No parkinsonism in SCA2 and SCA3 despite severe neurodegeneration of the dopaminergic substantia nigra

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The spinocerebellar ataxias types 2 (SCA2) and 3 (SCA3) are autosomal dominantly inherited cerebellar ataxias which are caused by CAG trinucleotide repeat expansions in the coding regions of the disease-specific genes. Although previous post-mortem studies repeatedly revealed a consistent neurodegeneration of the dopaminergic substantia nigra in patients with SCA2 and with SCA3, parkinsonian motor features evolve only rarely. As the pathophysiological mechanism how SCA2 and SCA3 patients do not exhibit parkinsonism is still enigmatic, we performed a positron emission tomography and a post-mortem study of two independent cohorts of SCA2 and SCA3 patients with and without parkinsonian features. Positron emission tomography revealed a significant reduction of dopamine transporter levels in the striatum as well as largely unaffected postsynaptic striatal D2 receptors. In spite of this remarkable pathology in the motor mesostriatal pathway, only 4 of 19 SCA2 and SCA3 patients suffered from parkinsonism. The post-mortem investigation revealed, in addition to an extensive neuronal loss in the dopaminergic substantia nigra of all patients with spinocerebellar ataxia, a consistent affection of the thalamic ventral anterior and ventral lateral nuclei, the pallidum and the cholinergic pedunculopontine nucleus. With the exception of a single patient with SCA3 who suffered from parkinsonian motor features during his lifetime, the subthalamic nucleus underwent severe neuronal loss, which was clearly more severe in its motor territory than in its limbic or associative territories. Our observation that lesions of the motor territory of the subthalamic nucleus were consistently associated with the prevention of parkinsonism in our SCA2 and SCA3 patients matches the clinical experience that selective targeting of the motor territory of the subthalamic nucleus by focal lesions or deep brain stimulation can ameliorate parkinsonian motor features and is likely to counteract the manifestation of parkinsonism in SCA2 and SCA3 despite a severe neurodegeneration of the dopaminergic substantia nigra.

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Degeneration of the compact part of the substantia nigra and loss of dopaminergic nigrostriatal projections, as well as the presence of alpha-synuclein immunopositive neuronal Lewy bodies and Lewy neurites are currently considered as the neuropathological hallmarks of idiopathic Parkinson’s disease (Braak et al., 2003a; Obeso et al., 2008; Dickson et al., 2009).

Together with a variety of other brain structures the dopaminergic substantia nigra is integrated into the excitatory ‘direct’ and inhibitory ‘indirect’ basal ganglia pathways, which together are crucial for the performance of voluntary movements. The motor cortices represent the subordinate structures of the ‘direct’ pathway and ‘indirect’ pathways. They send glutamatergic signals to the striatum (i.e. caudate nucleus and putamen), which normally receive excitatory dopaminergic projections from the substantia nigra and send inhibitory GABAergic signals to the internal pallidum and reticulate part of the substantia nigra. This inhibition of the output structures of the ‘direct’ pathway results in the activation of the glutamatergic motor ventral anterior and ventral lateral nuclei of the thalamus and of the motor areas in the neocortex, enables the initiation and execution of intended voluntary movements and guarantees their fine tuning and adjustment. This cortical circuit by means of the ‘hyperdirect’ pathway can also activate the subthalamic nucleus, which ensures an increased excitation of the internal pallidum and inhibition of the motor nuclei of the thalamus thus inhibiting undesired movements (Benarroch, 2008; Obeso et al., 2008). The ‘indirect’ pathway operates together with the direct pathway, decreases the effects of the ‘direct’ pathway, reduces the neuronal activity in the motor cerebral cortices and eventually helps to prevent unwanted voluntary movement (Benarroch, 2008; Obeso et al., 2008).

It is widely believed that functional consequences of the decline in striatal dopamine content caused by progressive degeneration of the compact part of the substantia nigra can be initially compensated for by adaptations of the remaining dopaminergic nigral neurons, as well as by modulations and adjustments accomplished by the ‘direct’ and ‘indirect’ pathways. Such mechanisms of compensation could explain why loss of dopaminergic neurons of the substantia nigra can occur for a long time without reduction of basal ganglia output and manifestation of parkinsonism. However, it is widely held that (i) the involved basal ganglia circuits lose their normal physiological tuning activity when loss of dopaminergic neurons in the substantia nigra surpasses the threshold of ~50–60%; and (ii) that this may be tantamount with the emergence of parkinsonian motor features in affected patients (Marsden, 1990; Benarroch, 2008; Obeso et al., 2008).

