Recessively Inherited Parkinsonism

Effect of ATP13A2 Mutations on the Clinical and Neuroimaging Phenotype

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Objective: To determine clinical features and to identify changes in brain structure and function in compound heterozygous and heterozygous ATP13A2 mutation carriers.

Design: Prospective multimodal clinical and neuroimaging study.

Setting: University of Lübeck, Lübeck, Germany.

Participants: Eight family members of a large Chilean pedigree with Kufor-Rakeb syndrome (KRS).

Interventions: Clinical characterization, dopamine transporter (DAT) imaging, voxel-based morphometry (VBM), and transcranial sonography (TCS).

Main Outcome Measures: Frequency of parkinsonian signs, brain structure, and functional alterations.

Results: The only available patient with compound heterozygous KRS showed a markedly reduced striatal DAT density bilaterally. Magnetic resonance imaging revealed severe global brain atrophy as well as iron deposition in the basal ganglia. The heterozygous mother had definite parkinsonism with reduced DAT density in both putamina. While all asymptomatic heterozygous siblings displayed subtle extrapyramidal signs, DAT imaging revealed striatal tracer uptake within physiological levels. Voxel-based morphometry revealed an increase in gray matter volume in the right putamen and a decrease in the cerebellum of the heterozygous carriers. In all mutation carriers, the substantia nigra had a normal appearance on TCS.

Conclusions: Single ATP13A2 heterozygous mutations may be associated with clinical signs of parkinsonism and contribute to structural and functional brain changes. Lack of hyperechogenicity in the substantia nigra may be a distinctive feature of this form of genetic parkinsonism. This, along with the finding of iron in the basal ganglia in our patient with KRS, implies a different underlying pathophysiology compared with other monogenic forms of parkinsonism and idiopathic PD and may place KRS among the syndromes of neurodegeneration with brain iron accumulation (NBIA).

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UFOR-RAKEB SYNDROME (KRS) is a rare autosomal recessive form of juvenile-onset parkinsonism with subacute levodopa-responsive bradykinesia, severe dementia, supranuclear gaze palsy, and spasticity.1,2 Recently, mutations in the ATP13A2 gene (PARK9 locus) were found to be responsible for KRS.3 Postmortem analysis of patients with idiopathic Parkinson disease (PD) indicated a 10-fold increased ATP13A2 expression in dopaminergic substantia nigra (SN) neurons compared with control brains, suggesting a possible role of this gene in the etiology also of sporadic, clinically typical PD.4

Single heterozygous mutations in other recessive PD genes such as Parkin (PARK2 locus) and PINK1 (PARK6 locus) have been discussed as susceptibility factors for late-onset PD5,6 and tend to be associated with a later age at PD onset than homozygous mutations.6 For ATP13A2, only 2 articles described a total of 5 patients with early-onset PD who harbored heterozygous ATP13A2 mutations.7,8

The central aim of the study was to determine whether heterozygous ATP13A2 mutations have a pathophysiological relevant effect on brain function. We used a multimodal approach and clinical and neuroimaging methods to shed light on the role of single ATP13A2 mutations. The clinical assessment comprised established motor and nonmotor features of PD. The neuroimaging part included structural and functional brain imaging. Second, we performed a multimodal examination in the affected patient for better characterization of KRS and classification among the atypical parkinsonian syndromes.
SUBJECTS AND CLINICAL EVALUATION

