Dopamine Transporter Positron Emission Tomography in Spinocerebellar Ataxias Type 1, 2, 3, and 6

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Background: The spinocerebellar ataxias (SCAs) are a genetically heterogeneous group of autosomal dominant ataxias: some mutations, including SCA1, SCA2, and SCA3, are multisystemic disorders characterized by a variety of noncerebellar symptoms while others, like SCA6, give rise to a pure cerebellar syndrome.

Objective: To identify impairments of the dopaminergic system and regional changes of glucose metabolism in SCA1, SCA2, SCA3, and SCA6.


Results: The binding potential of [11C]d-threo-methylphenidate was reduced in the striatum in SCA2 and SCA3; in contrast to patients with Parkinson disease, no increased susceptibility of the putamen was evident. Decreased regional cerebral glucose metabolism was found in the cerebellum of all patients with SCA, the brainstem of SCA1, SCA2, SCA3, the thalamus and putamen of SCA3, and the parietal cortex of patients with SCA2. A trend toward increased regional cerebral glucose metabolism was found in the temporal cortex of all patients with SCA, pronounced in SCA6.

Conclusions: Specific biochemical patterns point to different mechanisms of neuronal dysfunction in SCA1, SCA2, SCA3, and SCA6; dopamine terminal loss is severe in SCA2 but distinct from Parkinson disease.

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THE SPINOCEREBELLAR ATAXIAS (SCAs) are a heterogeneous group of autosomal dominantly inherited progressive ataxias with more than 20 identified gene loci (SCA1-8, SCA10-17, SCA19-21, SCA25, dentatorubral-pallidolysian atrophy [DRPLA], and fibroblast growth factor 14 [FGF14]). Most mutations are expanded repeats; in 6 (SCA1-3, SCA6, SCA7, and SCA17) the mutation is a translated CAG repeat expansion coding for an elongated polyglutamine tract within the respective protein.1

Although ataxia is the prominent symptom in all SCAs, their clinical presentation is diverse; few mutations are characterized by an almost pure cerebellar syndrome and isolated degeneration of the cerebellar cortex. The most frequent disorder of this group is SCA6.2 Other SCAs are multisystemic disorders. In particular, the most common forms SCA1, SCA2, and SCA3 present with a variety of additional symptoms.3-5 Correspondingly, neuropathological studies show neurodegeneration not only in the spinocerebellar system, but also in the basal ganglia and midbrain.5-7 Parkinsonian features have been reported early in SCA3 and more recently also in SCA2.8-14

Positron emission tomography (PET) studies in degenerative ataxias prior to molecular diagnoses found [18F]fluorodeoxyglucose ([18F]FDG) hypometabolism in the cerebellum and brainstem.15 In contrast, in a study of γ-aminobutyric acid type A (GABA_A)/benzodiazepine receptors using [11C]Flumazenil, patients suffering from dominant ataxia had normal ligand influx in most brain areas investigated, in particular the cerebellum and brainstem, indicating a functional rather than a structural deficit.16 More recently, normal striatal dopamine D2 receptors and variable degrees of dopamine terminal damage and metabolic impairment have been reported in SCA2 and SCA3.17-22

We analyzed the dopamine transporter using [11C]d-threo-methylphenidate ([11C]dMP) and regional cerebral glucose metabolism (rCMRglu) using [18F]FDG in patients with SCA1, SCA2, SCA3, and SCA6 in comparison with patients with Parkinson disease (PD) and healthy volunteers.
We studied 21 patients with SCA1, SCA2, SCA3, and SCA6; 10 patients with PD in Hoehn and Yahr stage II (Unified Parkinson’s Disease Rating Scale III: mean ± SD, 23 ± 10); and 10 healthy volunteers without any overt neurological or psychiatric symptoms (Table 1). All patients’ conditions were diagnosed at the movement disorder clinics of the Universities of Bonn or Tübingen, Germany; genetic testing was performed as reported previously. Patients with SCA were evaluated using an ataxia rating score, measuring ataxia of stance and gait, kinetic functions, speech, and oculomotor disturbances on a 5-point scale and the International Cooperative Ataxia Rating Scale.