This pathophysiological mechanism has been approved in idiopathic Parkinson’s disease but may not apply to the spinocerebellar ataxias type 2 (SCA2) and type 3 (SCA3). SCA2 and SCA3 are comparatively rare movement disorders, belong to the group of autosomal dominantly inherited cerebellar ataxias and are caused by CAG trinucleotide repeat expansions in the coding regions of the disease-specific genes that are translated into enlarged polyglutamine stretches in the disease proteins ataxin 2 and ataxin 3 (Kawaguchi et al., 1994; Pulst et al., 1996; Schöls et al., 2004; Dürr, 2010; Orr, 2012; Seidel et al., 2012; Rüb et al., 2013). Although cerebellar signs and symptoms (i.e. ataxia, dysarthria) are the key clinical features of both diseases, SCA2 and SCA3 patients commonly also present with non-cerebellar symptoms (e.g. oculomotor symptoms, dysphagia, pyramidal signs, sensory deficits and cognitive impairment) (Dürr et al., 1995, 1996; Iwabuchi et al., 1999; Gilman, 2000; Schöls et al., 2004; Dürr, 2010; Rüb et al., 2013).

Along with neurodegeneration of the cerebellum, thalamus, spinal cord and a large variety of brainstem nuclei, neuropathological studies repeatedly revealed and pointed to a consistent involvement of the midbrain dopaminergic substantia nigra in SCA2 and SCA3 patients (Dürr et al., 1995, 1996; Estrada et al., 1999; Iwabuchi et al., 1999; Gilman, 2000; Schöls et al., 2004; Seidel et al., 2012; Rüb et al., 2013). Although the extent of neuronal loss in the compact part of the substantia nigra in many of the SCA2 and SCA3 patients previously studied post-mortem even went beyond that present in patients with clinically diagnosed idiopathic Parkinson’s disease with advanced Braak stage 5 or 6 brain pathologies (Braak et al., 2003a), the occurrence of parkinsonian motor features is...
only exceptionally described in the clinical SCA2 and SCA3 literature and is only registered as a predominant phenotype in some patients (Tuite et al., 1995; Dürr et al., 1996; Estrada et al., 1999; Iwabuchi et al., 1999; Gilman, 2000; Gwinn-Hardy et al., 2001; Shan et al., 2001; Lu et al., 2004; Schöls et al., 2004; Dürr, 2010; Seidel et al., 2012; Rübs et al., 2013).

Because the exact pathophysiological mechanisms why and how the majority of SCA2 and SCA3 patients can escape parkinsonian motor symptoms despite even severe neurodegeneration of the compact part of the substantia nigra remain enigmatic (Gilman, 2000; Schöls et al., 2004), and the classical models of basal ganglia neural circuits have provided a conceptual framework for better understanding the pathophysiological background of parkinsonian motor features in various neurological diseases (Hamani et al., 2004; Benarroch, 2008; Lambert et al., 2012), we evaluated the mesostriatal dopaminergic system in SCA2 and SCA3 patients with and without parkinsonian motor features. We used two complementary approaches including an in vivo study by PET and a post-mortem analysis of the compact part of the substantia nigra and associated basal ganglia nuclei.

Subjects and methods

The present study includes a combination of (i) an in vivo neuroimaging study including patients with SCA2, SCA3, idiopathic Parkinson’s disease and healthy control subjects (Supplementary Table 1); and (ii) a post-mortem pathoanatomical study of an independent cohort of clinically diagnosed and genetically confirmed SCA2 and SCA3 patients and healthy control subjects without neuropsychiatric diseases in their medical records (Supplementary Table 2).