The 5 youngest siblings from a previously described noncon- sanguineous Chilean family with 17 children had typical KRS. Four died of pneumonia between 33 and 43 years of age. As part of the present study, a detailed clinical examination was performed in 5 ATP13A2-heterozygous mutation carriers (MCs), the compound heterozygous affected son (II.14), and 2 non–mutation carriers (NMCs) (Figure 1). After giving informed consent, all subjects underwent a neurological examination by 2 experienced movement disorders specialists (J.H. and N.B.) who were blinded to the genetic status, established a consensus diagnosis by 2 experienced movement disorders specialists (J.H. and A.M., who were also blinded to the genetic status, established a consensus diagnosis by 2 experienced movement disorders specialists (J.H. and A.M., who were also blinded to the genetic status, established a consensus diagnosis by 2 experienced movement disorders specialists). Because the term PD usually refers to the diagnosis of the idiopathic (uninherited) disease, we will use parkinsonism instead, to avoid confusion in terminology. Assessment of nonmotor signs of PD included the Mini-Mental State Examination, Montreal Cognitive Assessment, screening for axis I and II disorders, the Epworth Sleepiness Scale, and a PD risk factor questionnaire assessment. Smell function was determined using the University of Pennsylvania Smell Identification Test. The Farnsworth Munsell 100 Hue Test was applied to estimate individual color perception, stratified according to superior (total error score, 0-16), average (20-100), and low (<100) discrimination according to normative data.

The study protocol was approved by the local ethics committee at the University of Lübeck.

DOPAMINE TRANSPORTER SCAN

Striatal N-ω-fluoropropyl-2β-carbomethoxy-3β-[4-iodophenyl]nortropane (FP-CIT) single-photon emission computed tomography were performed in 6 of 7 siblings, whereas the heterozygous mother underwent 2β[N,N'-bis(2-mercaptoethyl)-ethylenediamino]methyl-3β-[4-chlorophenyl]tro-

pane (TRODAT) single photon emission computed tomography. The images were taken 4 to 5 hours after intravenous injection of fluopane labeled with iodine 123 ([123I-fluopane; 85 MBq) or TRODAT-1 labeled with technetium 99 (38 mCi), which was given 60 minutes after thyroid blockade with sodium perchlorate. The scans were analyzed by region-of-interest–based semiquantitative analysis of the caudate nucleus and putamen in reference to the occipital uptake (FP-CIT) and by visual assessment (FP-CIT, TRODAT) established by a consensus panel of 3 nuclear medicine specialists who were blinded to the clinical and genetic status.

STRUCTURAL MAGNETIC RESONANCE IMAGING

Four asymptomatic MCs (I.I.1, II.8, II.11, II.12; mean [SD] age, 52.0[6.7] years) were compared with 16 age- and sex-matched healthy volunteers (mean [SD] age, 52.0[7.6] years). The symptomatic MC II.14 (age, 44 years) was compared with 10 controls (mean [SD] age, 47.5[4.5] years). There were no significant age differences between the groups. The different control group assignment used for heterozygous MCs and the compound heterozygous MC was owing to the varying ages of the probands and the fact that one of the heterozygous MCs was female.

Structural brain magnetic resonance images were acquired on a 3.0-T whole-body scanner (Philips, Achieva, the Netherlands) using a 3-dimensional T1-weighted fast low-angle shot sequence. Magnetic resonance images were processed with the Statistical Parametric Mapping software (SPM5, www.fil.ion.ucl.ac.uk/spm) implemented in Matlab Version 7.1 (Mathworks, Sherborn, Massachusetts). Using the voxel-based morphometry toolbox, images were bias corrected, tissue classified, and registred using linear and nonlinear transformations within the same generative model. Analyses were performed on gray matter (GM) segments that were multiplied by the nonlinear components derived from the normalization matrix (modulated GM volumes). Finally, modulated GM images were smoothed with a Gaussian kernel of 12 mm full width at half maximum.

Using a general linear model, voxelwise GM differences between the 2 groups were examined using independent-sample t tests. To avoid possible edge effects around the border between gray and white matter and cerebrospinal fluid, an absolute GM threshold of 0.25 was used.

