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PARK7 and Nucleoside Diphosphate Kinase A as Plasma Markers for the Early Diagnosis of Stroke

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Background: **Plasma markers for stroke could be useful in diagnosis and prognosis and in prediction of response of stroke patients to therapy. PARK7 and nucleoside diphosphate kinase A (NDKA) are increased in human postmortem cerebrospinal fluid (CSF), a model of global brain insult, suggesting that measurement in CSF and, more importantly, in plasma may be useful as a biomarker of stroke.**

Methods: **We used ELISA to measure PARK7 and NDKA in plasma in 3 independent European and North American retrospective studies encompassing a total of 622 stroke patients and 165 control individuals.**

Results: **Increases in both biomarkers were highly significant, with sensitivities of 54%–91% for PARK7 and 70%–90% for NDKA and specificities of 80%–97% for PARK7 and 90%–97% for NDKA. The concentrations of both biomarkers increased within 3 h of stroke onset.**

Conclusions: **PARK7 and NDKA may be useful plasma biomarkers for the early diagnosis of stroke. In addition, this study demonstrated the utility of analysis of postmortem CSF proteins as a first step in the discovery of plasma markers of ischemic brain injury.**

The prognosis of acute stroke has been improved by the use of very early therapeutic interventions such as systemic or intraarterial thrombolysis. Consequently, establishing a rapid and accurate diagnosis has recently emerged as a pivotal issue. At present, the diagnosis of stroke relies on historical data, neurologic examination, and neuroimaging techniques, including brain computerized tomography (CT)[5] and magnetic resonance imaging (MRI) scans. These assessments have limitations, particularly within the first hours after a stroke. CT scanning, the major tool, can detect most hemorrhagic strokes, but not until 6–24 h after the event *(1)*. The extent of CT ischemia predicts vascular occlusion *(2)*. MRI can detect features of stroke within a few hours and even within 15 min of vascular occlusion in an experimental model *(3)*. Such expensive tools, however, are available only in specialized hospitals. Beyond the issue of diagnosis, other crucial issues are frequently raised during the acute phase of stroke; these include the early distinction between transient ischemic attack (TIA) and established stroke, the degree of ischemic penumbra (as opposed to necrosis), the extent of final brain tissue damage, the measurable effects of thrombolytic therapies, and ultimately, the prognosis and risk of death of individual patients.

By analogy with biological markers of acute myocardial infarction, we hypothesized that a reliable plasma marker of stroke would provide early, quantitative information about the extent of brain tissue damage. Previously studied plasma markers have not been found to have utility in the routine assessment of stroke patients in a large cohort. For example, tau *(4–6)*, neuron-specific enolase, and B-type neurotrophic growth factor *(7–9)* have been reported as neuronal markers of stroke, and astroglial protein S-100b *(7, 10, 11)* and glial fibrillary acidic protein *(12)* have been described as glial markers of stroke. Inflammation mediators such as C-reactive protein *(13–18)*, serum amyloid A *(19)*, matrix metalloproteinase-9 *(10, 11, 20)*, vascular and intracellular cell adhesion molecules *(11, 18)*, tumor necrosis factor α, and interleukins (interleukin-1, -6, and -8) *(14, 16–18)* have also been

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[5] Nonstandard abbreviations: CT, computerized tomography; MRI, magnetic resonance imaging; TIA, transient ischemic attack; CSF, cerebrospinal fluid; NDKA, nucleoside diphosphate kinase A; CI, confidence interval; and NDP, nucleoside diphosphate.

investigated as surrogate markers for the diagnosis of stroke. In general, most, if not all, of these molecules have not fully met the major requirements for a useful diagnostic biomarker. They display relatively low sensitivity and specificity, their plasma concentrations tend to increase rather late in the course of the brain insult (beyond 6 to 12 h), they are poor indicators of lesion size, and they have been assessed in small populations only.

The main objectives of the present study were to identify, assess, and validate new diagnostic markers of stroke ideally exhibiting the following features: (*a*) "brain-specific", i.e., released by damaged or suffering brain tissue and therefore considered scientifically plausible as stroke biomarkers; (*b*) easily detected in blood samples; and (*c*) exceed reference limits as early as possible after the onset of symptoms and within the time frame required to perform thrombolytic interventions, i.e., 3 to 6 h. With respect to the first criterion, we recently explored the concept of postmortem cerebrospinal fluid (CSF) as a model of massive and global brain insult *(21)*. CSF is expected to mirror changes that take place in the brain. When these changes are of sufficient magnitude, they eventually become detectable in the blood. Supporting this view, heart-type fatty acid–binding protein, which was identified from postmortem CSF, may be reliably used as a serum or plasma marker of stroke *(9)* and Creutzfeld–Jakob disease *(22)*.

