Evolution of striatal degeneration in McLeod syndrome

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Abstract

McLeod neuroacanthocytosis syndrome is an X-linked multisystem disorder with central nervous system manifestations resembling Huntington disease. Neuroimaging studies revealed striatal atrophy with predominance of the caudate nucleus. Our previous cross-sectional magnetic resonance imaging study showed an association of volume loss in the caudate nucleus and putamen with the disease duration. In the present study, we longitudinally examined three brothers with genetically confirmed diagnosis of McLeod syndrome using an observer-independent and fully-automated subcortical segmentation procedure to measure striatal volumes. In comparison with 20 healthy age-matched control males, the volumes of the caudate nucleus of the three patients were significantly smaller as confirmed by z-score transformations. On an individual basis, volumes in the two more severely affected and older patients were smaller than in the less affected, younger brother. Longitudinal MRI-based measurements over seven years demonstrated a significant decrease of both caudate nucleus volumes in each patient. Our findings indicate that structural magnetic resonance imaging combined with fully-automated computational morphometric analyses represents an objective and observer-independent imaging tool for the representation of progressive striatal degeneration in McLeod syndrome and might be a valuable methodology for cross-sectional as well as longitudinally volumetric studies in other rare neurodegenerative diseases, even on individual patients.

Keywords: McLeod syndrome · neuroacanthocytosis · striatal degeneration · longitudinal MRI-based volumetry · automated subcortical segmentation
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Introduction

McLeod neuroacanthocytosis syndrome (MLS) is an X-linked multisystem disorder which is characterized by the association of erythrocyte acanthocytosis with central nervous system (CNS) symptoms resembling Huntington disease as well as neuromuscular and cardiac abnormalities [1,2]. CNS symptoms are caused by striatal dysfunction and neurodegeneration and include chorea, other hyperkinetic movement disorders, psychiatric abnormalities, and cognitive decline as well as epileptic seizures [1,3]. Neuropathological examinations revealed unspecific neuronal loss and astrocytic gliosis of the caudate nucleus, putamen and, to a lesser degree, of the globus pallidum, but not of cortex, thalamus, subthalamic nucleus, or brainstem [4,5]. Structural neuroimaging studies of the brain including computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated atrophy of the caudate nucleus and the putamen at a variable degree [3]. In line with these findings, positron emission tomography (PET) studies in MLS invariably revealed impaired striatal glucose metabolism with reduced 2-fluoro-2-deoxy-glucose (FDG) uptake in caudate nucleus and anterior putamen, even in patients without psychiatric or movement abnormalities and in female heterozygotes [3,6].

MRI-based volumetry and quantitative PET examinations in a series of MLS patients suggested that striatal atrophy and glucose hypometabolism were more pronounced in patients with longer disease duration [3]. However, the progression of striatal neurodegeneration has not yet been followed by serial neuroradiological examinations. In this study we aimed at assessing the evolution of striatal degeneration by longitudinal MRI-based volumetry using a fully-automated and observer-independent subcortical segmentation procedure in order to associate these volumetric findings with the clinical disease course.
Patients and Methods

Subjects

We examined three brothers who have been suffering from McLeod syndrome originating from the German-speaking part of Switzerland. Their clinical, neuroimaging, and genetic findings have been presented in part in previous studies [3,7]. All patients carried the McLeod blood group phenotype characterized by absent Kx erythrocyte antigen and weak expression of Kell antigens, had erythrocyte acanthocytosis, and elevated serum creatine kinase (CK) levels ranging from 600 to 2300 U/L (normal for our laboratory, < 270). All patients harbouring the identical mutation of the XK gene, namely a point mutation in exon 3 (977C>T), which introduces a premature stop codon in the eighth of ten putative transmembrane domains of the XK protein [3].

Magnetic Resonance Imaging

The three MLS patients (mean age across both time points 44.8 years, standard deviation 4.9 years) were examined two times within an interval of seven years on a 1.5 T MRI system (General Electric Medical Systems, Milwaukee, Wisconsin). A T1-weighted sequence [3D spoiled gradient echo, repetition time (TR) = 50 msec, echo time (TE) = 9 msec, flip angle (FA) = 45°, matrix 256 x 256 pixels, in-plane resolution = 0.94 x 0.94 mm, slice thickness = 2 mm] was applied to acquire structural MR images with a high grey-white matter contrast in all three MLS cases. 20 healthy age-matched control males (mean age 45.1 years, standard deviation 9.4 years) were examined one time in order to compare the striatal and pallidal volumes of the MLS patients with that of the healthy males. These control subjects were scanned with a similar pulse sequence as the patients, but on a different MRI system (3.0 T Philips Intera whole body scanner; Philips Medical Systems, Best, The Netherlands). A volumetric 3D T1-weighted turbo field echo scan was obtained with a measured spatial
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resolution of 1 x 1 x 1.5 mm³ (acquisition matrix 224 x 224 pixels, 180 slices). Further imaging parameters were: Field of view FOV = 220 x 220 mm², echo-time TE = 2.3 ms, repetition-time TR = 20 ms, flip-angle FA = 20°.