Figure 1. Family pedigree. Filled symbols indicate the subjects with Kufor-Rakeb syndrome. Slashes indicate deceased relatives. Arrows mark the examined individuals: the heterozygous mother (I.2), 4 heterozygous children (II.1, II.8, II.11, II.12), the compound-heterozygous affected son (II.14), and 2 non–mutation carriers (II.2, II.6). The genetic variations are included in the arrow box. wt indicates wild-type allele.
Table. Demographic Data of Members of the Chilean Family With KRS

<table>
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<tr>
<th>Characteristic</th>
<th>II.14</th>
<th>I.2</th>
<th>II.1</th>
<th>II.8</th>
<th>II.11</th>
<th>II.12</th>
<th>II.2</th>
<th>II.6</th>
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<td>Wild-type</td>
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<td>TES/color discrimination</td>
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<td>68/A</td>
<td>80/A</td>
<td>60/A</td>
<td>248/L</td>
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<tr>
<td>Symptom status</td>
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<td>15</td>
<td>Anosmia</td>
<td>29</td>
<td>Moderate hyposmia</td>
<td>31/Mild hyposmia</td>
<td>28/Moderate hyposmia</td>
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<td>6</td>
<td>1</td>
<td>5</td>
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<td>TES/color discrimination</td>
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<td>276/L</td>
<td>68/A</td>
<td>80/A</td>
<td>60/A</td>
<td>248/L</td>
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</tbody>
</table>

Abbreviations: A, average discrimination; ellipses, information not applicable because the individual is completely unaffected; ESS, Epworth Sleepiness Scale; KRS, Kufor-Rakeb syndrome; L, low discrimination; MMSE, Mini-Mental State Evaluation; MoCA, Montreal Cognitive Assessment; NA, data not available owing to severe dementia; TES, total error score; UPDRS, Unified Parkinson Disease Rating Scale; UPSIT, University of Pennsylvania Smell Identification Test.

For the statistical analysis, an explorative threshold of \( P < .001 \) (uncorrected) and clusters \( P < .05 \) (uncorrected at the cluster level) with a cluster extent greater than 80 voxels (the expected number of voxels per cluster) was applied.

**TRANSCRANIAL ULTRASOUND**

Brain parenchyma transcranial sonography (TCS) was used to determine the area of echogenicity in the SN (aSN) typically found in patients with PD and monogenic parkinsonism.\(^{14-16}\) Transcranial sonography was performed with the Acuson Antares ultrasound system (Siemens, Erlangen, Germany) using a 2.0- to 2.5-MHz transducer (PX4-1) by a blinded examiner. The temporal bone window of both sides was used to visualize the SN. Only the ipsilateral SN was evaluated in a standardized axial mesencephalic plane with a maximum depth of 12 cm. The aSN was manually encircled and measured by an independent rater using a computer-based analysis. Values of less than 0.2 cm\(^2\) were considered within the reference range, whereas values greater than 0.25 cm\(^2\) were classified as marked hyperechogenicity.\(^{16}\) The lenticular and caudate nucleus as well as the thalamus were also evaluated and considered hyperechogenic when the intensity was higher than that of the surrounding white matter.\(^{17}\)

**RESULTS**

**CLINICAL FINDINGS**

Based on the clinical findings, the 2 videotape raters predicted the genetic status correctly in 6 of 8 (1 false positive [II.12], 1 false negative [II.11]) and 8 of 8 family members, respectively.

The heterozygous mother I.2 presented with parkinsonism according to Hoehn/Yahr stage 2. All heterozygous siblings showed subtle extrapyramidal signs such as slight upper limb rigidity (II.1, II.11), reduced arm swing (II.1, II.8, II.11, II.12), postural (II.8) and action tremor (II.8, II.11), and mild unilateral shoulder elevation (II.12). The signs tended to be more pronounced in older family members. One NMC presented with mild postural and kinetic arm tremor. The second NMC had an unremarkable neurological examination. Pyramidal and frontal release signs were absent in all heterozygous MCs and NMCs. All siblings were asymptomatic for the reported mild signs.

Case reports of all subjects are provided in the supplemental appendix (www.neuro.uni-luebeck.de/arch.neuro.bruggemann.et.al); also see detailed case report of II.14). Clinical characteristics and test scores are summarized in the Table.