In vivo imaging studies

Six patients with SCA2 (four females, two males; median age at investigation: 44 years, range 37–68; median length of the CAG repeats in the mutated SCA2 allele: 39, range 35–39; median age at SCA2 onset: 34 years, range 27–51 years) (Supplementary Table 1) and 13 patients with SCA3 (three females, 10 males; median age at investigation: 45 years, range 27–66; median length of the CAG repeats in the mutated SCA3 allele: 70, range 56–76; median age at SCA3 onset: 35 years, range 19–59 years) (Supplementary Table 1) underwent clinical assessment and PET imaging (Ehrin et al., 1985; Ding et al., 1994; Brockmann et al., 2012). Patients were recruited from the ataxia clinic in Tübingen. Additionally, 19 patients who fulfilled the UK Brain Bank criteria for the diagnosis of idiopathic Parkinson’s disease (nine females, 10 males; median age at investigation: 65 years, range 43–77 years; mean disease duration 5 ± 3 years) (Hughes et al., 1992) were clinically and neuroradiologically investigated. Twenty-six healthy age- and gender-matched individuals without a medical history of neuropsychiatric diseases served as control subjects. Severity of ataxia was assessed using the Scale for the Assessment and Rating of Ataxia (SARA) (Schmitz-Hubsch et al., 2006). The Unified Parkinson’s Disease Rating Scale (UPDRS III) was used to estimate the severity of parkinsonian motor features (Fahn et al., 1987) (Supplementary Table 1). All individuals gave their written informed consent for the study. Clinical examination and functional brain imaging in patients with SCA2, SCA3 and idiopathic Parkinson’s disease as well as in control subjects was approved by the Ethical Review Board of the Medical Faculty, University of Tübingen (Vote 122/2004).

In the patients with SCA2, the median severity of ataxia in the SARA score was 12 points (range 6.5–18.5 points) (Supplementary Table 1) and in the SCA3 patients, 13.0 points (range 3.0–19.0 points) (Supplementary Table 1).

None of the SCA patients showed a resting tremor (Supplementary Table 1), whereas one of our six patients with SCA2 (Patient 5; Supplementary Table 1) and 3 of our 13 patients with SCA3 (Patients 9, 12 and 13; Supplementary Table 1) suffered from rigidity and akinesia with reduced arm swing and hesitations in repetitive hand movements, which responded well to L-DOPA treatment. Bradydysdiadokinesia and postural instability were observed in a subset of our SCA2 and SCA3 patients and contributed to increased UPDRS scores, but were regarded as unspecific signs as they are well known to occur in almost all types of ataxic disorders and to evolve independently of degeneration of the dopaminergic substantia nigra.

All SCA2, SCA3 and idiopathic Parkinson’s disease patients underwent one PET scan using the presynaptic dopamine transporter ligand 13C-d-threo-methylphenidat (dMP) (Ding et al., 1994) and one PET scan using the postsynaptic dopamine D2 receptor ligand 11C-raclopride (RAC) (Ehrin et al., 1985) (Table 1). Due to radiation safety considerations, control individuals underwent only either dMP or RAC PET scan (dMP: 13 controls, median age at investigation: 51 years; RAC: 13 controls, median age at investigation: 45 years) (Table 1).

Table 1. Patients’ characteristics and disease duration

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Gender</th>
<th>Age (years)</th>
<th>CAG repeats</th>
<th>Disease duration (years)</th>
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<td>SCA2</td>
<td>Female</td>
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<td>8</td>
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<td>SCA2</td>
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<td>45</td>
<td>39</td>
<td>5</td>
</tr>
<tr>
<td>SCA3</td>
<td>Female</td>
<td>42</td>
<td>40</td>
<td>7</td>
</tr>
<tr>
<td>SCA3</td>
<td>Male</td>
<td>47</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>Parkinson's</td>
<td>Female</td>
<td>50</td>
<td>42</td>
<td>2</td>
</tr>
<tr>
<td>Parkinson's</td>
<td>Male</td>
<td>55</td>
<td>43</td>
<td>3</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Female</td>
<td>40</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>Male</td>
<td>45</td>
<td>39</td>
<td>5</td>
</tr>
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</table>

Acquisition and analysis of PET data was performed as previously described (Brockmann et al., 2012). Subsequent to the attachment of three fiducial markers to the skull for correction of head movements, a 12.5 bolus injection of 700 MBq dMP or 700 MBq RAC was performed intravenously and dynamic data were acquired over 60 min by a PET scanner (GE Advance, General Electric Medical System). For attenuation correction a transmission scan with 500,000 kilocounts was performed at the end of each scan. All PET images were reconstructed with filtered back projection (Hanning filter with 4.6 mm cut-off). The statistical parametric mapping (SPM) software package SPM2 (Functional Imaging Laboratory, University College London, UK) was used for realignment and spatial normalization by comparing summation images 0–5 min after injection with the standard SPM perfusion template. A locally established set of 3D region of interest (dorsal putamen: 2 × 0.67 ml; caudate nucleus: 2 × 0.40 ml; occipital cortex as reference region) was used for region of interest analysis and applied to each frame. Binding potential (Innis et al., 2007) was quantified using Logan graphical analysis (dMP: regression interval 18–60 min; reference tissue: occipital cortex, k2’ = 0.05 min−1) (Logan et al., 1996) and the simplified reference tissue model (RAC) (Lammertsma et al., 1996).