PARK7 (also called DJ-1) and nucleotide diphosphate kinase A (NDKA) are overexpressed in human postmortem CSF compared with antemortem CSF. Because these proteins were found previously in brain and described in neurodegenerative disorders *(23–33)*, we hypothesized a potential regulation in stroke.

Patients and Methods

PLASMA SAMPLES FOR BIOMARKER VALIDATION

Plasma samples obtained from 1 European and 2 North American cohorts of patients were used for the assessment and validation steps. The samples were collected and tested in Geneva and San Diego, respectively, by different laboratories and different ELISA techniques. For the European patients, the local institutional ethics committee approved the clinical protocol. For the American cohorts, see Reynolds et al. *(10)*.

Seventy-five serial European stroke and control patients admitted to the Geneva University Hospital emergency unit were enrolled in this study from August 1996 to January 1997. Two stroke patients were excluded: one actually had not suffered a stroke, and the other was diagnosed after 9 days of hospitalization. Two control patients were also excluded from the study because they suffered stroke a few times after the blood collection. For each patient, a blood sample was collected in dry heparin-containing tubes at the time of admission. Stroke and control patients were matched for age and sex. Of the 71 consecutive patients enrolled, 35 were primarily diagnosed with nonneurologic conditions and classified as control samples (25 men and 10 women; mean age, 71.1 years; range, 28–91 years) and 36 were diagnosed with stroke (27 men and 9 women; mean age, 71.3 years; range, 25–92 years), including 33 ischemic (among them 6 with TIAs and 27 with established strokes) and 3 hemorrhagic strokes. Thirty-five stroke and 35 age/sex-matched control patients were retained for PARK7 evaluation. Because of a lack of samples, 31 stroke and 31 age/sex-matched controls patients were retained for NDKA detection; among them 31 stroke patients and 30 controls were common to both proteins.

After centrifugation at 1500*g* for 15 min at 4 °C, plasma samples were aliquoted and stored at −20 °C until analysis. For the patients in the stroke group, the mean time interval between the neurologic event and the first blood draw was 1012 min (i.e., 16 h and 51 min; range, 30 min to 5 days). The diagnosis of stroke was established by a trained neurologist and was based on the sudden appearance of a focal neurologic deficit and the subsequent delineation of a lesion consistent with the symptoms on brain CT or MRI images, with the exception of TIAs, for which a visible lesion was not required for the diagnosis. The stroke group was separated according to the type of stroke (ischemia or focal hemorrhage), location of the lesion (brainstem or hemisphere), and clinical evolution over time (TIA when complete recovery occurred within 24 h, or established stroke when the neurologic deficit was still present after 24 h). The control group included patients with various medical or surgical conditions, including cancer (n = 12), acute renal failure (n = 3), and gastrointestinal disorders. A few of the control patients suffered from chronic neurologic conditions as secondary diagnoses, including meningioma (n = 1), Parkinson disease (n = 1), and dementia (n = 3). None of them had a past or recent history of cerebrovascular event.

To assess the performance of our tests on non-European populations, we included 2 North American cohorts, which were studied as described by Reynolds et al. *(10)*. The analyses were performed on frozen samples. The American population was less characterized than the European cohort. Briefly, sample set 1 was composed of 30 non–age/sex-matched controls and 53 stroke patients (including 6 with hemorrhagic strokes, 23 with TIAs, and 24 with established ischemic strokes) from whom samples were collected within 24 h after the onset of symptoms. Sample set 2 was composed of 100 control patients age-matched with 533 stroke patients (226 with hemorrhagic strokes, 124 with TIAs, and 183 with established ischemic strokes).

SANDWICH ELISA IMMUNOASSAY PROCEDURE

Because no commercial assay is currently available for the detection of our biomarkers, a home-made ELISA test was developed. A trained laboratory technician carried out, not blind, all of the European assays; the overall CV was ~15%. Sandwich ELISAs were performed as described by Allard et al. *(19)* in 96-well Reacti-Bind™

NeutrAvidin™-coated Black Plates (Pierce). The European cohort was measured in Geneva (Switzerland) and the North American cohorts in San Diego (CA). Briefly, 50 μL of plasma sample was used without dilution for NDKA measurements and diluted 2-fold for PARK7 measurements. Each plasma sample was assayed in duplicate and distributed randomly on the plate. Calibrator samples (corresponding recombinant proteins) for calibration curves were run in the same plate. Recombinant proteins were diluted to concentrations of 100, 50, 25, 12.5, 6.25, 3.25, 1.56, and 0 μg/L in the dilution buffer in Geneva and in control plasma in San Diego. The calibration curves were analyzed by linear regression in the linear range of the curve. Protein concentrations in plasma samples were calculated from the calibration curve.