**OLFACTION AND COLOR DISCRIMINATION**

The affected heterozygous mother I.2 had a markedly decreased sense of smell in keeping with anosmia. No differences were observed between the heterozygous siblings and NMCs, with a mean score of 29 of 40 in both groups. Sex-adjusted scores revealed mild hyposmia in 1 MC and 1 NMC as well as moderate hyposmia in 3 MCs and 1 NMC. Color discrimination was markedly impaired in the heterozygous mother and 1 NMC. The remaining siblings had average color discrimination. Smell and color testing could not be performed in the compound heterozygous proband II.14 owing to dementia.

**PRESYNAPTIC DOPAMINE TRANSPORTER IMAGING**

Striatal \(^{123}\)I-fluopane uptake was markedly reduced in the caudate nucleus and putamen bilaterally in patient II.14 predominantly on the right (Figure 2).

Dopamine transporter (DAT) imaging in the mother (I.2) demonstrated considerably reduced tracer uptake.
in both putamina. The 3 heterozygous MCs (II.1, II.11, II.12) showed physiological levels of tracer uptake but the only examined carrier of the heterozygous 1306 + 5G->A mutation (II.11) had an asymmetric tracer distribution with a significant difference of greater than 10% reduction toward the right caudate nucleus (Figure 2; supplemental table 1; www.neuro.uni-luebeck.de/arch.neurol_bruggemann.et.al/). The 2 NMCs had no reduction of striatal tracer uptake or asymmetry.

STRUCTURAL MRI

Patient II.14 showed a marked T2 hypointensity in the caudate nucleus and putamen bilaterally. The heterozygous MCs and NMCs had no T2 signal alterations (supplemental table 1; www.neuro.uni-luebeck.de/arch.neurol_bruggemann.et.al/).

On voxel-based morphometry, the main findings in the symptomatic ATP13A2 MC were 3 large clusters demonstrating a decrease in GM volume in the cerebellum. Furthermore, we observed pronounced bilateral loss of GM volume in the premotor and supplementary motor cortex, caudate, thalamus, prefrontal cortex, cingulate, and somatosensory association cortex (Figure 3; supplemental table 2; www.neuro.uni-luebeck.de/arch.neurol_bruggemann.et.al/).

In asymptomatic ATP13A2 MCs, we detected 1 large cluster of significantly increased GM volume in the right putamen when compared with healthy controls. We found an increase in GM volume in the bilateral somatosensory and motor cortex as well as in the parietal association cortex and the middle occipital gyrus. In contrast, the analysis revealed a decrease in GM volume in the cerebellum bilaterally and the right hippocampus (Figure 3; supplemental table 3; www.neuro.uni-luebeck.de/arch.neurol_bruggemann.et.al/).

BRAIN PARENCHYMA TCS

The mother had an insufficient temporal bone window bilaterally. None of the examined probands showed nigral hyperechogenicity of greater than 0.25 cm² (supplemental table 1; www.neuro.uni-luebeck.de/arch.neurol_bruggemann.et.al/). One MC (II.11) demonstrated a slightly increased aSN between 0.20 and 0.25 cm². No abnormal echogenicity was found in any other parts of the basal ganglia nor in the thalamus in any of the subjects.

COMMENTS

This detailed multimodal clinical and neuroimaging study collectively provides evidence for a possible pathogenic role of single ATP13A2 mutations in the development of parkinsonian signs. Heterozygous ATP13A2 mutations may cause an age-dependent measurable impairment of nigrostriatal function and altered brain plasticity. Second, we demonstrate that the neurodegenerative process in classical KRS involves several brain structures including the basal ganglia, cortical areas, and cerebellum. The presence of iron accumulation in the basal ganglia of patients with KRS may place this syndrome among the disorders of neurodegeneration with brain iron accumulation (NBIA).