The non-parametric Mann-Whitney U-test was used for statistical analyses of differences of the in vivo PET data from SCA2 and SCA3 patients with the control individuals studied (Table 1). In addition, Spearman’s rank correlation tau was
applied for the evaluation of potential relationships between the clinical data of the patients and the variables revealed by the performed in vivo imaging analyses.

**Post-mortem pathoanatomical study**

The autopsy brains of four patients with clinically diagnosed SCA2 (two females, two males; median age at SCA2 onset: 31 years, range 6–60 years; median age at death: 33 years, range 25–88 years; median length of the CAG-repeats in the mutated SCA2 allele: 40, range 36–52; median interval between the last neurological examination and death: 2.5 years, range 0.5–3 years; median post-mortem interval: 19 h; range 6–36 h) (Supplementary Table 2) and 13 control individuals (median age at death: 48 years, range 21–84 years) without neuropsychiatric diseases in their medical records (median post-mortem interval: 14 h, range 6–48 h) (Supplementary Table 2) and 13 control individuals (median age at death: 48 years, range 21–84 years) without neuropsychiatric diseases in their medical records underwent post-mortem pathoanatomical analyses. All 26 brains that came to autopsy at the University of Groningen (The Netherlands) were examined macroscopically, fixed in 4% non-buffered formaldehyde, and investigated for purposes of neuropathological diagnosis by an experienced neuropathologist (W.d.D.). Post-mortem pathoanatomical examination of the SCA2, SCA3 and control brains was approved by the Ethical board of the Faculty of Medicine at the Goethe University of Frankfurt/Main.

All of the SCA2 and SCA3 patients were repeatedly examined by experienced neurologists (E.R.B., K.B., G.A., L.S.) and suffered from progressive gait, stance and limb ataxia, dysarthria, dysphagia and showed a variable combination of oculomotor symptoms. Parkinsonian motor features were present only in one of the SCA3 patients and included a low frequency 4 Hz resting tremor at age 44 years that responded well to L-DOPA and trihexyphenidyl (Patient 7; Supplementary Table 2). Molecular genetic examination disclosed expanded CAG repeats in the diseased alleles of all SCA2 and SCA3 patients (Supplementary Table 2) and routine neuropathological examination revealed widespread damage to the cerebellum, pallidum and brainstem as described recently (Scherzer et al., 2012; Seidel et al., 2012; Rüb et al., 2013b).

For purposes of pathoanatomical examination, polyethylene glycol embedded serial 100-μm thick frontal tissue sections through the right cerebral hemispheres and midbrain were stained according to the Braak pigment-Nissl method or immunolabelled with the anti-synuclein antibody syn-1 to highlight idiopathic Parkinson’s disease-related neuronal inclusions bodies (1:2000, BD Biosciences) (Braak et al., 2003a, b; Seidel et al., 2012; Rüb et al., 2013).

Along with the compact part of the substantia nigra, we systematically analysed additional nuclei of the basal ganglia circuitry and investigated neuronal loss, alpha-synuclein immunopositive neuronal Lewy pathology and concomitant reactive astrogliosis in the internal and external pallidum, GABAergic subthalamic nucleus, midbrain cholinergic pedunculopontine nucleus, as well as in the thalamic ventral anterior and ventral lateral nucleus (Table 2). The thalamic ventral anterior nucleus represents the pallidal relay of the human motor thalamus and the ventral lateral nucleus of the thalamus the cerebellar afferent territory of the thalamus. In its ventral posterior region the ventral lateral nucleus contains the electrophysiologically defined ventro-intermediate nucleus, which is populated by nerve cells with tremor synchronous rhythmic burst activity and represents the most popular target for surgery and deep brain stimulation for tremor reduction in idiopathic Parkinson’s disease (Hirai and Jones, 1989; Krack et al., 2002).