Automated Segmentation Procedure and Volumetry

Cortical surface reconstruction and volumetric segmentation was performed with the FreeSurfer image analysis suite, which is documented and freely available for download online (http://surfer.nmr.mgh.harvard.edu/). The automated methods for measuring the volumes of the different brain structures are described in more detail elsewhere [8-13]. These procedures automatically assign a neuroanatomical label to each voxel in an MRI volume. This assignment is based on probabilistic information automatically estimated from a manually labeled training set. The training set is comprised by healthy persons in the age range 18–87 years and a group of Alzheimer’s disease patients in the age range 60–87 years. Briefly, the volumetric segmentation is realized in the following steps. First, a linear transformation is computed that maximizes the likelihood of the input image relative to an atlas constructed from manually labeled images. Second, a nonlinear transformation is estimated and the image is further deformed to better match the atlas. Third, segmentation is carried out following a Bayesian theorem, and the maximum a posteriori (MAP) probability of the labeling is computed.

This segmentation procedure takes into account three different kinds of information in order to disambiguate labels: (1) the prior probability that a given tissue class occurs at a specific location in the atlas, (2) the likelihood of the image given that tissue class, and (3) the probability of the local spatial configuration of labels given the tissue class. This latter term represents constraints on the space of allowable segmentations, and prohibits label configurations that never occur in the training set (e.g., the hippocampus is never located
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anterior to the amygdala). This technique has previously been shown to be comparable in
accuracy to manual labeling [8]. The segmentations were visually inspected for accuracy, and
none of the subjects and no segmentation had to be excluded. Finally, the volumes of the
labeled subcortical structures as well as global cortical measures were computed based on the
segmentations.

Statistics

In order to derive individual z-scores and corresponding p-values of the volumetric measures
we compared the three MLS patients with 20 male control subjects. We are aware that the
optimal control group should be comprised of healthy subjects scanned also twice within the
same time interval on the same MR system as used for measuring the patients. Unfortunately,
such control subjects were not available. From the control subjects, we derived the mean
volume and standard deviation of the subcortical structures and global cortical measures in
order to z-transform the patients’ volumetric measures and assign error probabilities to this z-
values. The z-scores and error probabilities are corrected for intracranial volume. To control
for potential confounding inter-scanners effects such as different geometric distortions, we
also used 10 control subjects scanned on the same 1.5 T systems as the patients. These
subjects (6 males; mean age 70.0 years, standard deviation 5.1 years) were significantly older
than the MLS patients. However, this analysis (results not shown) revealed similar results as
the comparisons with the age-matched control group. Hence, there were no inter-scanner
effects that confound the measured volumes.
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Results

Clinical findings

The oldest brother (patient IV-4) had a first psychotic depression when he was 26 years old. Thereafter, he had recurrent episodes of severe depression necessitating antidepressive medication and one hospitalization in a psychiatric ward. At the age of 49 years, the first neurological examination revealed absent tendon reflexes but no other abnormalities, in particular no clear atrophy, weakness or movement disorders. Formal cognitive testing was normal [3]. At the time of the second examination three years later, the patient had developed marked motor restlessness. Seven years later, at the time of the second MRI acquisition, he had moderate generalized chorea but no other movement disorders. Tendon reflexes were absent, plantar responses were flexor, but there were no sensory disturbances. He had a slight general muscular hypotrophy but no overt muscular palsies. Cardiac examination including holter electrocardiography (ECG) and echocardiography revealed no abnormalities [14].

The middle brother (patient IV-6) was reported to have motor restlessness and a tic-disorder developing after the age of 20 years which had been interpreted in the context of a primary psychiatric disorder by the treating physicians. The first neurological examination at age 40 years revealed a schizotypal personality disorder, moderate generalized chorea, and moderate subcortical cognitive deficits [3]. Tendon reflexes were absent and there was a slight general muscular atrophy but no overt muscular palsies. Cardiac examination including holter electrocardiography (ECG) and echocardiography revealed no abnormalities [14].

Neurological evaluation at the age of 44 years documented worsening of chorea and cognitive deficits as well as the muscular atrophy. In addition, a feeding dystonia developed with consecutive weight loss and a nutritional therapy as well as social support was initiated. The last neurological examination at the age of 49 years documented a clinically stable situation.
The youngest brother (patient IV-7) was reported to develop personality changes after the age of 25 years. The first neurological examination at the age of 35 years revealed mild motor restlessness and absent tendon reflexes. Formal cognitive testing was normal [3]. Cardiac examination including holter ECG and echocardiography revealed no abnormalities [14]. The last neurological examination at the age of 42 years demonstrated mild to moderate generalized chorea without evidence for other movement disorders, muscular atrophy, or muscular palsies.

**Structural MRI findings**

Visual interpretation of the first MRI scan revealed mild caudate head and putamen atrophy without pathological signal alterations in patient IV-6. There were no gross abnormalities of the cerebral cortex, the white matter, thalamus, brain stem and cerebellum. The second cerebral MRI scan was performed 7 years after the first examination. Visual inspection revealed a moderate atrophy of the head of the caudate nucleus in patients IV-4 and IV-6 as well as a slight atrophy of this structure in patient IV-7 with consecutive enlargement of the lateral ventricles. The other cerebral structures appeared to be normal on visual inspection.

