial correlation. After controlling for age, education, National Institutes of Health Stroke Scale scores, Mini-Mental Status Examination scores at 3 months, diabetes mellitus, volume of infarcts, presence of basal ganglia infarcts, and white matter lesions volume, the correlation between the left dorsolateral prefrontal cortex volume and the Verbal Fluency Test in the female patients remained significant in partial correlation (partial coefficient = 0.453, P = 0.045).

Table 2 Comparisons of MRI variables between male and female patients with acute ischemic stroke

Variable	Female (n = 30)	Male (n = 30)	$t/z/\chi^2$	P
Location of infarcts				
Frontal lobe[a]	1(3.3)	3(10.0)	0.301	0.612
Parietal lobe[a]	2(6.7)	4(13.3)	0.741	0.671
Temporal lobe[a]	2(6.7)	2(6.7)	0.000	1.000
Occipital lobe[a]	0	1(3.3)	1.017	1.000
Subcortical white matter[b]	6(20.0)	9(30.0)	0.800	0.371
Thalamus[a]	3(10.0)	5(16.7)	0.577	0.706
Basal ganglia[b]	11(36.7)	18(60.0)	3.270	0.071
Infratentorial region[b]	11(36.7)	9(30.0)	0.300	0.584
Volume of infarcts (cm^3)[c]	1.0±1.1	4.1±9.0	–0.602	0.529
WMLs volume (cm^3)[c]	3.7±3.1	4.3±2.5	–1.301	0.193
Gray matter ratio (%)[d]	55.6±4.2	54.1±4.5	–1.361	0.179
Std. volume of the brain regions[e]				
Left PFC[f]	21.61±2.61	20.52±2.30	–1.705	0.094
Right PFC[f]	21.35±2.38	20.25±2.12	–1.891	0.064
Left DLPFC[f]	1.03±0.31	1.02±0.22	–0.122	0.928
Right DLPFC[f]	1.05±0.23	1.04±0.28	–0.164	0.870
Left OFC[f]	7.12±0.83	6.84±0.85	–1.304	0.197
Right OFC[f]	7.23±0.74	6.84±0.75	–2.032	0.047
Left ACC[f]	2.40±0.44	2.27±0.46	–1.187	0.240
Right ACC[f]	2.51±0.41	2.29±0.34	–2.235	0.029

WMLs: White matter lesions; PFC: prefrontal cortex; DLPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex; ACC: anterior cingulate cortex. [a]n(%), Fisher's exact test; [b]n(%), chi-square test; [c]mean ± SD, Mann-Whitney U test; [d]gray matter ratio(%) was calculated as the gray matter volume derived by the whole brain volume; [e]standardized (Std.) volumes were calculated as raw volume × 1 000/intracranial volume; [f]mean ± SD, t-test.

Associations between Verbal Fluency Test score and MRI variables (15 months poststroke)

At 15 months poststroke, the left dorsolateral prefrontal cortex and prefrontal cortex volumes were significantly correlated (Pearson's correlation) with the Verbal Fluency Test score in female patients but not in male patients (Table 4). There was still a significant association between the left dorsolateral prefrontal cortex and Verbal Fluency Test (partial coefficient = 0.661, P = 0.001; Figure 1B) and between the left prefrontal cortex and Verbal Fluency Test (partial coefficient = 0.573, P = 0.004) after the same adjustment (Table 5).

Table 3 Correlations between volumetric MRI variables and cognitive tests in male and female patients 3 months poststroke