The unequivocal identification and accurate delineation of the anatomical borders of the nuclei of interest represent the basic prerequisites for the application of the stereological methods of choice for obtaining reliable estimations of absolute nerve cell numbers (i.e. stereological methods using the point counting method in combination with the optical dissector; fractionator) (Rüb et al., 2002). Owing to a very severe neuronal loss in most instances the exact delineation of the anatomical borders of the basal ganglia nuclei under consideration was hardly possible (Figs 3, 5 and Supplementary Fig 1). As the extensive neuronal loss was demonstrable even without using advanced stereological approaches, we decided to use a semi-quantitative approach for the assessment of neurodegeneration and categorized neurodegeneration into: (i) not discernible (−); (ii) unequivocal and marked neuronal loss present (+); and (iii) only scarce numbers of remaining nerve cells present (++). This semi-quantitative assessment of neurodegeneration was performed by U.R. and repeated by K.S., who was blinded

**Table 1** Positron emission tomography findings in patients with SCA2 and SCA3

<table>
<thead>
<tr>
<th></th>
<th>SCA2 n = 6</th>
<th>SCA3 n = 13</th>
<th>Controls RAC n = 14</th>
<th>Controls dMP n = 15</th>
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<tbody>
<tr>
<td>Age</td>
<td>44.0 (37–68)</td>
<td>45.0 (27–66)</td>
<td>46.0 (37–57)</td>
<td>51.0 (34–61)</td>
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<tr>
<td>Raclopride (k3/k4)</td>
<td>1.71 (1.54–2.03)</td>
<td>1.65 (1.47–2.08)</td>
<td>1.73 (1.30–2.10)</td>
<td>n.p.</td>
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<tr>
<td>Caudeate nucleus</td>
<td>1.30 (0.99–1.57)</td>
<td>1.26 (1.01–1.56)</td>
<td>1.38 (1.01–1.76)</td>
<td>n.p.</td>
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<tr>
<td>Methylphenidate (k3/k4)</td>
<td>0.43**** (0.37–0.92)</td>
<td>0.57**** (0.21–0.96)</td>
<td>n.p.</td>
<td>1.34 (0.84–1.82)</td>
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<tr>
<td>Caudeate nucleus</td>
<td>0.38**** (0.29–0.87)</td>
<td>0.58**** (0.15–0.81)</td>
<td>n.p.</td>
<td>1.21 (0.87–1.70)</td>
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***p < 0.001, ****p < 0.0001: Mann-Whitney U-test. n.p. = not performed.
to the results of the first rating. To demonstrate the objectivity, reproducibility and reliability of this less time-consuming approach we performed an evaluation of its inter-rater reliability by calculating Cohen’s weighted kappa coefficient $K_w$ for Windows, version 9.14, Epsilon, Darmstadt, Germany).

The presence and extent of idiopathic Parkinson’s disease-related alpha-synuclein immunoreactive Lewy bodies and Lewy neurites, likewise, was semi-quantitatively assessed and scored as follows: Lewy bodies and Lewy neurites not detectable (–), rare Lewy bodies and Lewy neurites present (+), an abundance of Lewy bodies and Lewy neurites present (++).

**Results**

**Positron emission tomography imaging study**

For functional *in vivo* analyses of the dopamine system we performed dMP PET to trace presynaptic dopamine transporter (DAT) levels and RAC PET for postsynaptic D2 receptor imaging (Ehrin et al., 1985; Ding et al., 1994). Resembling typical findings in patients with idiopathic Parkinson’s disease, these PET investigations in the SCA2 and SCA3 patients revealed pronounced presynaptic dopaminergic deficits and largely unaffected postsynaptic D2 receptors (Figs 1 and 2).

Compared to control individuals, dMP binding potential was bilaterally significantly reduced in SCA2 patients (putamen: $P < 0.001$; caudate: $P < 0.001$; Mann-Whitney U-test) as well as in the SCA3 patients (putamen: $P < 0.0001$; caudate: $P < 0.0001$; Mann-Whitney U-test) (Table 1). Specifically, putaminal DAT levels in five of the six SCA2 and in 11 of 13 SCA3 patients were below the lowest level observed in healthy controls (Fig. 2A). In five of the SCA2 patients and in eight of the SCA3 patients, putaminal DAT levels were within the range of the levels of the patient with idiopathic Parkinson’s disease (Fig. 2A). Reduction of DAT level was more severe in the caudate nucleus of the SCA2 and SCA3 patients than in the patients with idiopathic Parkinson’s disease (Fig. 2B). Whereas patients with idiopathic Parkinson’s disease typically presented with a putaminal-caudate gradient of presynaptic dopaminergic deficits with the caudate nucleus showing only later deficits the affection of the striatum of the SCA2 and SCA3 patients was rather homogenous (Figs 1 and 2A, B and D).

In our patients with SCA2 and SCA3, parkinsonism was associated with a pronounced putaminal DAT deficit. With the exception of one patient, the SCA2 and SCA3 patients that presented with motor symptoms of parkinsonism were those with the lowest putaminal dMP binding (Fig. 2A–C).