**MRI volumetry findings**

After segmentation of the different neural compartments (Figure 1), MRI-based volumetry was performed on the segmentations of global cerebral grey matter, global cerebral white matter, caudate nucleus, putamen, pallidum, thalamus, and on the cerebrospinal fluid (CSF) of the lateral ventricle. Comparison of the grey matter volumes (corrected for intracranial volume) of the different compartments demonstrated a decrease of the volume of the caudate nucleus with a consecutive increase of the CSF volume over the observation period in all three patients (Figure 2). These volume reductions were statistically significant when
Striatal degeneration in McLeod syndrome compared with the volumes of the control group as revealed by z-score transformations (Table 1). On a global scale and compared with 20 control males, cortical grey matter volume was slightly yet significantly reduced in two patients (IV-4 and IV-6). Cortical white matter volume did not differ between patients and controls. Volumes of putamen were significantly smaller in the patients compared with the control subjects, irrespective whether or not we controlled for intracranial volume. In the most severely affected patient (IV-4), the volumes of both thalami appeared to be significantly reduced at the second time point. In the patients, using Wilcoxon’s signed ranks tests, we found a volume reductions of the caudate nucleus, which, however, was not statistically significant (for both caudate nuclei: z = -1.604, p = 0.055, one-tailed). The volumes of the putamen and pallidum did not show significant changes between the two time points in all three patients (z = -1.604, p = 0.109, two-tailed).
Discussion

We describe the natural progressive course of striatal atrophy in a small series of patients with McLeod neuroacanthocytosis syndrome (MLS). Although only three individual patients were available for follow-up examinations, we were able to demonstrate a significant decrease of the caudate nucleus volumes over a time period of seven years using a fully-automated segmentation analysis and volumetry based on structural MRI. This methodology implemented in the FreeSurfer software suite was previously evaluated against manual labelling [8] and was successfully used in cerebral volumetric studies in normal aging, Huntington disease, schizophrenia, mesial temporal lobe epilepsy, and the effects of prenatal poly-substance exposure and early human diet on brain structures [15-20].

Several neuroimaging and pathological studies in MLS revealed atrophy of caudate nucleus and putamen [3,5]. Our previous cross-sectional study showed an association of volume loss in the caudate nucleus and putamen with the disease duration [3]. The present longitudinal volumetric analysis demonstrated a significant volume loss of the caudate nucleus of the MLS patients in comparison to the healthy control subjects, even though the mean age of the control group was higher. Although interpretation is limited due to the small number of patients examined we observed that the patients with the more pronounced neurological signs and symptoms, i.e., the clinically more affected and older patients, had smaller volumes of the caudate nucleus compared to their younger and less affected brother.

Volumes of putamen and pallidum bilaterally were significantly smaller in the three patients compared to the controls without significant volume change over the observation period of seven years. In contrast to previous neuroimaging and pathological findings in MLS, which did not demonstrate extrastriatal pathological alterations, we found evidence for a global volume reduction of grey matter [3,5]. Also a previous magnetic resonance spectroscopy (MRS) study showed subtle metabolic alterations in cortical areas corresponding...
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to the symptoms of the patients [21]. Therefore, minor extrastriatal pathology might be
present in MLS. Based on the neuropathological data available, however, these alterations
seem to be much less pronounced compared to Huntington disease (HD) [4,5,22]. Taken
together and in line with the estimated disease duration of the MLS ranging between 20 and
30 years, our findings reflect a slowly progressive neurodegenerative process affecting
predominantly the caudate nucleus [1,3].

Volumetric imaging studies focusing on the basal ganglia have been performed in
other neurodegenerative choreatic disorders, namely in HD. Using manual labelling, HD
patients had smaller volumes of caudate nucleus and putamen, not only in symptomatic
patients but also in presymptomatic disease stage [23]. After disease onset, volumes of frontal
cortex were also found to be reduced [24]. Longitudinal MRI studies showed significant
decreases of the caudate volume over a period of only 10 months [25,26]. The pattern of
atrophy with predominance of the head of the caudate nucleus is similar in HD compared to
MLS, but disease progression appears to be faster in HD. In the studies with HD patients,
however, manual labelling was used, and significantly higher numbers of patients had to be
examined to obtain significant results.

Our findings are in line with the results of a recent cross-sectional MRI-based
volumetric study in patients with choreoacanthocytosis [27] at single subject level and
indicate that the fully automated, subcortical segmentation procedure used in the present study
represents an objective and observer-independent imaging tool for representation of
progressive striatal degeneration in MLS patients, even on individual patients. Moreover, this
methodology might be valuable for volumetric studies in other rare neurodegenerative
disorders and should be considered as a promising alternative approach instead of the time
consuming and error-prone manual in-vivo morphometry.
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Contributions

Research project: A. Conception (JH, MM, HHJ), B. Organization (POV, JH, MM, HHJ), C. Execution (POV, JH);

Statistical Analysis: A. Design (JH, MM), B. Execution (JH, MM), C. Review and Critique (POV, JH, MM, HHJ);

Manuscript: A. Writing of the first draft (POV, HHJ), B. Review and Critique (POV, JH, MM, HHJ).
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References


Table 1  Volumes of subcortical structures, cerebrospinal fluid, and cortical grey and white matter

Shown are the absolute volumes of the structures of interest in the three patients and the mean volumes of a control group consistent of 20 age-matched males. The z-scores and error probabilities (p) are derived from the comparison of the three patients’ volumes at each time point with the mean volumes of these structures in the control group. These values are corrected for intracranial volume. Significant p-values are printed in bold and statistical trends toward significance (0.05 < p < 0.10) are printed in italic. Note that cortical grey and white matter does not include the cerebellar volumes.
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Figure Legends

Figure 1  Automated subcortical segmentations
Raw structural T1-weighted MR images and the corresponding subcortical parcellations of the brain of patient IV-4 demonstrate a normal aspect of the caudate nucleus and putamen at the time of the first examination (left panel) and moderate atrophy of the caudate nucleus seven years later with consecutive enlargement of the lateral ventricles (right panel).