Volumetric variable	Female			Male		
	MMSE[a]	VFT[b]	FAB[a]	MMSE[a]	VFT[b]	FAB[a]
Gray matter ratio	0.212	0.088	0.072	0.293	0.305	0.498[c]
Std. volume of WMLs or brain regions[d]						
WMLs	–0.408	–0.437	–0.538[c]	0.048	–0.188	–0.089
Left PFC	0.191	0.326	0.135	0.241	0.259	0.371
Right PFC	0.059	0.309	0.285	0.239	0.208	0.319
Left DLPFC	0.064	0.499[c]	0.079	0.133	–0.162	0.104
Right DLPFC	–0.101	0.067	–0.085	–0.034	0.055	0.129
Left OFC	0.150	0.008	0.072	0.348	0.304	0.416
Right OFC	0.262	0.086	0.275	0.208	0.232	0.284
Left ACC	0.210	0.173	0.457	0.079	0.088	0.238
Right ACC	–0.183	0.052	0.028	–0.249	0.037	–0.002
Location of infarcts						
Frontal lobe	–0.025	–0.054	–0.141	–0.204	0.098	–0.078
Parietal lobe	0.055	0.062	0.265	0.116	0.108	0.160
Temporal lobe	0.331	0.186	0.039	0.029	–0.261	–0.094
Occipital lobe	–	–	–	–0.041	–0.215	–0.293
Subcortical white matter	–0.122	–0.299	–0.117	0.034	0.000	0.115
Thalamus	0.037	–0.367	0.143	0.260	0.213	0.057
Basal ganglia	–0.201	0.116	0.282	0.137	–0.439	–0.044
Infratentorial region	0.163	0.092	–0.044	0.441	0.326	0.055

[a]Spearman's correlation. [b]Pearson's correlation (correlations with gray matter ratio and ratio, standardized (Std.) volume of WMLs and brain regions); Spearman's correlation (correlations with location of infarcts). [c]$P < 0.01$. [d]Std. volumes were calculated as raw volume × 1 000/ intracranial volume. MMSE: Mini-Mental State Examination; VFT: Verbal Fluency Test; FAB: Frontal Assessment Battery; WMLs: white matter lesions; PFC: prefrontal cortex; DLPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex; ACC: anterior cingulate cortex.

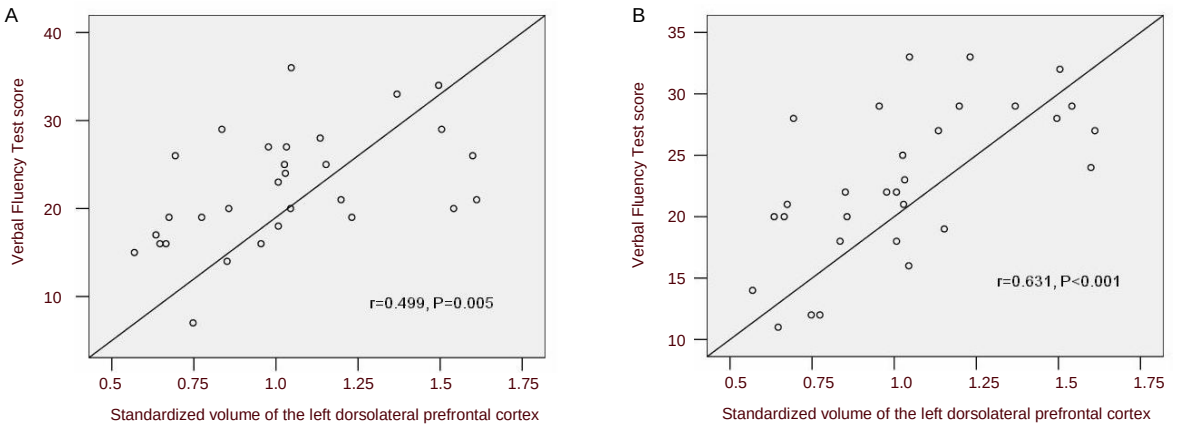

Figure 1 Scatter plots of the correlation between standardized volume of the left dorsolateral prefrontal cortex and Verbal Fluency Test score.

(A) 3 months after stroke; (B) 15 months after stroke.

Associations between Frontal Assessment Battery score and MRI variables

The Frontal Assessment Battery score was significantly correlated with the volume of white matter lesions, but not with the volume of the prefrontal cortex or its subdivisions; this was at both 3 and 15 months poststroke in elderly female patients. However, partial correlations for these associations, with the same adjustments as above, became insignificant (3 months poststroke: partial correlation coefficient = –0.406, P = 0.076; 15 months poststroke: partial correlation coefficient = –0.222, P = 0.347). In elderly male patients with ischemic stroke, Frontal Assessment Battery and Verbal Fluency Test scores did not correlate with any MRI variables in bivariate correlations (Tables 4, 5).