There was no correlation between the presence of parkinsonian motor features and the severity of cerebellar ataxia in the SCA2 and SCA3 patients studied *in vivo* (data not shown).

Upon RAC PET investigation neither relevant postsynaptic dopaminergic deficits nor a compensatory upregulation of D2 receptors was found in the SCA2 and SCA3 (Fig. 2C and Table 1).

**Post-mortem investigation**

The pathoanatomical assessment revealed a considerable loss of melanin-containing dopaminergic neurons in the compact part of the substantia nigra in all SCA2 and SCA3 patients without a consistent topographical preference. In six of the SCA patients, neuronal loss of the compact part of the substantia nigra was similar to or even more severe than in clinically advanced patients with idiopathic Parkinson’s disease with neuropathological findings corresponding to Braak stages 5 and 6 (Braak et al., 2003a) (Fig. 3 and Table 2). The ventral anterior and ventral lateral thalamic nuclei, the internal and external pallidum likewise were consistently affected and showed an evenly

### Table 2 Distribution and extent of neuronal loss in nuclei of the basal ganglia circuits in the SCA2 and SCA3 patients studied post-mortem

<table>
<thead>
<tr>
<th>Patients</th>
<th>VA</th>
<th>VL</th>
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Extent of neuronal loss in the basal ganglia nuclei of the SCA2 and SCA3 patients studied: not discernible (–), unequivocal and marked neuronal loss present (+), only scarce numbers of remaining nerve cells present (++). In all SCA patients with neuronal loss in the subthalamic nucleus, neuronal loss was either confined to or clearly more severe in its dorsolateral somatomotor territory than in its limbic and associative territories (see also Figs 4 and 5). PE = external pallidum; PI = internal pallidum; PPN = pedunculopontine nucleus; SNc = substantia nigra, compact part; VA = ventral anterior thalamic nucleus; VL = ventral lateral thalamic nucleus.
distributed marked to severe neuronal loss in all SCA patients (Table 2). The pedunculopontine nucleus was involved in all of our SCA2 and SCA3 patients and mostly showed a severe loss of its cholinergic neurons (Supplementary Fig. 1 and Table 2).

The subthalamic nucleus was affected by neuronal loss in all SCA2 and in eight of nine SCA3 patients (Figs 4, 5 and Table 2). Neuronal loss of the subthalamic nucleus was either confined to or clearly more severe in its dorsolateral motor territory than in its medial limbic and ventrolateral associative territories (Figs 4, 5 and Table 2) (Benarroch, 2008; Lambert et al., 2012). In all of our SCA patients no neuronal loss could be observed in the caudate nucleus and putamen. In all basal ganglia nuclei of the SCA2 and SCA3 patients studied a concomitant reactive astrogliosis was present, while alpha-synuclein immunopositive neuronal Lewy bodies and Lewy neurites were completely absent.

Calculation of the weighted kappa coefficient revealed a high inter-rater reliability and an almost perfect agreement between the semi-quantitative assessments of neuronal loss in the basal ganglia nuclei studied between the authors U.R. and K.S. (κw = 0.82; P < 0.0001).

The medical records of our patients SCA revealed that only the SCA3 patient with a well-preserved subthalamic nucleus and destroyed compact part of the substantia nigra (Patient 7; Supplementary Table 2) suffered from parkinsonian motor features during his lifetime. In this patient a 4 H resting tremor responding to L-DOPA and trihexyphenidyl was initially diagnosed at age 44 years.

Discussion
We performed an in vivo neuroimaging study of the mesostriatal dopamine system in patients with SCA2, SCA3 and idiopathic Parkinson’s disease as well as a post-mortem pathoanatomical study of the dopaminergic substantia nigra and associated basal ganglia nuclei in an additional, independent cohort of patients with SCA2 and SCA3. Our PET investigations of SCA2 and SCA3 patients revealed a pronounced reduction of dopamine transporter levels in the striatum and largely unaffected postsynaptic striatal D2 receptors resembling typical findings in patients with idiopathic Parkinson’s disease (Shinotoh et al., 1997; Boesch et al., 2004; Varrone et al., 2004; Wüllner et al., 2005).
In spite of these remarkable changes in the motor mesostriatal pathway, only a minority of the SCA2 and SCA3 patients studied by PET suffered from motor signs of parkinsonism. The short disease duration of most of these SCA patients of <10 years shows that degeneration of the dopaminergic substantia nigra and the resulting nigrostriatal dopaminergic deficit are not restricted to late disease stages of the disease.