### METHODS

#### PATIENTS

To synthesize [11C]dMP, the free acid of N-protected d-threo-methylphenidate was alkalized using [11C]methyliodide. High specific activity [11C]methyliodide was prepared in an automated module (PETTrace MeI Microlab; General Electric Medical Systems, Uppsala, Sweden). The radio labeling was performed in a PET tracer synthesizer for [11C]methylations (PETtrace MeI Microlab; General Electric Medical Systems, Uppsala, Sweden). The product was obtained in 45% to 65% radiochemical yield with specific activities of 30 to 50 Gbq/μmol at the end of synthesis (60 minutes). Chemical and radiochemical purities of the final radiotracers were greater than 95% as determined by high-performance liquid chromatography. [18F]FDG was prepared in a PETtrace FDG Microlab (General Electric Medical Systems).

#### RADIOCHEMISTRY

Quantiﬁcation of dMP and FDG binding

Binding of [11C]dMP and FDG were analyzed by a standardized ROI technique. Additionally, group differences of SCA3, SCA6, and control subjects were assessed on a voxel basis (SPM99 perfusion template, 12-mm smooth mask; isocontour 65% of maximum in SPM template; threshold: P < .001 uncorrected, t > 3.93). The ROI template consists of 31 three-dimensional regions defined in stereotactic standard space, including 2 × 3 striatal regions with small volumes (eg, dorsal putamen 2 × 0.67 mL). For this study, we analyzed cerebellum, brainstem, thalamus, putamen, caudate nucleus, parietal, and temporal cortex. The position of all ROIs was compared with the early summation images of each patient and adjusted manually. Dopamine transporter availability (dMP binding potential, BP\textsubscript{DAT}) in the 2 striatal regions of interest was calculated with Logan’s graphical analysis and the occipital cortex as a reference region. The washout from the occipital cortex (k\textsubscript{3}) was assumed to be 0.05 per minute and 18 to 60 minutes postinjection was chosen as the interval for linear regression. Binding potential for dMP was calculated from (slope−1), aiming at quantification of binding potential = k\textsubscript{r}/K\textsubscript{D} = (f\textsubscript{r} × B\textsubscript{max})/K\textsubscript{D} with k\textsubscript{r}, k\textsubscript{D}, and B\textsubscript{max} being transfer rate constants in the 2-tissue compartment model, f\textsubscript{r} the free fraction of tracer in the first tissue compartment, B\textsubscript{max} the density of binding sites, and K\textsubscript{D} the equilibrium dissociation constant. Asymmetry indices (specific binding) were calculated with standard SPM99 settings including 4 × 5 × 4 basis functions and with affine transformation only for region of interest (ROI) analysis.

#### PET ACQUISITION AND IMAGE RECONSTRUCTION

The patient’s head was ﬁxed in an elastic mold with 3 markers for correction of head movements. After automated intravenous bolus injection (12 seconds) of 700 MBq [11C]dMP or 400 MBq [18F]FDG, respectively, dynamic data were acquired from 0 to 60 minutes. Post injection, in 2-dimensional mode with a full-ring PET scanner (GE Advance; General Electrics Medical System, Milwaukee, Wis), followed by a transmission scan with 500,000 kilo counts for attenuation correction. Attenuation corrected images were reconstructed with filtered back projection (128 × 128 pixels corresponding to 30 × 30 cm, Hanniﬁng ﬁlter with a 4.6-mm cutoff). Statistical parametric mapping (SPM) software (SPM 99; Welcome Department of Cognitive Neurology, London, England) was used for realignment and spatial normalization by comparing summation images 0 to 5 minutes postinjection with the standard SPM perfusion template. For [11C]dMP, normalization parameters were estimated from early summation images 0 to 5 minutes postinjection. Normalized images were calculated with standard SPM99 settings including 4 × 5 × 4 basis functions and with afﬁne transformation only for region of interest (ROI) analysis.
binding putamen – caudate/[specific binding][mean putamen + caudate] were calculated and assessed with 2-tailed unpaired t tests.