Figure 2  Caudate nuclei volumes
The volumes of the caudate nuclei are decreased in each patient within the study period of seven years. In addition, there is an absolute and significant decrease in caudate nucleus volumes compared to 20 age-matched control subjects as revealed by z-score transformations. The z-scores and error probabilities (p) are derived from the comparison of three patients’ caudate volumes at each time point with the mean caudate volumes of the control group. * p < 0.05, ** p < 0.01, *** p < 0.001, ♦ 0.05 < p < 0.1.
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246x150mm (72 x 72 DPI)
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Abstract

Background: McLeod neuroacanthocytosis syndrome is an X-linked multisystem disorder with central nervous system manifestations resembling Huntington disease. Neuroimaging studies revealed striatal atrophy with predominance of the caudate nucleus. Our previous cross-sectional magnetic resonance imaging study showed an association of volume loss in the caudate nucleus and putamen with the disease duration.

Patients and Methods: In the present study, we examined three brothers with genetically confirmed diagnosis of McLeod syndrome using an observer-independent and fully-automated subcortical segmentation procedure to measure striatal volumes.

Results: In a cross-sectional comparison with 20 healthy age-matched control males, the volumes of the caudate nucleus of the three patients were significantly smaller as confirmed by z-score transformations. On an individual basis, volumes in the two more severely affected and older patients were smaller than in the less affected, younger brother. Longitudinal MRI-based measurements over seven years demonstrated a statistical trend toward significant decreased caudate volumes in McLeod patients.

Conclusions: Our findings indicate that structural magnetic resonance imaging combined with fully-automated computational morphometric analyses represents an objective and observer-independent imaging tool for the representation of progressive striatal degeneration in McLeod syndrome and might be a valuable methodology for cross-sectional as well as longitudinally volumetric studies in other rare neurodegenerative diseases, even on individual patients.

Keywords: McLeod syndrome · neuroacanthocytosis · striatal degeneration · longitudinal MRI-based volumetry · automated subcortical segmentation
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Introduction

McLeod neuroacanthocytosis syndrome (MLS) is an X-linked multisystem disorder which is characterized by the association of erythrocyte acanthocytosis with central nervous system (CNS) symptoms resembling Huntington disease as well as neuromuscular and cardiac abnormalities [1,2]. CNS symptoms are caused by striatal dysfunction and neurodegeneration and include chorea, other hyperkinetic movement disorders, psychiatric abnormalities, and cognitive decline as well as epileptic seizures [1,3]. Neuropathological examinations revealed unspecific neuronal loss and astrocytic gliosis of the caudate nucleus, putamen and, to a lesser degree, of the globus pallidum, but not of cortex, thalamus, subthalamic nucleus, or brainstem [4,5]. Structural neuroimaging studies of the brain including computed tomography (CT) and magnetic resonance imaging (MRI) demonstrated atrophy of the caudate nucleus and the putamen at a variable degree [3]. In line with these findings, positron emission tomography (PET) studies in MLS invariably revealed impaired striatal glucose metabolism with reduced 2-fluoro-2-deoxy-glucose (FDG) uptake in caudate nucleus and anterior putamen, even in patients without psychiatric or movement abnormalities and in female heterozygotes [3,6].

MRI-based volumetry and quantitative PET examinations in a series of patients with MLS suggested that striatal atrophy and glucose hypometabolism were more pronounced in patients with longer disease duration [3]. However, the progression of striatal neurodegeneration has not yet been followed by serial neuroradiological examinations. In this study we aimed at assessing the evolution of striatal degeneration by longitudinal MRI-based volumetry using a fully-automated and observer-independent subcortical segmentation procedure in order to associate these volumetric findings with the clinical disease course.
Patients and Methods

Subjects

This prospective study was conducted at the Department of Neurology of the University Hospital and the Institute of Psychology of the University of Zurich. The study protocol was approved by the local ethics committee, and all patients gave written informed consent.

We examined three brothers who have been suffering from MLS originating from the German-speaking part of Switzerland. Their clinical, neuroimaging, and genetic findings have been presented in part in previous studies [3,7]. All patients carried the McLeod blood group phenotype characterized by absent Kx erythrocyte antigen and weak expression of Kell antigens, had erythrocyte acanthocytosis, and elevated serum creatine kinase (CK) levels ranging from 600 to 2300 U/L (normal for our laboratory, < 270). All patients harboured the identical mutation of the \( XK \) gene, namely a point mutation in exon 3 (977C>T), which introduces a premature stop codon in the eighth of ten putative transmembrane domains of the XK protein [3].