In accordance with previous studies, our pathoanatomical assessment revealed an extensive loss of melanin-containing dopaminergic neurons in the dopaminergic substantia nigra of SCA2 and SCA3 patients (Dürr et al., 1995, 1996; Estrada et al., 1999; Schöls et al., 2004; Seidel et al., 2012; Rüb et al., 2013), which was comparable to or even greater than in the advanced clinical stage of idiopathic Parkinson’s disease corresponding to Braak stages 5 and 6 (Braak et al., 2003a). However, in the SCA2 and SCA3 patients of our post-mortem study nigral neuronal loss was accompanied by neurodegeneration of the pallidum and the pallidal territory of the thalamus (i.e. thalamic ventral anterior nucleus). Furthermore, nerve cell loss was also present in the brain nuclei of our SCA2 and SCA3 patients that represent acknowledged targets for stereotactic lesions and deep brain stimulation to ameliorate parkinsonian motor symptoms in patients with idiopathic Parkinson’s disease (i.e. cholinergic pedunculopontine nucleus; cerebellar territory of the thalamus including its ventro-intermediate nucleus; and glutamatergic subthalamic nucleus with its three functionally and connectionally defined territories) (Hirai and Jones, 1989; Krack et al., 2002; Hamani et al., 2004; Breit et al., 2006; Benarroch, 2008; Obeso et al., 2008; Fasano et al., 2012; Lambert et al., 2012; Fournier-Gosselin et al., 2013). The subthalamic nucleus underwent severe neuronal loss in all SCA patients except one SCA3 patient, who suffered from parkinsonian motor features during his lifetime. Neuronal
The severe neuronal loss in the dopaminergic substantia nigra represents the unifying hallmark of all currently known parkinsonian syndromes (e.g. Parkinson’s disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration) (Rüb et al., 2014) and according to our and others experience, its extent in our SCA2 and SCA3 patients was definitively sufficient to provoke clinically relevant Parkinsonism (Rüb et al., 2014). Accordingly, the question arises which compensatory mechanisms are operating in the brains of SCA2 and SCA3 patients and help to escape Parkinsonism despite severe nigral neuronal loss.

Conceivably, the parallel development of cerebellar symptoms (e.g. hypotonia) may be among such potential compensatory mechanisms suitable to prevent parkinsonism. However, our following findings may argue against a compensatory effect of cerebellar disease symptoms as a universally valid pathophysiological explanation for the striking discrepancy between the severe impairments in the dopaminergic nigrostriatal pathway and the rare manifestation of parkinsonian motor features in SCA2 and SCA3: (i) The presence or absence of motor parkinsonian features in our SCA2 and SCA3 patients studied in vivo was not correlated with the clinical severity of ataxic symptoms. (ii) Despite a clear presynaptic dopamine transporter deficit, the SCA3 patients of our PET study with only mild ataxic symptoms and low scores on the Scale for the Assessment and Rating of Ataxia (SARA) did not suffer from concomitant or concurrent parkinsonism (Schmitz-Hübsch et al., 2006). (iii) Parkinsonian motor symptoms were present in the SCA3 patient of our PET study with the highest SARA score. (iv) The only SCA patient of our post-mortem study with manifest parkinsonian motor features suffered from early occurring and progressive cerebellar symptoms and developed a
4 H resting tremor, which was confirmed by means of electromyographical investigations 1 year before his death.

PET findings differ between patients with idiopathic Parkinson’s disease and those with SCA2 and SCA3 in the caudate-putaminal distribution of dopamine transporter signalling. Whereas patients with idiopathic Parkinson’s disease showed the well-known rostro-caudal gradient, SCA2 and SCA3 patients presented a more homogeneous loss of dopamine transporter binding within the striatum. This difference resulted from a more severe reduction of dopamine transporter signalling in the caudate nucleus of the SCA2 and SCA3 patients. However, it is important to see that the loss of putaminal dopamine transporter was similar between SCA2 and SCA3 and idiopathic Parkinson’s disease patients. We do not believe that the pronounced loss of dopamine transporter binding in the caudate nucleus can explain the lack of parkinsonian motor symptoms in most patients with SCA2 and SCA3 as caudate dopamine transporter binding decreases in the course of idiopathic Parkinson’s disease as well and is most pronounced in advanced stages of idiopathic Parkinson’s disease. Additionally, Varrone et al. (2004) reported similar findings in patients with SCA2 parkinsonism. However, the caudate-putaminal gradient may show variable expression in SCA2 and SCA3 patients as other groups found a rostro-caudal gradient typical for idiopathic Parkinson’s disease in