DISCUSSION

To the best of our knowledge, this is the first study to investigate sex differences in the association between the prefrontal cortex and cognitive function in stroke patients.

Table 4 Correlations between the volumetric MRI variables and cognitive tests in male and female patients 15 months poststroke

Volumetric variable	Female			Male		
	MMSE[a]	VFT[b]	FAB[a]	MMSE[a]	VFT[b]	FAB[a]
Gray matter ratio	0.122	0.375	0.338	0.439	0.267	0.434
Std. volume of WMLs or brain regions[d]						
WMLs	–0.477[c]	–0.514[c]	–0.612[c]	–0.046	–0.098	–0.147
Left PFC	0.049	0.617[c]	0.348	0.402	0.030	0.295
Right PFC	0.274	0.537[c]	0.433	0.332	0.180	0.204
Left DLPFC	0.045	0.631[c]	0.331	0.201	–0.181	0.053
Right DLPFC	0.084	0.216	0.068	0.082	0.128	0.095
Left OFC	0.045	0.423	0.210	0.480[c]	0.092	0.242
Right OFC	0.343	0.402	0.359	0.151	0.056	0.218
Left ACC	0.226	0.179	0.298	0.260	–0.021	0.126
Right ACC	0.124	0.225	0.226	–0.109	0.012	0.130
Location of infarcts						
Frontal lobe	0.075	–0.226	–0.162	–0.066	0.081	0.007
Parietal lobe	–0.039	0.132	0.233	0.221	0.138	0.091
Temporal lobe	0.000	0.077	–0.039	–0.008	–0.082	–0.115
Occipital lobe	–	–	–	–0.253	–0.102	–0.289
Subcortical white matter	0.073	0.121	0.082	–0.030	0.188	–0.144
Thalamus	–0.058	–0.502[c]	–0.329	0.170	–0.109	–0.072
Basal ganglia	0.241	0.032	0.177	0.032	–0.362	–0.125
Infratentorial region	–0.144	0.136	0.113	0.250	0.371	0.357

[a]Spearman's correlation. [b]Pearson's correlation (correlations with gray matter ratio and ratio, standardized (Std.) volume of WMLs and brain regions); Spearman's correlation (correlations with location of infarcts), [c]$P < 0.01$. [d]Std. volumes were calculated as raw volume × 1 000/ intracranial volume. MMSE: Mini-Mental State Examination; VFT: Verbal Fluency Test; FAB: Frontal Assessment Battery; WMLs: white matter lesions; PFC: prefrontal cortex; DLPFC: dorsolateral prefrontal cortex; OFC: orbitofrontal cortex; ACC: anterior cingulate cortex.

Our main finding is that left dorsolateral prefrontal cortex volume is positively correlated with verbal fluency performance in female patients at 3 and 15 months poststroke, even after adjusting for possible confounders. This correlation was insignificant in male stroke patients of the same age group.

Table 5 Partial correlation of left DLPFC and left PFC standardized volumes and Verbal Fluency Test scores in elderly poststroke women

Stardardized volume[c]	Verbal Fluency Test (3 months)		Verbal Fluency Test (15 months)	
	Partial coefficient	*P*	Partial coefficient	*P*
Left DLPFC	0.453[a]	0.045	0.661[b]	0.001
Left PFC	–	–	0.573[b]	0.004

[a]Controlled by age, education years, diabetes mellitus, NIHSS, MMSE (3 months), volume of infarcts, volume of white matter lesions and presence of basal ganglia infarcts. [b]Controlled by age, education years, diabetes mellitus, NIHSS, MMSE (15 months), volume of infarcts, volume of white matter lesions and presence of basal ganglia infarcts.

[c]Standardized volumes were calculated as raw volume × 1 000/ intracranial volume). DLPFC: Dorsolateral prefrontal cortex; PFC: prefrontal cortex; NIHSS: the National Institutes of Health Stroke Scale; MMSE: Mini-Mental State Examination.