STATISTICAL ANALYSIS

Protein concentrations were initially expressed in relative fluorescence units, and the concentration was calculated via a calibration curve obtained by means of the recombinant proteins on the same plate. Statistical analyses were performed with GraphPad Prism® software, Ver. 4.0 (GraphPad Software), and graphs were produced with Aabel 1.5.8 software (Gigawiz Ltd.). Notched box-and-whisker charts were used to show the 10th, 25th, 50th, 75th, and 90th percentiles and outliers. The diamond shape in each box indicates the mean, and the notches indicate the confidence interval around the median of a sample. Boxes whose notches do not overlap indicate that the medians of the 2 groups differ at the 5% significance level. To assess the ability of the protein concentrations to differentiate between different populations, nonparametric tests were performed. A Wilcoxon matched-pairs test was performed for age- and sex-matched European data and a Mann–Whitney test for the first set of North American data. For analysis based on time after onset of symptoms in the European patients and North American data containing more than 2 groups, we used a one-way ANOVA Kruskal–Wallis test followed by a Dunn multiple comparison test. ROC curves were drawn, and areas under curves were determined for each biomarker. Cutoffs were chosen at specificity ideally above 90% because this parameter is the most clinically relevant for the diagnosis of stroke.

Results

Using 2-dimensional gel electrophoresis, we previously identified 12 proteins that showed an increase in postmortem CSF compared with antemortem CSF samples ($P < 0.05$) *(21)*. Specific antibodies were then engineered and tested for PARK7, also called DJ-1 and previously identified as RNA-binding regulatory subunit (RNA-BP; SwissProt accession no. Q99497; 189 amino acids; 19 847 Da; theoretical pI 6.33), and NDKA (SwissProt accession no. P15531; 152 amino acids; 17 149 Da; theoretical pI 5.83) encoded by the *nm23* gene. PARK7 and NDKA were measured in plasma samples from distinct cohorts of stroke patients and controls.

We first studied a European cohort of 71 consecutive patients (Table 1). As shown in Fig. 1, both biomarkers were highly significantly increased in the stroke population ($P < 0.0001$, nonparametric Wilcoxon matched-pairs test) compared with controls. The ROC curve for PARK7 (Fig. 2) indicated that the sensitivity and specificity, at a cutoff of 9.33 μg/L, were 91% and 80%, respectively (corresponding to 3 false-negative and 7 false-positive results out of 35). For NDKA at a cutoff of 2 μg/L, the sensitivity and specificity were both 90% (corresponding to 3 false-negative and 3 false-positive results out of 31 samples tested). The areas under the ROC curves for PARK7 and NDKA were 0.88 [95% confidence interval (CI), 0.80–0.97] and 0.94 (0.86–1.0), respectively ($P < 0.0001$ for both). No stroke patient was negative by both tests, and only 3 controls were positive by both tests. Five controls suffered from various neurologic conditions, including dementia (n = 3), Parkinson disease (n = 1), and meningioma (n = 1). None of these patients showed positive results with either of the 2 biomarkers, strengthening the specificity of the stroke markers tested.

All 3 control individuals who had increased results for both markers suffered from metastatic prostate cancer extending toward the spinal cord and lumbosacral roots. Each of the 3 had also been subjected to radiotherapy and

Table 1. Demographic data for European and American patients for the sample set in which blood was collected within 0 and 24 h after arrival at the emergency room.

	Stroke	Control
European study 1 (age/sex-matched)		
n	36	35
Age, years		
Mean (SD)	71.3 (16)	71.1 (15.6)
Minimum–maximum	25–92	28–91
F/M, n (%)	9 (25)/27 (75)	10 (28.6)/25 (71.4)
Time after onset of symptoms, min		
Mean (SD)	1012 (1663)	
Minimum–maximum	30–7200	
Hemorrhagic stroke, n (%)	3 (8.3)	
TIA, n (%)	6 (16.7)	
Ischemic stroke, n (%)	27 (75)	
American study 2		
n	53	30
Hemorrhagic stroke, n (%)	6 (11.3)	
TIA, n (%)	23 (43.4)	
Ischemic stroke, n (%)	24 (45.3)	
American study 3 (age-matched)		
n	533	100
Hemorrhagic, n (%)	226 (42.4)	
TIA, n (%)	124 (23.3)	
Ischemic, n (%)	183 (34.3)	