EFFECT OF SINGLE ATP13A2 MUTATIONS

Detailed clinical assessment revealed mild extrapyramidal signs in all examined heterozygous MCs and parkinsonism in the mother. Determining subtle motor findings is a diagnostic challenge, especially in genetic studies in which family members are examined for mild signs, which may be indicative of milder phenotypes or forms frustes. Such subtle signs do not necessarily equate parkinsonism and could alternatively be attributed to dystonia, or may still be within the physiological spectrum. One of the 2 NMCs presented with mild symmetric action tremor. The response to alcohol and β-blocking agents was not tested. Family history for tremor was negative. Rest tremor as a cardinal PD sign, and other PD-defining signs were absent in this subject.
Unlike his heterozygous siblings, tremor was the only sign. We conclude that this subject presented with either an accentuated physiological tremor or a mild essential tremor. In keeping with her clinical status, the mother had a clear reduction in putaminal tracer uptake on both sides, whereas none of the remaining investigated heterozygous MCs had pathologically decreased values. It remains questionable whether the observed asymmetric tracer uptake in subject II.11 represents a true abnormality indicative of the first evidence of a possible nigrostriatal decline. Alternatively, some carriers may display a subtle nigrostriatal dopaminergic abnormality that can only be detected with more sensitive mapping techniques such as positron emission tomography. Although the sensitivity of DAT scans is reported to be high, a certain proportion of probands with degenerative parkinsonism demonstrates results in the reference range and may fall below the detection threshold. A recent single-photon emission computed tomographic study revealed that 22 of 112 clinically diagnosed patients with PD had DAT imaging that appeared normal initially, a fraction of whom showed abnormal results on follow-up scans. Only long-term clinical and neuroimaging follow-up investigations will help determine whether these at-risk subjects will develop definite parkinsonism later in life, like their mother.

Former DAT scan and fluorine-18-L-dihydroxyphenylalanine positron emission tomographic studies in patients with mutations in the more frequently mutated recessive genes Parkin and PINK1 revealed bilaterally reduced striatal uptake, particularly in the posterior putamen, comparable with idiopathic PD. Progression of the nigrostriatal decline in both monogenic forms was slower compared with idiopathic PD. Also, asymptomatic carriers of heterozygous Parkin mutations showed subclinical striatal PET changes, suggesting a correlation between nigrostriatal dysfunction and the number of mutated alleles. A subset of these individuals who were at risk exhibited unequivocal extrapyramidal signs and, in part, a parkinsonian phenotype. Of note, striatal...
PET changes were given as group differences between several carriers of single mutations and healthy controls and therefore need to be interpreted with caution when referring to individual probands, as in our study.

Structural neuroimaging suggests that single ATP13A2 mutations lead to unilateral putaminal hypertrophy and to increased GM volume in the precentral and postcentral gyrus as well as the precuneus/cuneus bilaterally. The side of putaminal GM increase matches the clinically affected side in 3 of 4 heterozygous MCs that were included in the analysis. These findings are consistent with an effect of heterozygous mutations in modifying the structural brain organization. The described alterations support a previously demonstrated hypertrophy in the putamen and internal globeus pallidus in carriers of heterozygous Parkin and PINK1 mutations.23,26 The morphometric changes may result from a latent striatal dopamine deficiency. Evidence supporting this hypothesis is gleaned from a study on long-term treatment with antiparkinsonian drugs over a period of 2 years. The mean basal ganglia volume increased in patients who were treated with typical neuroleptics and decreased after the change to atypical neuroleptics.23 Hypertrophy in different brain regions, especially in the putamen, may point toward compensatory plasticity in the younger and middle-aged heterozygous MCs, leading to a preserved striatal dopaminergic innervation, as shown by DAT imaging. However, the underlying biochemical alterations and the type of the involved tissue of these adaptive mechanisms remain to be elucidated.