Results from this study showed that verbal fluency was predominantly impaired when left frontal lesions were present in stroke patients. This is consistent with previous studies on patients with traumatic brain injuries and other brain lesions[9, 30-32]. Verbal fluency impairment is not only a manifestation of speech disturbance, but also an important component of executive dysfunction[2]. In patients with ischemic stroke, severe non-fluent aphasia, such as Broca's aphasia and transcortical motor aphasia, always occurs because of acute infarction involving the dominant hemisphere, especially the frontal lobe or frontal subcortical white matter[4]. However, in non-aphasic stroke patients, especially at the chronic stage of stroke, verbal fluency performance may also be attributed to chronic brain lesions, such as regional brain atrophy. Our study suggests that other than focal damage or acute injures, chronic (*e.g*. atrophy or ischemic changes) lesions in the left dorsolateral prefrontal cortex may also contribute to the impairment of verbal fluency in non-aphasic stroke in female patients.

The consistently significant association between the performance of verbal fluency and volume of the left dorsolateral prefrontal cortex at both 3 and 15 months poststroke strengthens the reliability of this finding. A possible reason for why the association at 15 months was stronger than at 3 months may be the fact that 3 months poststroke is still in the relatively acute phase and further recovery in cognitive functioning may still occur in the following months. Thus, at 15 months

poststroke, the effect of the infarction on verbal fluency was lessened and chronic lesions might play a more important role.

However, global prefrontal cortex function was not found to be associated with volume of the prefrontal cortex or a subdivision in our study. We propose several possible explanations for this finding. First, executive functions comprise a variety of cognitive functions, such as sequencing, planning, abstracting, switching, as well as verbal fluency. In this study, we evaluated executive function only with Frontal Assessment Battery. Frontal Assessment Battery is commonly used as a screening tool for executive functioning[33] and may not reflect every aspect of this domain. Second, cognitive reserve (*e.g*. education level, occupational activities) may also play an important role in prefrontal cortex functioning[34]. Third, the absence of correlations between executive function and atrophy of the prefrontal cortex and its subdivisions in female patients may be partly due to the coexisting white matter lesions. White matter lesions, features of subcortical ischemic vascular disease[35], have been linked to poststroke executive dysfunction[35-37] and may lessen the effect of prefrontal cortex atrophy. The relationship between prefrontal cortex and dorsolateral prefrontal cortex atrophy and executive function warrants further studies with more sensitive neuropsychological tests for executive function. More sensitive and specific tests include the Stroop test, the Go/No-Go test, and the Wisconsin Card Sorting Test.

It is interesting that this finding was only found in elderly poststroke female patients and not in male patients. It indicates that the left dorsolateral prefrontal cortex and left prefrontal cortex may have different roles in the neuropsychological processing of verbal fluency in male and female elderly stroke patients. To preclude the effect of possible confounders, we controlled for important demographic factors and those that differed between males and females. Although elderly poststroke females scored significantly lower in tests of global cognition and executive function than males, they had a verbal fluency performance comparable to their male counterparts. This corresponds with the notion that language function is predominant in women[12-13].

Sex differences in prefrontal cortex functions and volume may be linked to gonadal hormones that could modulate the function of the prefrontal cortex[11]. Animal experiments have demonstrated that gonadal hormones are responsible for the sexual differentiation of the central nervous system at the structure and function levels *via* organizational and activation effects[38]. That gonadal hormones may influence prefrontal cortex functions in humans is supported by the finding that the prefrontal cortex has one of the highest binding sites for estrogen in female brain specimens[39]. In view of plasticity mechanisms, men may have higher cognitive reserve due to a higher level of education and more complicated occupational activities[40]. Thus, it can be speculated that women's verbal fluency performance is more likely to be dependent on biological factors (*e.g*. lesions in the dorsolateral prefrontal cortex). Additionally, in this study, male patients had a significantly lower volume of right orbitofrontal cortex and right anterior cingulate cortex than women, and a trend for a lower volume of both sides of the prefrontal cortex. However, male and female patients had a similar dorsolateral prefrontal cortex size. There may be selective atrophy in brain regions in both male and female patients.