Magnetic Resonance Imaging

The three patients (mean age across both time points 44.8 years, standard deviation 4.9 years) were examined two times within an interval of seven years on a 1.5 T MRI system (General Electric Medical Systems, Milwaukee, Wisconsin). A T1-weighted sequence [3D spoiled gradient echo, repetition time (TR) = 50 msec, echo time (TE) = 9 msec, flip angle (FA) = 45°, matrix 256 x 256 pixels, in-plane resolution = 0.94 x 0.94 mm, slice thickness = 2 mm] was applied to acquire structural MR images with a high grey-white matter contrast in all three patients. 20 healthy age-matched control males (mean age 45.1 years, standard deviation 9.4 years) were examined one time in order to compare the striatal and pallidal volumes of the MLS patients with that of the healthy males. These control subjects were scanned with a
similar pulse sequence as the patients, but on a different MRI system (3.0 T Philips Intera
whole body scanner; Philips Medical Systems, Best, The Netherlands). A volumetric 3D T1-
weighted turbo field echo scan was obtained with a measured spatial resolution of 1 x 1 x 1.5
mm³ (acquisition matrix 224 x 224 pixels, 180 slices). Further imaging parameters were: Field
of view FOV = 220 x 220 mm², echo-time TE = 2.3 ms, repetition-time TR = 20 ms, flip-
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Automated Segmentation Procedure and Volumetry

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FreeSurfer image analysis suite, which is documented and freely available for download
online (http://surfer.nmr.mgh.harvard.edu/). The automated methods for measuring the
volumes of the different brain structures are described in more detail elsewhere [8-13]. These
procedures automatically assign a neuroanatomical label to each voxel in an MRI volume.

This assignment is based on probabilistic information automatically estimated from a
manually labeled training set. The training set is comprised by healthy persons in the age
range 18–87 years and a group of Alzheimer’s disease patients in the age range 60–87 years.

Briefly, the volumetric segmentation is realized in the following steps. First, a linear
transformation is computed that maximizes the likelihood of the input image relative to an
atlas constructed from manually labeled images. Second, a nonlinear transformation is
estimated and the image is further deformed to better match the atlas. Third, segmentation is
carried out following a Bayesian theorem, and the maximum a posteriori (MAP) probability
of the labeling is computed.

This segmentation procedure takes into account three different kinds of information in
order to disambiguate labels: (1) the prior probability that a given tissue class occurs at a
specific location in the atlas, (2) the likelihood of the image given that tissue class, and (3) the
probability of the local spatial configuration of labels given the tissue class. This latter term represents constraints on the space of allowable segmentations, and prohibits label configurations that never occur in the training set (e.g., the hippocampus is never located anterior to the amygdala). This technique has previously been shown to be comparable in accuracy to manual labeling [8]. The segmentations were visually inspected for accuracy, and none of the subjects and no segmentation had to be excluded. Finally, the volumes of the labeled subcortical structures as well as global cortical measures were computed based on the segmentations.

**Statistics**

In order to derive individual z-scores and corresponding p-values of the volumetric measures we compared the three MLS patients with 20 male control subjects that were scanned only once (cross-sectional comparison). From these control subjects, we derived the mean volume and standard deviation of the subcortical structures and global cortical measures in order to z-transform the patients’ volumetric measures and assign error probabilities to this z-values. The z-scores and error probabilities are corrected for intracranial volume. For the comparison of the volume change over time, Wilcoxon’s signed ranks tests were applied (longitudinal comparison).

In order to control for potential confounding inter-scanners effects such as different tissue contrasts that might affect segmentation, we also used 10 control subjects scanned on the same 1.5 T GE MRI system and pulse-sequence as used for the patients. These subjects (6 males; mean age 70.0 years, standard deviation 5.1 years) were significantly (25 years) older than the MLS patients. However, this analysis (results not shown) revealed similar results as the comparisons with the 20 age-matched control subjects, the MR images of whom were acquired on a different MR system. Furthermore, we also computed the contrast-to-noise ratio.
(CNR), the most important quality measure for MR-based morphometric procedures, of the repeated as well as single time point MR acquisitions in order to control other than disease-related sources of measurement variability.

Results

Clinical findings

The clinical and epidemiological data of the three brothers are summarized on Table 1. Their medical histories, neurological findings and the results of ancillary tests are published elsewhere in more detail [2,3,7,14].

Structural MRI findings

Visual interpretation of the first MRI scan revealed mild caudate head and putamen atrophy without pathological signal alterations in patient IV-6. There were no gross abnormalities of the cerebral cortex, the white matter, thalamus, brain stem and cerebellum. The second cerebral MRI scan was performed 7 years after the first examination. Visual inspection revealed a moderate atrophy of the head of the caudate nucleus in patients IV-4 and IV-6 as well as a slight atrophy of this structure in patient IV-7 with consecutive enlargement of the lateral ventricles. The other cerebral structures appeared to be normal on visual inspection.

MRI volumetry findings

After segmentation of the different neural compartments (Figure 1), MRI-based volumetry was performed on the segmentations of global cerebral grey matter, global cerebral white matter, caudate nucleus, putamen, pallidum, thalamus, and on the cerebrospinal fluid (CSF) of the lateral ventricle. Comparison of the grey matter volumes (corrected for intracranial
volume) of the different compartments demonstrated a decrease of the volume of the caudate nucleus with a consecutive increase of the CSF volume over the observation period in all three patients (Table 2). These volume reductions were statistically significant when compared cross-sectionally with the volumes of the control group as revealed by z-score transformations (Table 2). On a global scale and compared with 20 age-matched control males, cortical grey matter volume was slightly yet significantly reduced in two patients (IV-4 and IV-6). Cortical white matter volume did not differ between patients and controls. Volumes of putamen were significantly smaller in the patients when cross-sectionally compared with the control subjects, irrespective whether or not we controlled for intracranial volume. In the most severely affected patient (IV-4), the volumes of both thalami appeared to be significantly reduced at the second time point and the volumes of both pallida were reduced at the first time point (cross-sectional comparison). In the MLS patients, using Wilcoxon’s signed ranks tests, we found a longitudinal volume reduction of the caudate nucleus, which, however, was statistically only significant on a trend level (for both caudate nuclei: \( z = -1.604, p = 0.055 \), one-tailed). The volumes of the putamen and pallidum did not show significant changes between the two time points in all three patients (\( z = -1.604, p = 0.109 \), two-tailed).