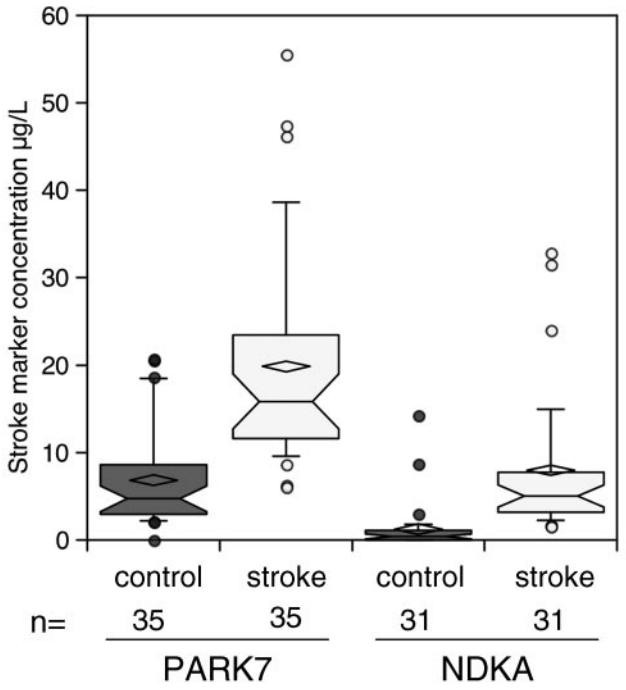
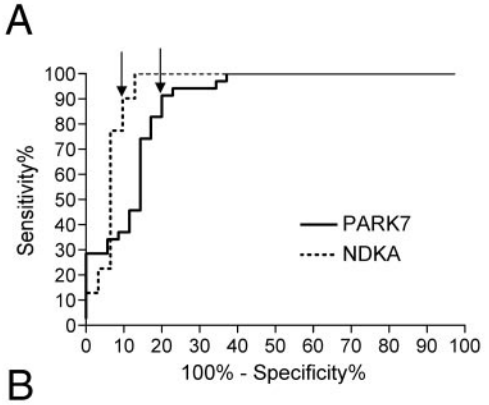


Fig. 1. European study 1: PARK7 and NDKA concentrations in stroke and control plasma samples.

Notched box-and-whisker charts show 10th, 25th, 50th, 75th, and 90th percentiles and outliers (*circles*). The *diamond shapes* indicate the means.

chemotherapy. This suggests that meningeal carcinomatosis or prostate metastases involving neural structures may also increase the concentration of these 2 biomarkers. To explore the possibility that prostate cancer may be an unrelated cause of PARK7 and NDKA increase, plasma samples from 25 patients with nonmetastatic and 13 with metastatic prostate cancer were tested. None of these patients had neurologic complications related to the cancer extension. The concentrations of both biomarkers were below the cutoff values in all patient samples compared with controls (data not shown), excluding prostate cancer itself as a direct source of these biomarkers in plasma.



	P	CO	SE%	95% CI	SP%	95% CI
PARK7	<0.0001	9.33	91.4	76.9% to 98.2%	80.0	63.1% to 91.6%
NDKA	<0.0001	2	90.3	74.2% to 98%	90.3	74.2% to 98%

Fig. 2. European study 1: PARK7 and NDKA ROC curves (*A*), and statistics associated with PARK7 and NDKA (*B*).

(*A*), *arrows* indicate cutoffs. (*B*), *CO*, cutoff; *SE*, sensitivity; *SP*, specificity.

Concentrations of the 2 proteins increased in plasma within the first 3 h after stroke onset (Fig. 3) compared with the control population ($P < 0.001$ when comparing early and late patients with the control population; one-way ANOVA, Kruskal–Wallis test, and post hoc Dunn multiple comparison test). Of the 14 early patients, all were positive with both PARK7 and NDKA. Plasma obtained 30 min after the onset of stroke symptoms from an individual suffering from an established stroke was detected as positive with both PARK7 and NDKA. Concentrations of the 2 biomarkers remained increased in plasma up to 5 days after onset of symptoms compared with the control population ($P < 0.0001$, Mann–Whitney test).

The second and third studies were performed on 2 unrelated cohorts of North American patients (described in Table 1). All blood specimens were collected within 24 h after the onset of the symptoms. Again, PARK7 and NDKA were higher (Mann–Whitney, $P < 0.01$) in stroke patients than in controls (Fig. 4). The areas under the ROC curves were not statistically significantly different [0.97 (95% CI, 0.94–0.9997) for PARK7 and 0.94 (0.89–0.99) for NDKA]. At a cutoff of 1.55 µg/L for PARK7, the sensitivity and specificity for stroke (hemorrhagic plus TIA plus ischemic) were 85% and 97%, respectively, and at a cutoff of 2.55 µg/L for NDKA, they were 73% and 97%, respectively (Fig. 5B). As was apparent in the European study, the proteins were also significantly increased in plasma samples that were taken more than 24 h after the onset of stroke symptoms ($P < 0.0001$, Mann–Whitney test; data not shown).