Notwithstanding these findings, the study had significant limitations. First, the subjects had a relatively mild neurological deficit (National Institutes of Health Stroke Scale score was about 5), which limits the generalization of the findings. In addition, patients with aphasia (more likely to have left frontal lobe infarction) had to be excluded because of the purpose of the study. Furthermore, the determination of aphasia based on a score of zero in best language score of National Institutes of Health Stroke Scale might not be very accurate. Second, MRI volumetry was not conducted in many regions of the cerebral cortex, especially the medial frontal cortex, which may also correlate with executive function. Third, only the Frontal Assessment Battery was used to assess executive functions. It is commonly used as a screening tool and may not be able to reflect every aspect of executive functions, thus there was possibly a ceiling effect in our sample. Moreover, phonemic verbal fluency was not tested. Fourth, the relatively small sample size limits the generalization of the findings in our study.

In conclusion, atrophy of the left dorsolateral prefrontal cortex is associated with semantic verbal fluency impairment in elderly stroke female patients, but not in their male counterparts. Sex differences may be an associated factor in the neuropsychological pathomechanism of poststroke verbal fluency impairment. In other words, verbal fluency impairment may be more likely associated with biological factors in elderly stroke patients, suggesting they may have poorer cognitive reserve.

Future studies with a larger sample size and a series of comprehensive neuropsychological tests on executive functions should be performed to confirm this finding. Moreover, to study the effects of sex differences and chronic brain lesions (including regional brain atrophy) on executive function and verbal fluency, recruitment of a sample of stroke-free patients with transient ischemic attack is preferential.

MATERIALS AND METHODS

Design

A cross-sectional observational study.

Time and setting

Assessments were performed at a psychiatric clinic in Prince of Wales Hospital in Hong Kong between June 2006 and June 2007.

Subjects

Eighty-three elderly patients with acute ischemic stroke, aged ≥ 60 years old, were admitted to the Acute Stroke Unit of the Prince of Wales Hospital in Hong Kong between June 2006 and June 2007 and were consecutively recruited in a longitudinal study on poststroke cognitive impairment. All participants were right-handed, of Chinese descent and fluent in the Cantonese dialect. Each participant underwent an MRI examination. To exclude the aphasic patients, all participants were required to have a score of zero in the best language item of National Institutes of Health Stroke Scale[41]. Scores on the best language item of National Institutes of Health Stroke Scale range from 0–3, with a higher score indicating worse language disturbance. Participants did not have dementia or depression or any other central nervous system disease other than stroke, and they were free of significant dysarthria and visual and hearing impairment. Among them, 33 elderly female patients with ischemic stroke participated in the neuropsychological testing 3 months poststroke, but three of these patients dropped out within 1 year following the baseline assessment. Eventually, 30 elderly poststroke female patients were included in the study. Simultaneously, a group of 30 age-matched male patients with ischemic stroke who completed the follow-up assessment were selected from the same cohort; these participants comprised the sex-matched group. Each female case was age-matched (≤ 3 years) to a male patient with the closest admission date. Thus, in this cohort of 83 subjects initially, 20 elderly stroke male patients were excluded. All participants signed a consent form.

Basic sociodemographic and clinical data including age, sex, education (years), hypertension, diabetes mellitus, previous stroke history, and National Institutes of Health Stroke Scale score on hospital admission were retrieved from the Stroke Registry at the Acute Stroke Unit of the Prince of Wales Hospital. Hypertension was defined as repeated blood pressure measures of ≥ 140/90 mmHg (1 mmHg = 0.133 kPa) or the need for chronic antihypertensive medication. Diabetes mellitus was defined as a fasting blood glucose ≥ 7.0 mM, postprandial blood glucose ≥ 11.1 mM, or on glucose-lowering treatment.