Potential sources of other than disease-related measurement variability were controlled for by using an additional control group scanned on the same scanner as the patients and by computing the contrast-to-noise ratio (CNR) of the different neural tissue classes of the repeated as well as single MR acquisitions. Total CNRs (CNR of grey-white and grey-CSF borders) of the three groups were CNR = 1.502 / 0.153 (mean / standard deviation) for the patients, CNR = 1.482 / 0.129 for the 10 elderly controls measured on the same scanner as the patients, and CNR = 1.483 / 0.182 for the 20 age-matched controls measured on a different scanner. These CNRs were neither significantly different between the two time points nor between the images acquired on different MRI systems.
Discussion

We describe the natural progressive course of striatal atrophy in a small series of patients with McLeod neuroacanthocytosis syndrome (MLS). Although only three individual patients were available for follow-up examinations, we were able to demonstrate a strong statistical trend ($p = 0.055$) towards significantly decreased caudate nucleus volumes over a time period of seven years using a fully-automated segmentation analysis and volumetry based on structural MR images. This methodology implemented in the FreeSurfer software suite was previously evaluated against manual labelling [8] and was successfully used in cerebral volumetric studies in normal aging, Huntington disease, schizophrenia, mesial temporal lobe epilepsy, and the effects of prenatal poly-substance exposure and early human diet on brain structures [15-20].

Several neuroimaging and pathological studies in MLS revealed atrophy of caudate nucleus and putamen [3,5]. Our previous cross-sectional study showed an association of volume loss in the caudate nucleus and putamen with the disease duration [3]. The volumetric analyses in the present study demonstrated a significant volume loss of the caudate nucleus of the patients in comparison with 20 healthy age-matched control subjects scanned once on a different MRI system and also in comparison with 10 healthy elderly subjects scanned once on the same MRI system as used for the patients. Furthermore, the longitudinal analysis revealed a statistical trend toward significant caudate volume reductions in the patients over the seven years period. Although interpretation is limited due to the small number of patients examined we observed that the patients with the more pronounced neurological signs and symptoms, i.e., the clinically more affected and older patients, had smaller volumes of the caudate nucleus compared to their younger and less affected brother.

Volumes of putamen and pallidum bilaterally were significantly smaller in the three patients compared cross-sectionally to the 20 age-matched controls without a significant
longitudinal volume change over the observation period of seven years. In contrast to previous neuroimaging and pathological findings in MLS, which did not demonstrate extrastriatal pathological alterations, we found evidence for a global volume reduction of grey matter [3,5]. Also a previous magnetic resonance spectroscopy (MRS) study showed subtle metabolic alterations in cortical areas corresponding to the symptoms of the patients [21]. Therefore, minor extrastriatal pathology might be present in MLS. Based on the neuropathological data available, however, these alterations seem to be much less pronounced compared to Huntington disease (HD) [4,5,22]. Taken together and in line with the estimated disease duration of the MLS ranging between 20 and 30 years, our findings reflect a slowly progressive neurodegenerative process affecting predominantly the caudate nucleus [1,3].

Volumetric imaging studies focusing on the basal ganglia have been performed in other neurodegenerative choreatic disorders, namely in HD. Using manual labelling, HD patients had smaller volumes of caudate nucleus and putamen, not only in symptomatic patients but also in presymptomatic disease stage [23]. After disease onset, volumes of frontal cortex were also found to be reduced [24]. Longitudinal MRI studies showed significant decreases of the caudate volume over a period of only 10 months [25,26]. The pattern of atrophy with predominance of the head of the caudate nucleus is similar in HD compared to MLS, but disease progression appears to be faster in HD. In the studies with HD patients, however, manual labelling was used, and significantly higher numbers of patients had to be examined to obtain significant results.

Potential sources of other than disease-related measurement variability, which might have been occurred due to the use of two different MRI systems, were controlled for in the following way: (i) We used an additional control group of 10 healthy elderly controls scanned once on the same MRI system with the same pulse sequence as used for the patients. Although the mean age of these control subjects was 25 years higher than the one of the
patients, their caudate volumes were still significantly larger compared with the caudate volumes of the patients, suggesting that volume differences due to normal healthy aging are very unlikely. (ii) We computed the contrast-to-noise ratio (CNR) of the different neural tissue classes of the repeated and single MR acquisitions. These CNRs were comparable between all acquired MRI scans; hence, changes due to MR system software/hardware changes during the 7-year period as well as MR system differences between vendor, field strength, and image contrast can be ruled out. The intrinsic test-retest reliability of the morphometric procedures used in our study was evaluated in prior publications [9,11,27].

Our findings are in line with the results of a recent cross-sectional MRI-based volumetric study in patients with choreoacanthocytosis [28] and indicate that the fully automated, subcortical segmentation procedure used in the present study represents an objective and observer-independent imaging tool for representation of progressive striatal degeneration in patients with MLS, even on an individual patient level. Moreover, this methodology might be valuable for volumetric studies in other rare neurodegenerative disorders and should be considered as a promising alternative approach instead of the time consuming and error-prone manual in-vivo morphometry.