NEURODEGENERATIVE PROCESS AND BRAIN IRON ACCUMULATION IN KRS

In the index patient, presynaptic DAT imaging revealed a marked striatal uptake decrease, indicating severely impaired nigrostriatal function, with the most prominent reduction in the dorsal portions of the putamen. Accordingly, fluorine-18-L-dihydroxyphenylalanine positron emission tomography showed diminished bilateral striatal uptake in a previously described Japanese patient with a homozygous ATP13A2 mutation.28 We detected marked iron deposition in the basal ganglia of the patient with KRS and pronounced bilateral loss of GM volume in several brain regions on observer-independent voxel-based morphometry. Unlike the heterozygous MCs, the patient with KRS did not show an increase in GM volume in the basal ganglia but a marked decline. Apparently, regions with an accumulation of iron seem to be associated with neuronal death in this neurodegenerative syndrome. The compensatory capacity for avoiding neuronal death is obviously lost in the patient with KRS in contrast to younger and middle-aged heterozygous subjects. Whether the increased iron content in KRS reflects a pathophysiologically relevant mechanism or a secondary epiphenomenon remains to be elucidated in future studies. Interestingly, the yeast orthologue of the human ATP13A2 protein, YPK9p, might play a role in the sequestration of divalent heavy metals.29 Despite the lack of post mortem data, ATP13A2-associated parkinsonism may be considered the latest member in the growing list of neurodegenerative diseases with brain iron accumulation such as pantothenate kinase-associated neurodegeneration.30

Unlike patients with idiopathic PD and carriers of heterozygous and homozygous Parkin and PINK1 mutations, subjects with ATP13A2-associated parkinsonism and heterozygous ATP13A2 mutations showed no increased aSN in TCS.14,15 Substantia nigra hyperechogenicity in idiopathic PD is thought to be associated with increased iron content, bound to proteins other than ferritin.31 The lack of this TCS sign in the present study may be explained by the putative presence of different iron compounds and binding partners in KRS and other atypical forms of parkinsonism.16 Only limited data are currently available regarding the hyperechogenicity of the lenticular and caudate nuclei. Apart from increased metal tissue content, signal alterations may result from minor calcifications, enlarged perivascular spaces, and gliosis. The underlying neurodegenerative process in ATP13A2-associated atypical parkinsonism may differ from other forms of inherited as well as uninherited PD.

The pathophysiological role of heterozygous mutations in recessive parkinsonism genes currently remains a matter of vivid debate.32 Their potential effect on the development of parkinsonism is influenced by a variety of parameters including changes in other genes or gene-regulating elements as well as epigenetic and environmental factors. Our data from this multilayered investigation collectively provide evidence for a relevant pathophysiologic effect of single ATP13A2 mutations in the development of parkinsonian signs and demonstrate possible compensatory brain plasticity mechanisms in heterozygous subjects. The question whether the measured phenotypic effects are consistently caused by single ATP13A2 mutations should be clarified in an independent, larger sample of carriers with a different genetic and environmental background. This approach will help to better interpret the role of heterozygous mutations in putatively recessive parkinsonism genes.

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Author Contributions: Dr Klein had full access to all of the data in the study and takes responsibility for the integ-
rity of the data and the accuracy of the data analysis. Study concept and design: Brüggemann, Hagenah, Münchau, Siebner, Binkofski, and Klein. Acquisition of data: Brüggemann, Hagenah, Schmidt, Kasten, Buchmann, Eckerle, Bähr, Münchau, Djarmati, van der Vegt, Behrens, and Klein. Analysis and interpretation of data: Brüggemann, Hagenah, Reetz, Buchmann, Bähr, Münchau, Siebner, Binkofski, Ramirez, Behrens, and Klein. Drafting of the manuscript: Brüggemann, Eckerle, and Bähr. Critical revision of the manuscript for important intellectual content: Hagenah, Reetz, Schmidt, Kasten, Buchmann, Münchau, Djarmati, van der Vegt, Binkofski, Ramirez, Behrens, and Klein. Statistical analysis: Reetz and Siebner. Obtained funding: Siebner and Klein. Administrative, technical, and material support: Brüggemann, Reetz, Schmidt, Kasten, Buchmann, Eckerle, Bähr, van der Vegt, and Klein. Study supervision: Buchmann, Münchau, Binkofski, Ramirez, and Klein. Financial Disclosure: None reported.

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