PARK7 and NDKA were finally assessed on a third, much larger cohort encompassing 633 stroke patients and controls. The stroke population was divided into hemorrhagic (n = 226), TIA (n = 183), and established (n = 124) strokes. The significant increase in the 2 biomarkers in the 3 types of stroke are illustrated in panels A and B of Fig. 6, and Fig. 7 shows the ROC curves and the corresponding sensitivities and specificities obtained for PARK7 and NDKA when comparing different stroke subtypes. The areas under the ROC curves were 0.74 (95% CI, 0.69–0.78) and 0.83 (0.79–0.87), respectively ($P < 0.0001$). For PARK7, at a cutoff of 14.1 µg/L, the sensitivity and specificity for stroke (hemorrhagic plus TIA plus established stroke) were 54% and 90%; for NDKA, at a cutoff of 22 µg/L, they were 70% and 90%. An increase in marker concentration was also observed in each type of stroke, hemorrhagic, TIA and ischemic, compared with controls ($P < 0.001$ for both PARK7 and NDKA, Kruskal–Wallis test and post hoc Dunn multiple comparison test).

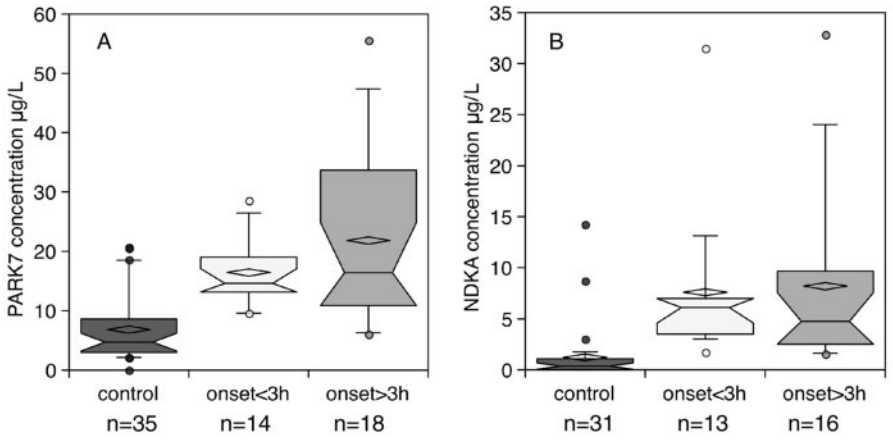


Fig. 3. Stroke marker concentrations as a function of time after the onset of the symptoms (European ischemic and TIA patients).

(*A*), PARK7; (*B*), NDKA. Notched box-and-whisker charts show 10th, 25th, 50th, 75th, and 90th percentiles and outliers (*circles*). The *diamond shapes* indicate the means.

Discussion

In this study, we have demonstrated a highly significant early increase in plasma concentrations of PARK7 and NDKA after a stroke event. These results were consistent across 3 independent studies in different laboratories using different immunoassay platforms in Europe and the United States and encompassing up to 622 patients with different types of stroke and 165 control individuals. The European population used for the study encompassed very well characterized patients (n = 71). On the other hand, the populations that were used for the American studies were less well characterized but contained a higher number of patients and allowed us to confirm the results obtained with the European population. The results were not influenced by patient demographics, including age, sex, ethnic group (Caucasian or African American), and origin (Europe or United States). There was no apparent center effect because the analyses were performed on different technologic platforms and in different laboratories using slightly different techniques of assessment. These facts can explain the differences in cutoff values observed between the different studies and prevent generalization of the results. Nevertheless, the sensitivities and specificities remained similar among the 3 studies, which supports the results. The slight decrease in the second American study is mainly attributable to the enlargement of the population. PARK7 and NDKA exhibited a very early increase above the cutoff values after stroke onset, in some cases after only 30 min, making these biomarkers applicable for use within a short timeframe, which may allow thrombolytic therapies to be considered. The 2 biomarkers appeared similarly increased in all 3 types of patients evaluated in the large American cohort. This result is consistent with those