**Acknowledgements**

We thank the patients and their families for the participation and S. Kollias and his team for performing the magnetic resonance imaging acquisitions.
Striatal degeneration in McLeod syndrome

Contributions

Research project: A. Conception (JH, MM, HHJ), B. Organization (POV, JH, MM, HHJ), C. Execution (POV, JH);

Statistical Analysis: A. Design (JH, MM), B. Execution (JH, MM), C. Review and Critique (POV, JH, MM, HHJ);

Manuscript: A. Writing of the first draft (POV, HHJ), B. Review and Critique (POV, JH, MM, HHJ).
References


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### Table 1  Clinical Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Presenting symptoms</th>
<th>Signs and symptoms at last examination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Age at onset, years)</td>
<td>(Age, years)</td>
</tr>
<tr>
<td>IV-4</td>
<td>Psychotic depression (26)</td>
<td>Depression</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slight generalized chorea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Areflexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slight muscular hypotrophy</td>
</tr>
<tr>
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<td></td>
<td>(57)</td>
</tr>
<tr>
<td>IV-6</td>
<td>Motor restlessness Tic-disorder (20)</td>
<td>Personality disorder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pronounced generalized chorea</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Subcortical cognitive deficits</td>
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<tr>
<td></td>
<td></td>
<td>Areflexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pronounced muscular hypotrophy and moderate weakness</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(47)</td>
</tr>
<tr>
<td>IV-7</td>
<td>Personality changes (25)</td>
<td>Personality disorder</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Motor restlessness</td>
</tr>
<tr>
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<td>Areflexia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(42)</td>
</tr>
</tbody>
</table>
Table 2  Volumes of subcortical structures, cerebrospinal fluid, and cortical grey and white matter

Shown are the absolute volumes of the structures of interest in the three patients and the mean volumes of a control group consistent of 20 age-matched males. The z-scores and error probabilities (p) are derived from the cross-sectional comparison of the three patients’ volumes at each time point with the mean volumes of these structures in the control group that was measured only once. These values are corrected for intracranial volume. Significant p-values are printed in bold and statistical trends toward significance (0.05 < p < 0.10) are printed in italic. Note that cortical grey and white matter does not include the cerebellar volumes.
Striatal degeneration in McLeod syndrome

Figure Legend

Figure 1  Automated subcortical segmentations

Raw structural T1-weighted MR images and the corresponding subcortical parcellations of

The brain of patient IV-4 demonstrate a normal aspect of the caudate nucleus and putamen at

The time of the first examination (left panel) and moderate atrophy of the caudate nucleus

Seven years later with consecutive enlargement of the lateral ventricles (right panel).
Reply to the reviewer

Evolution of striatal degeneration in McLeod syndrome

Philipp O. Valko, Jürgen Hänggi, Martin Meyer, Hans H. Jung

Dear Prof. Juvela,

Thank you very much to reconsider our above-mentioned revised manuscript for publication in European Journal of Neurology. Based on the comments of the reviewers we performed the following changes (changes in the manuscript are indicated with red color). In addition, we have now provided a structured abstract and mentioned that the study protocol was approved by the local ethics committee and written informed consent was obtained from each patient.

Reviewer #1

This paper estimates longitudinal brain morphometric changes in a group of three McLeod subjects from two measurements done 7 years apart. A cross-sectional comparison of morphometric results is done with a group of 20 age-matched controls (mean age 45 years) scanned using a different scanner system (vendor and field strength). The main result reported is that relative to the controls, caudate structures of the McLeod patients show significant reduced volumes both longitudinally and cross-sectionally.

The paper is clear and well written. The main methodological issue of this study is reported by the authors themselves: the group of controls should have ideally been scanned also twice, 7-years apart on the same MR system used for the patient group. The authors try to use a third dataset to support the equivalence of the measurement conditions (single time measure of a healthy group of subjects with mean age 70 years on the patient's scanner).

Ideally, the group of controls should have been scanned also twice 7-years apart on the same MR system and with the same pulse sequence as used for the patient group. Unfortunately, such data were not available.

To strengthen the claim that the observed effects are due mainly to the disease I would recommend that in the discussion section the authors discuss further the various sources of variability that may potentially confound the interpretation of the results:

We discuss the potential sources of variability in more details in the discussion section and explain why other than disease-related sources of measurement variability can be ruled out.

Interpretation of longitudinal results: longitudinal morphometric changes of the patient group could be due to several causes, including: changes specific to the disease, changes due to healthy aging (even with intracranial volume normalization), changes due to MR system software/hardware changes during the 7-year period, changes due to intrinsic test-retest reproducibility error.

Based on the observation that the caudate volumes of the patients were reduced, not only when compared with an age-matched control group of 20 male subjects (mean age 45 years) but also when compared with a group of 10 healthy older subjects (mean age 70 years), suggests that changes due to normal healthy aging are very unlikely.