Methods

Neuropsychological tests

All subjects completed a series of neuropsychological tests both 3 and 15 months after the index stroke. We chose the time point of 3 months poststroke, as stroke survivors can have a relatively quick natural recovery within 3 months after the stroke has occurred. These two time points were often adopted by studies on poststroke cognitive impairment[42-44]. Global cognitive function was assessed using the Cantonese version of the Mini-Mental Status Examination[45]. It contains assessments covering 5 cognitive domains, which are orientation, memory, attention and calculation, recall and language, and its score can range from 0–30 points. The Mini-Mental Status Examination is a quick and easy measure of cognitive functioning that has been widely used in clinical evaluation and research involving patients with dementia, as well as in poststroke patients[43]. The Chinese-version of Frontal Assessment Battery[46-47] was used to evaluate executive function. A high Frontal Assessment Battery score indicates good performance. The Chinese Frontal Assessment Battery contains six items, which assess conceptualization, lexical fluency, motor programming, sensitivity-to-interference, go/no-go, and environmental autonomy. A maximum of 3 is scored for each item and the total test score is 18. The Frontal Assessment Battery takes about 10–15 minutes to administer depending on the patient's level of impairment. It has been widely used in the evaluation of executive function in elderly stroke and degenerative dementia patients[48-49]. In the Semantic Verbal Fluency Test[50], patients were asked to retrieve words belonging to the categories of 'food' and 'animal'. The time limit was 60 seconds for both tasks. The Verbal Fluency Test score was the sum of both category tests. The severity of depressive symptoms was also evaluated with a short form of the Geriatric Depression Scale, Chinese version[51], with scores that ranged 0–15, where a higher indicates more depressive symptoms.

MRI measurements

MRI assessments were performed in a 1.5T MR scanner (Sonata, Siemens Medical, Erlangen, Germany) for each subject within 7 days of the initial stroke. Imaging sequences included diffusion weighted imaging, T1 weighted imaging, T2 weighted imaging, fluid attenuation inversion recovery, and T2* weighted gradient echo. Whole brain volume was acquired using a T1-weighted FLASH sequence. Diffusion weighted imaging spin echo echo planar imaging (repetition time/echo time/ excitation = 180/122/4, matrix = 128 × 128, field of view = 230 mm, slice thickness/gap = 5 mm/1 mm, echo planar imaging factor = 90, acquisition time = 55 seconds) with three orthogonally applied gradients were used with a *b* value of 1 000 and 500. Axial gradient echo images were acquired as the second sequence with imaging parameters of repetition time/echo time/excitation = 350/30/2, flip angle of 30°, slice thickness/gap = 5 mm/0.5 mm, field of view = 230 mm, matrix = 256 × 256 and acquisition time = 5 minutes 4 seconds. Axial SE T1 (repetition time/echo time/excitation = 425/14/2, field of view = 230 mm, slice thickness/gap = 5 mm/0.5 mm, matrix = 256 × 256 and acquisition time = 4 minutes 28 seconds) and TSE T2 (repetition time/echo time/excitation = 2 500/120/1, turbo factor of 15, field of view = 230 mm, slice thickness/gap = 5 mm/0.5 mm, matrix of 256 × 256 and acquisition time = 1 minute 39 seconds) images were also acquired.

Identification of brain infarcts

Brain infarctions were identified on T1 weighted images and confirmed on the corresponding T2-weighted images, with the volumetric measurements being carried out on T1-weighted images[48]. They included acute and old infarcts on the MRI images in the acute phase of stroke. Brain infarcts that affected the frontal, temporal, parietal and occipital lobes, corona radiata, centrum semiovale, internal capsule, basal ganglia, thalamus, brainstem and cerebellum were recorded. Multiple infarcts or infarct(s) involving more than one location were counted in all locations they occurred. The area of infarcts in each visible slice was measured with manual outlines. The total volume of infarcts was calculated by multiplying the total area by the sum of the slice thickness and gap[48].