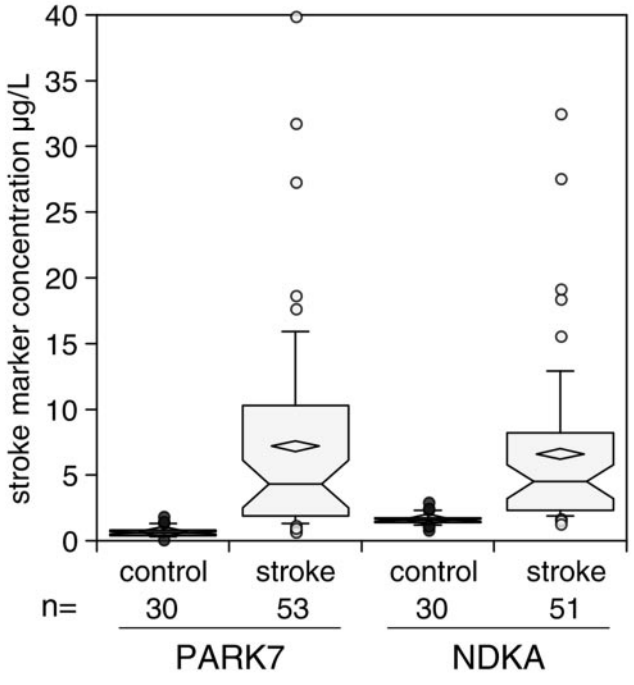
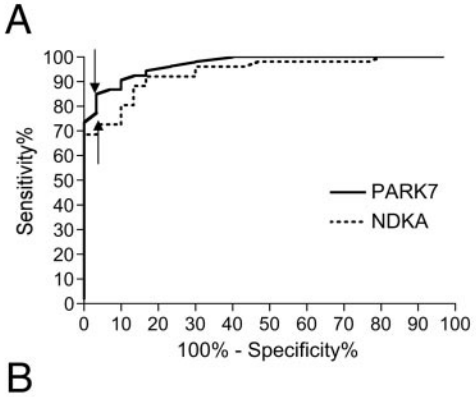


Fig. 4. American study 2: PARK7 and NDKA concentrations in stroke and control plasma samples.

Notched box-and-whisker charts show 10th, 25th, 50th, 75th, and 90th percentiles and outliers (*circles*). The *diamond shapes* indicate the means.



	P	CO	SE%	95% CI	SP%	95% CI
PARK7	<0.0001	1.55	84.9	72.4% to 93.2%	96.7	82.8% to 99.9%
NDKA	<0.0001	2.55	72.6	58.3% to 84.1%	96.7	82.8% to 99.9%

Fig. 5. American study 2: PARK7 and NDKA ROC curves (*A*), and statistics associated with PARK7 and NDKA (*B*).

(*A*), *arrows* indicate cutoffs. (*B*), *CO*, cutoff; *SE*, sensitivity; *SP*, specificity.

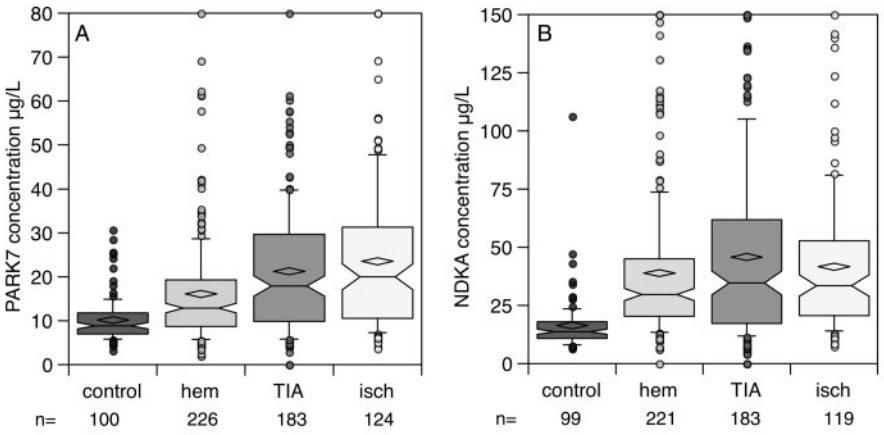
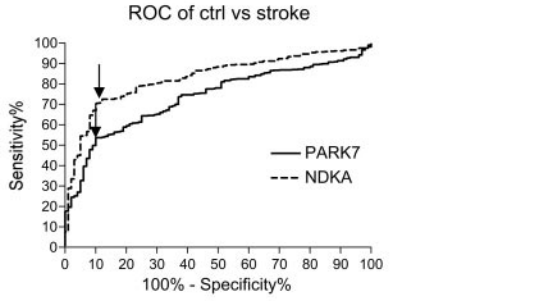


Fig. 6. American study 2: PARK7 (*A*) and NDKA (*B*) concentrations in 633 and 622 stroke and age-matched control plasma samples, respectively.