The index of regional cerebral metabolic rates of glucose (rCMRglu) was calculated as the ratio of the average $^{18}$F-FDG concentration 42 to 54 minutes postinjection over the average concentration in a modified whole-brain mask created from a standard whole-brain mask by manually excluding the cerebellum, brainstem, diencephalon, and striatum. Voxel-wise group differences were calculated as percentage change of the index of rCMRglu. Areas with average FDG concentrations below threshold, eg, white matter, were excluded and a gaussian smoothing filter (12 mm) was applied.

### STATISTICS

Statistical analysis of the ROI data was performed using analysis of variance and post hoc Tukey tests for group comparisons and multiple testing, and 2-tailed paired $t$ tests for intraindividual asymmetries (JMP501; SAS Institute Inc, Cary, NC).

#### RESULTS

**$^{[11C]}$D-THREO-METHYLPHENIDATE**

Striatal $^{[11C]}$dMP binding potential (BP$_{dMP}$) was markedly reduced in SCA2, SCA3, and patients with PD ($P<.01$), but no significant changes were found in SCA1 and SCA6 (Figure 1).

Putamen and caudate nucleus displayed the same degree of BP$_{dMP}$ decrease in SCA2 (57% and 55%) and SCA3 (29% and 20%), whereas a more severe loss of BP$_{dMP}$ was evident in PD putamen (69% vs 46%; asymmetry index [mean±SD], 44±17 vs 10±7 in SCA2, 15±11 in SCA3, and 14±11 in control, $P<.001$). Similarly, a pronounced side-to-side asymmetry was found in the more affected vs less affected putamen in PD (asymmetry index, 38±23 vs 5±5 in control, $P<.001$), but not in either SCA.

No correlation was found between striatal BP$_{dMP}$ and repeat length in SCA2 or SCA3, although it must be noted that the patients studied had repeat lengths in very narrow ranges (42±3 and 72±5, respectively). Also, no significant correlation between striatal BP$_{dMP}$ and age or (apparent) disease duration was observed in SCA2 and SCA3.

#### $^{[18F]}$FLUORODEOXYGLUCOSE

Reduced rCMRglu was found in the cerebellum of all patients with SCA, the brainstem of SCA1, SCA2, SCA3, and the thalamus of patients with SCA3 (Table 2). Patients with SCA2 and PD displayed reduced rCMRglu in the parietal cortex. A trend toward reduced rCMRglu was found in the putamen in SCA3 and SCA6, whereas no changes were present in putamen or caudate nucleus of patients with PD or SCA2. A trend toward increased rCMRglu was noted in the temporal cortical ROI in SCA2 and SCA3, which theoretically may be a normalization artifact because of reduced parietal rCMRglu, especially in patients with SCA2.

The SPM analysis confirmed the results obtained with the ROI analysis and revealed a distinctive pat-
tern of changes for either disease: in SCA3, we found areas of decreased metabolism extending from cerebellar midline structures to adjacent pons and midbrain; in SCA6, we found decreased metabolism confined to the cerebellum (**Figure 2** and **Figure 3**). Both patients with SCA3 and SCA6 display areas of increased metabolism (>$\text{control}$, bottom) in the superior and middle temporal gyri.

**Figure 2.** Statistical parametric mapping analysis of [$^{18}$F]fluorodeoxyglucose in SCA3 and SCA6 compared with healthy volunteers. Note areas of decreased metabolism ($<$ control, top) in SCA3 extending from cerebellar midline structures to adjacent pons and midbrain but confined to the cerebellum in SCA6. Both patients with SCA3 and SCA6 display areas of increased metabolism ($>$ control, bottom) in the superior and middle temporal gyri.

We found decreased $\text{BP}_{\text{dop}}$ in the striatum of patients with SCA2 and SCA3 (and PD), but not in those with SCA1 or SCA6. The pattern of dopamine terminal loss in SCA2 and SCA3 differed from PD because no increased susceptibility of the putamen or a significant asymmetry could
be detected. The FDG study in addition to the expected reductions of rCMRglu in the cerebellum and brainstem identified the thalamus as an affected brain region in patients with SCA3.