Determination of volumetry of brain regions

Volumetric analysis of brain regions was performed using an automatic image analysis program, Insight Segmentation and Registration Toolkit (http://www.itk.org). The N3 algorithm[52] was employed to automatically correct the non-uniform image intensity for the volume data. The brain extraction tool[53] was used to segment the brain from the MRI data. Tissue classification was performed using the supervised k-nearest neighbor classifier for classifying the entire 3-D image[54]. The volumes of the white matter and gray matter were calculated as the number of voxels multiplied by the size of each voxel. A fully automated clustering-based quantitative white matter lesion volume detection technique was adopted to analyze fluid attenuation inversion recovery intensities on the white matter mask, which is generated from the segmentation result from T1 images co-aligned with fluid attenuation inversion recovery data. The whole-brain segmentation was achieved using an atlas-based approach that automatically adjusts the prior atlas intensity model to new input data and has good robustness[55]. The brain atlas (*i.e.*, Talairach atlas[56]) was constructed by manually delineating the deep brain structures and intracranial region on a single subject. The intracranial volume was calculated by automatically adjusting the prior atlas intensity model to new input data. The label of the intracranial region was transformed to the input data non-rigidly (using demon registration)[57].

Parcellation of the cerebral cortex into different cortical regions was made by non-rigid registration to a cortical surface atlas[58]. The registration to the spherical atlas was performed using the cortical folding patterns to match the cortical geometry between each subject and the surface atlas. This registration algorithm modeled the warping of gray scale in the images as the flow of fluid using the partial differential equations of the Navier-Stokes equations[59]. The labels of the anterior cingulate cortex and orbitofrontal cortex were given by the atlas[57], whereas the labels of the dorsolateral prefrontal cortex and prefrontal cortex were defined manually according to a previous reference[60]. All brain region volumes were standardized by the intracranial volume (1 000 × raw volume/intracranial volume). The volumes described below refer to the standardized volumes. The gray matter ratio was defined as the gray matter volume divided by the whole brain volume. Examples of parcellation of the cerebral cortex of a woman are shown in Figure 2.

Statistical analysis

The difference in proportions between the groups was analyzed with the chi-square test or the Fisher's exact test. The continuous data were compared using *t*-tests (parametric data) or Mann-Whitney *U* tests (non-parametric data), as appropriate. The one-sample Kolmogorov-Smirnov test determined age, gray matter ratio and all standard volume of the brain regions as parametric data. Education years, National Institutes of

Health Stroke Scale score, Mini-Mental Status Examination score, Frontal Assessment Battery and Geriatric Depression Scale scores, volume of infarcts and volume of white matter lesions were determined as non-parametric data.

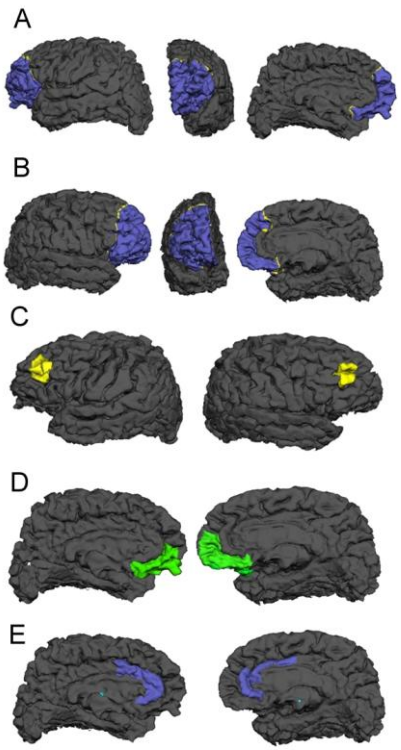

Figure 2 Parcellation of brain regions of a 63-year-old woman with an acute infarct in the right putamen.

(A) Left prefrontal cortex (from left to right: lateral view, coronary view and internal view).

(B) right prefrontal cortex (from left to right: lateral view, coronary view and internal view).

(C) left and right dorsolateral prefrontal cortex.

(D) left and right orbitofrontal cortex.

(E) left and right anterior cingulate cortex.

Spearman's correlations were performed between the standardized volumes of brain regions and white matter lesions and Mini-Mental Status Examination and Frontal Assessment Battery scores separately for female and male patients. Pearson's correlations were performed for the MRI volumetric variables and Verbal Fluency Test. For these univariate correlation analyses, the significance level was set at $P < 0.01$ (two-tailed) because of multiple comparisons. Then, significant correlations were retested by partial correlation after controlling for possible confounders. The level of significance in partial correlations was set at $P < 0.05$ (two-sided). The statistical analyses were performed using SPSS (version 16.0; SPSS, Chicago, IL, USA).

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