Figure 5 The glutamatergic subthalamic nucleus in SCA2 and SCA3. (A) Frontal section at the level of the rostral cerebral peduncle (CP) of a healthy 21-year-old male control individual depicting the cigar-shaped glutamatergic subthalamic nucleus (STN): (1) limbic, (2) associative, and (3) motor territories of the STN. (B) Severe neuronal loss in the subthalamic nucleus of a clinically diagnosed and genetically confirmed SCA2 patient without motor parkinsonism (Patient 1; Supplementary Table 2 and Table 2) and (C) in the subthalamic nucleus of a clinically diagnosed and genetically confirmed SCA3 patient without motor parkinsonism (Patient 8; Supplementary Table 2 and Table 2). (B and C) Note that the dorsolateral motor territory (asterisks) of the subthalamic nucleus is predominantly affected by nerve cell loss in both SCA patients. Their medial limbic and ventrolateral associative territories are widely spared by neuronal loss (Benarroch, 2008; Lambert et al., 2012). (D) Intact STN without discernable neuronal loss of a clinically diagnosed and genetically confirmed SCA3 patient with motor parkinsonian features (Patient 7; Supplementary Table 2 and Table 2). (A–D) Aldehyde-fuchsin Darrow red staining; 100 μm polyethylene glycol sections.
limited numbers of patients with SCA2 (Furtado et al., 2002; Yen et al., 2002).

As the extent and distribution of neurodegeneration in the thalamic ventral anterior and ventral lateral nuclei, internal and external pallidum, and in the pedunculopontine nucleus did not discriminate unequivocally between the SCA patients of our post-mortem study with or without parkinsonian motor features, the key to a plausible pathophysiological explanation of the clinical phenomenon that most SCA2 and SCA3 patients could escape parkinsonism despite severe nigral neurodegeneration could lie in the characteristic topographical distribution of neurodegeneration in the subthalamic nucleus in SCA2 and SCA3. While selectively spared in our SCA3 patient with a 4 H resting tremor, the subthalamic nucleus of all other SCA3 and SCA2 patients without parkinsonian motor features underwent a predominant severe neuronal loss, which was either confined to or clearly more severe in its motor territory than in its limbic and/or associative territories (Figs 4 and 5) (Hamani et al., 2004; Benarroch, 2008; Lambert et al., 2012).

The subthalamic nucleus subserves multiple functional aspects in the human brain and exhibits a rhythmic activity at both beta (13–30 Hz) and tremor (4–10 Hz) frequencies in patients with idiopathic Parkinson’s disease. Based on its different intracerebral connections and on functional neuroanatomical criteria the subthalamic nucleus, like other basal ganglia nuclei (e.g. striatum), is subdivided into motor, associative and limbic territories (Fig. 4). The motor territory of the subthalamic nucleus plays a crucial role in the motor basal ganglia circuits, its ventrolateral associative territory is involved in oculomotor control and cognitive aspects of motor behaviour, and its limbic territory serves motivational and emotional aspects of motor behaviour (Hamani et al., 2004; Benarroch, 2008; Obeso et al., 2008; Lambert et al., 2012). This neuroanatomically based subdivision of the subthalamic nucleus is reflected by the differential clinical outcome of patients with idiopathic Parkinson’s disease after treatment of the subthalamic nucleus, which depends on the chosen location of the interventions within this nucleus. While selective high frequency stimulation or neurosurgical stereotactic lesions of the motor territory of the subthalamic nucleus can ameliorate motor parkinsonian symptoms in idiopathic Parkinson’s disease patients (Hamani et al., 2004; Benarroch, 2008; Lambert et al., 2012) and the absence of clinical parkinsonism in SCA2 and SCA3 patients with a severe nigral neuronal loss and a concomitant selective neurodegeneration of the motor territory of the subthalamic nucleus point to a common pathophysiological explanation of both phenomenon, i.e. selective targeting of the motor territory of the subthalamic nucleus can exert an anti-parkinsonian effect and is capable to counteract and to prevent the manifestation of parkinsonian motor features in patients with SCA2, SCA3 and idiopathic Parkinson’s disease despite an even severe neurodegeneration of the dopaminergic compact part of the substantia nigra.

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Supplementary material

Supplementary material is available at Brain online.

References