Our findings comply with recently published data of dopamine transporter single-photon emission computed tomography (DAT-SPECT) in patients with SCA2 and earlier [18F]FDG-PET studies in patients with SCA3.17-22 For SCA2, the first imaging studies of members of the Alberta family presenting clinically with parkinsonism revealed DAT loss similar to PD with a more severely affected putamen.11,13 None of our patients displayed parkinsonian signs, yet similar to patients with PD, all patients with SCA2 in our PET study and in the SPECT study of Varrone et al20 displayed severe DAT loss throughout the striatum, suggesting that the dopaminergic system is particular sensitive to the SCA2 mutation. In her study of clinical and neuropathological features in SCA2, Dürr et al6 already emphasize the striking discrepancy between the severe pathological changes observed in the substantia nigra and the lack of overt parkinsonian features. Thus, either additional factors are required for parkinsonian signs to appear or severe cerebellar dysfunction may mask parkinsonian signs.23 Interestingly, patients with SCA2 with parkinsonian signs reported to date all had relatively short repeat expansions and possibly less severe cerebellar pathology.14 Alternatively, although less likely, loss of DAT may represent a necessary but not a sufficient condition to elicit the typical parkinsonian motor features in PD.

In contrast to SCA2, loss of DAT was less severe in patients with SCA3. Only 1 patient showed a reduction of BP_{dop} in the striatum below 2.5 SDs, whereas 4 patients had BP_{dop} reductions below 1 SD (of the mean normal control value). Almost identical, in the study by Shi-notoh et al17 2 out of 6 patients with SCA3 showed a significant reduction of putaminal and caudate Ki below 2.5 SDs, while 4 patients had Ki values below 1 SD. Similar to SCA2, no patient with SCA3 in our study had overt parkinsonian signs (despite a BP_{dop} loss of 69% in 1 case). Thus, in SCA3, the available imaging data indicate a variable degree of damage of the dopaminergic system with little correlation to the clinical presentation.

Our PET data revealed no clear-cut difference of BP_{dop} between putamen and caudate nucleus in patients with SCA2 and SCA3, even though Taniwaki et al18 reported a significant reduction of [18F]florodopa uptake only in the putamen. In that study however, mean putamen and caudate values were in the same order of magnitude (70% vs 77% of control) and no individual results were reported. A uniform involvement of striatal dopaminergic terminals in patients with SCA2 and SCA3 as observed with the DAT ligand [11C]dMP is in line with diffuse nigral cell loss reported post mortem.6-8 In addition to dopaminergic cell loss, global synaptic impairment might contribute to the alterations of DATs in patients with SCA3; recent bioinformatics and experimental data suggested that ataxin-3 functions as a ubiquitin protease involved in the regulation of synaptic activity.29,30 Reduced synaptic activity could also ex-
plain the (minor) reductions of rCMRglu in the putamen of patients with SCA3.

Although SCA1, like SCA2 and SCA3, is considered a multisystemic disease, the dopaminergic system appears to be spared, which reflects the specific pattern of neuronal vulnerability encountered in each polyglutamine disorder. On the other hand, both ROI and SPM analyses pointed toward an increased rCMRglu at rest in temporal cortica areas, suggesting either a common compensatory mechanism or cortical hyperactivity as a consequence of the cerebellar and brainstem dysfunction. Similarly, Wessel et al.31 using a sequential finger movement paradigm and [15O]H2O PET found, that specific motor areas were more active in patients with cerebellar degeneration.

The complex pattern of rCMRglu, which increases and decreases in distinct brain regions, might allow the characteriza- tion of spatially distributed neural networks and compensatory changes in response to either mutation. Our findings reflect the specific pathology observed in patients with SCA1, SCA2, SCA3, and SCA6 and provide a noninvasive phenotype that might be useful as a quantitative marker in future trials of neuroprotective drugs.

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