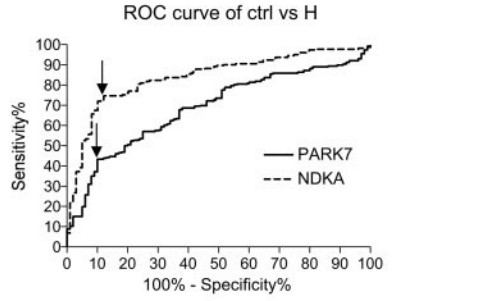
Notched box-and-whisker charts show 10th, 25th, 50th, 75th, and 90th percentiles and outliers (*circles*). The *diamond shapes* indicate the means. *hem*, hemorrhagic stroke; *isch*, ischemic stroke.

obtained by Reynolds et al. *(10)* on 5 different biomarkers: S-100, B-type neurotrophic growth factor, von Willebrand factor, matrix metalloproteinase-9, and monocyte chemotactic protein-1. Nevertheless, the TIA population is not as well characterized, and further investigations on a well-defined TIA group are required. The normal result in each of 5 controls with neurologic disorders (dementia, Parkinson disease, and meningioma) reinforces the specificity of the 2 markers. Nevertheless, studies need to be done in conditions that mimic a stroke, such as migraine or seizure, and metabolic disorders such as hypoglycemia.

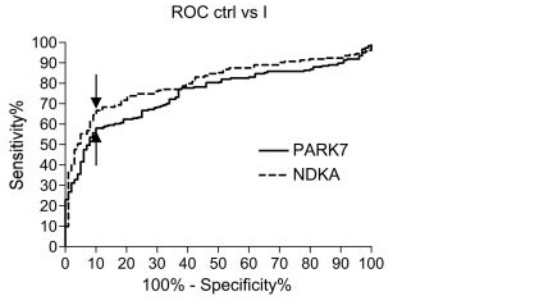
In the present study, the identification of PARK7 and NDKA was based on their increased concentrations in postmortem CSF, presumably as a result of global brain ischemia and necrosis after death. We previously explored the concept of postmortem CSF as a model of brain insult *(21)*. Fatty acid–binding protein, a potential biomarker of Creutzfeld–Jakob disease *(22)* and stroke



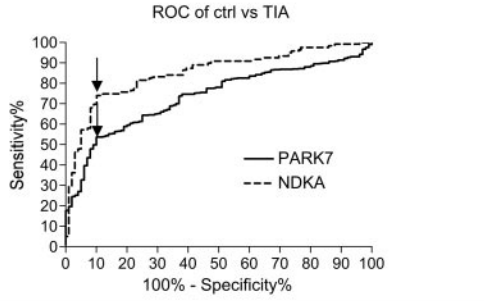
	P (CO)	SE%	95% CI	SP%	95% CI
PARK7	<0.0001 (14.1)	53.7	49.3% to 58%	90	82.4% to 95.1%
NDKA	<0.0001 (22)	70.5	66.4% to 74.4%	89.9	82.2% to 95%



	P (CO)	SE%	95% CI	SP%	95% CI
PARK7	<0.0001 (14.1)	43.4	36.8% to 50.1%	90	82.4% to 95.1%
NDKA	<0.0001 (21.2)	74.7	68.4% to 80.3%	87.9	79.8% to 93.6%



	P (CO)	SE%	95% CI	SP%	95% CI
PARK7	<0.0001 (14.2)	57.9	50.4% to 65.2%	90	82.4% to 95.1%
NDKA	<0.0001 (22.5)	66.7	59.3% to 73.5%	89.9%	82.2% to 95.1%



	P (CO)	SE%	95% CI	SP%	95% CI
PARK7	<0.0001 (14.2)	53.7	57.1% to 74.4%	90	82.4% to 95.1%
NDKA	<0.0001 (22)	74	65.1% to 81.6%	89.9%	82.2% to 95.1%

Fig. 7. American study 3: PARK7 and NDKA ROC curves.

Differentiation between the different types of stroke. Statistics associated with PARK7 and NDKA. *ctrl*, controls; *H*, hemorrhagic stroke; *I*, ischemic stroke; *CO*, cutoff; *SE*, sensitivity; *SP*, specificity. *Arrows* indicate cutoffs.