In order to exclude confounding effects due to MR system software and hardware changes during the 7-years period, we computed the signal-to-noise ratio (SNR) and the contrast-to-noise ratio (CNR) of the MR images. These ratios, which are the most important quality measures for structural MRI, were comparable between the two MR images acquired seven years apart suggesting that the effects we reported are not influenced by scanner software and/or hardware modifications.
The intrinsic test-retest reliability of the morphometric procedure used in our study was evaluated in a prior publication (Han & Fischl, 2007, IEEE Transaction of Medical Imaging). This study revealed very high test-retest reproducibility. Therefore we believe that the McLeod disease solely drives the morphological changes we observed in the three patients.

**Interpretation of cross-sectional results:** morphometric differences between the controls and the patients could be related to the disease but also to MR system differences (vendor and field strength) and image contrast differences (pulse sequences). It has been reported in the literature that brain morphometry biases may appear when the same group of subjects are scanned using different MR system vendor, field strengths, and pulse sequences.

With respect to the MR system manufacturer the 1.5 T Signa scanner, on which the three patients and the 10 elderly controls were measured, is from GE and the 3.0 T Intera scanner, on which the 20 age-matched controls were measured, is from Philips. We used corresponding gradient echo pulse sequences on both MR systems. The most important quality measure with respect to grey-white matter segmentation is the contrast-to-noise ratio (CNR), the difference between the signal-to-noise ratio (SNR) of grey and white matter. As for the longitudinal data, we also computed the SNRs and CNRs of the T1-weighted images of the cross-sectional data. The CNRs are comparable between the two different field strength applied in the present study.

Furthermore, it has been shown that the segmentation as implemented in the FreeSurfer analysis suite (the software used in our study) is sequence-independent (Fischl et al., 2004, Neuroimage) and reliable across scanner platforms (Han & Fischl, 2007, IEEE Transaction of Medical Imaging). The issue of morphometry biases that may appear when the same subjects are scanned using different MR system vendor, field strengths, and pulse sequences, was systematically investigated for the FreeSurfer analysis suite. Focusing on other brain features than subcortical volumes it has been shown that cortical thickness can reliably be measured across scanner vendor, field strength, and pulse sequence using FreeSurfer analysis suite (Han et al., 2006, Neuroimage).

**Reviewer #2**

The authors studied 3 patients with the rare McLeod syndrome and performed volumetric brain MRI studies at an interval of 7 years. They compared the results with an age-matched control sample, which was however, scanned on one occasion at another institution. They validated this control group by comparing with an older control group from their own institution.

Not surprisingly, the major finding was that the caudate nuclei and putamina were significantly smaller than controls at both time points. There was also a progressive decrease in the size of the caudate nucleus in subjects between the 2 time points which did not reach significance. Cortical grey matter volume was decreased in 2 subjects.

The major problem is that a main point of the paper, as stated in the Title, Abstract, Results, and Discussion, is that there was a significant decrease in the size of the caudate nucleus in the McLeod patients. However, this appears from their statistics (p9) to be only a trend. Thus the last sentence of the Abstract is rather an overstatement.

We now use the term “statistical trend toward significant” in our revised manuscript. The error probability of the Wilcoxon’s signed ranks test (based on the sign/direction of the change and not on its magnitude) used for analysing longitudinal volumetric changes cannot reveal a p-value smaller than p = 0.055 (one-tailed) with only three patients. When adding an additional patient with caudate volumes reductions between the two time points to our
sample, the error probability would decrease to $p = 0.034$ (one-tailed). It is not the effect size of our data that prohibits significant results; it is merely the nature of the Wilcoxon’s signed ranks test itself.

**What is true was that at both time points the caudate volumes were less than controls, and that this difference was greater at the later time point, but this does not constitute a true longitudinal study.**

We agree with the reviewer that we do not present a “true” longitudinal study. However, our longitudinal statistical analysis of the three patients revealed an error probability of $p = 0.055$ (one-tailed). This is a statistical trend toward significance. As stated above, smaller $p$-values can only be reached with larger sample sizes ($n > 3$).

**It would have been informative to compare controls studied over the same time interval, and this might have yielded more statistically satisfying data.**

Ideally, the group of controls should have been scanned also twice 7-years apart on the same MR system and with the same pulse sequence as used for the patient group. Unfortunately, such data were not available.

**The MRI volumetry results section is confusing to read. The 2nd sentence of this section (p.8) suggests that there was a significant decrease in the size of the caudate between the 2 time points, but from the 2nd to last sentence (p.9) it appears that this is just a trend.**

Since there is just a statistical trend ($0.05 < p < 0.1$) toward significance we now clarified this in our revised manuscript.

**The last line on this page refers to "volume reductions", referring to the change with time, but as the controls were studied at a single time point, this cannot be accurate. It should be clarified that the significant comparison is with the controls.**

We clarified that the significant results were obtained in the cross-sectional comparison with the 20 age-matched controls measured once.

**Fig. 2 is redundant as it merely presents in graphical form the data in Table 1 (as far as I could tell).**

We agree and have removed Fig. 2.

**In the Discussion, they mention a change in the size of the pallidum, however this is not mentioned in the Results section nor the table.**

We now mention the change in the pallidum size in the Results section. This volume reduction was only apparent in the most severely affected patient. The absolute volumes and its ICV-corrected $p$-values are also indicated in Table 2.

**The clinical details for these patients have been published elsewhere and do not need to be presented here in detail, thus should be summarized**

According the suggestion of the reviewer, we summarized the presentation of the clinical findings in a table (new Table 1).

Thank you very much again for reconsidering our manuscript for publication.

With kind regards,

Hans H. Jung, MD

Zurich, 13.08.2009