(9), was identified from 2-dimensional electrophoresis gels of postmortem CSF, which were compared with gels from antemortem CSF. Our results were recently reinforced by 3 independent studies, showing an increase of heart-type fatty acid–binding protein in patients suffering from mild traumatic brain injury *(34)*, stroke *(35)*, and neurodegenerative diseases with dementia *(36)*. We believe the model of postmortem CSF may partly reproduce mechanisms underlying the ischemic cascade of events leading to stroke lesions and, as such, may confer scientific plausibility to the biomarkers identified. Indeed, PARK7 and NDKA exhibit particular features that may reflect aspects of ischemic brain injury. Both proteins, although ubiquitous, are known to be expressed in neural structures and have been implicated in various neurologic conditions, including ischemia and neurodegeneration *(24, 25, 29, 31–33)*.

RNA-binding protein regulatory subunit is a protein that differs from PARK7/DJ-1 by a single amino acid change. Very few data are available about RNA-binding protein regulatory subunit. It has been identified from 2-dimensional electrophoresis gels of the human hepatocellular cell line HCC-M *(37)* and dermal fibroblast cells from healthy individuals *(38)*. An up-regulation of DJ-1 has been reported *(39)* in prostate tumor compared with matched healthy prostate tissue. DJ-1 also may be involved in the differential control of apoptosis in healthy and cancerous prostate cells *(40)*. DJ-1 is a conserved protein widely expressed in many tissues, including the brain and the heart *(23)*, and high expression of DJ-1 mRNA has been detected in neuronal and nonneuronal structures of the motor system in the mouse brain *(24)*. Mutations in the *DJ-1* gene have recently been linked to a form of autosomal recessive early-onset familial Parkinson disease *(28, 41)*. Moreover, it has also been demonstrated that mutations in *DJ-1* alter the cellular response to oxidative stress and proteosomal inhibition *(42)*. DJ-1 may be involved in the oxidative stress response *(25–29)* and in the detoxification of proteins through a chaperone function *(43)*. DJ-1 functions as a redox-sensitive molecular chaperone that is activated in an oxidative environment *(44)*. The same authors showed that familial Parkinson disease associated with the L166P mutation disrupts DJ-1 protein folding and function, and as a result, the mutant protein is selectively polyubiquitinated and rapidly degraded by the proteasome system.

Expression of the *NM23* gene, which encodes for NDKA, is decreased in highly metastatic murine melanoma cell lines. *NM23* has since been reported as a metastatic suppressor gene *(45)*, which is found in lower concentrations in tumor cells with high metastatic potential. Human *NM23-H1/NDKA* and *NM23-H2/NDKB* share 88% homology and encode for 2 subunits of nucleoside diphosphate (NDP) kinase. This kinase is a ubiquitous enzyme that catalyzes the transfer of the terminal phosphate from ATP to (deoxy)nucleotide triphosphates via the formation of a high-energy phosphorylated intermediate. Expression of *NM23-H1* is decreased in human hepatocellular carcinoma and hepatoma cell lines *(46)*. The presence of NM23 protein is a predictor of good prognosis in many cancers *(47–54)*. High specific activity of the enzyme was reported in the brain (150 kU/kg of protein) *(30)*, and its expression was highlighted in human brain *(31, 55)*. Forced expression of the murine gene coding for NDKA affects neuron proliferation and differentiation *(56, 57)*. *NM23*/NDKA has also been implicated in neurodegenerative disorders. For example, decreases in the expression and enzymatic activity of NDP kinase were detected in the brain of patients with Alzheimer disease and Down syndrome *(32)*, and NDP kinase protects mouse and rat cell lines from oxidative stress *(33)*.

On the basis of the known distributions and functions of PARK7 and NDKA, several hypotheses can be proposed regarding the mechanisms by which they may gain access to and be overexpressed in the plasma of stroke patients. A soluble protein produced by an injured neuron or glial cell may reach the blood through altered microvessels, notably venules, within or in the vicinity of the lesion. A leakage through a disrupted blood–brain barrier appears to be a likely, direct, and nearly immediate route for brain proteins to appear in the blood.

To date, many plasma markers have been unsuccessfully proposed for the diagnosis of stroke. In this study, 2 new biomarkers have been identified and appear to be useful for this purpose. Because the pathogenesis of stroke is complex, involving multiple mechanisms such as ischemia, thrombosis, inflammation, atherosclerosis, and neurodegeneration, the detection of stroke by use of markers may require a multiple markers to capture simultaneously all processes underlying the ongoing ischemic event. As a consequence, the approach of a panel of several biomarkers, as described previously *(10, 11)*, is currently under evaluation for the biomarkers presented in this study. Preliminary experiments using panel algorithms have given promising results when using a combination of the 2 biomarkers.

In conclusion, PARK7 and NDKA appear to be reliable biomarkers for the early diagnosis of stroke and, in the future, might be used in combination.

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