[¹⁸F]Fluorodopa PET shows striatal dopaminergic dysfunction in juvenile neuronal ceroid lipofuscinosis

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Abstract

Objectives—To investigate whether nigrostriatal dopaminergic hypofunction is related to the extrapyramidal symptoms in patients with juvenile neuronal ceroid lipofuscinosis (JNCL).

Methods—Nine patients with JNCL and seven healthy controls were studied using [¹⁸F]fluorodopa PET.

Results—In the patients with JNCL [¹⁸F]fluorodopa uptake (K_i^{occ}) in the putamen was 60% of the control mean and the corresponding figure in the caudate nucleus was 79%. There was a weak correlation between putamen K_i^{occ} values and extrapyramidal symptoms of the patients evaluated by the motor part of the unified Parkinson's disease rating scale ($r = -0.57$, $P < 0.05$). The overall severity of the disease also displayed a negative correlation with the K_i^{occ} values in the putamen ($r = -0.71$, $P < 0.05$).

Conclusion—In patients with JNCL there was reduced striatal [¹⁸F]fluorodopa uptake, which had a modest correlation with extrapyramidal symptoms. Dysfunction of nigrostriatal dopaminergic neurons is therefore not the only cause of the patients' extrapyramidal symptoms, but degenerative changes in other brain areas are also contributory.

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The neuronal ceroid lipofuscinoses (NCLs) are a group of recessively inherited progressive encephalopathies characterised by neural and extraneural accumulation of cytosomes storing ceroid and lipofuscin.¹ The diseases are considered to be among the most common progressive encephalopathies during childhood in the western world.² Three major childhood types are known as infantile (INCL), late infantile (LINCL), and juvenile types (JNCL). Synonyms for JNCL are Spielmeyer-(Vogt)-Sjögren disease and late onset Batten disease.

JNCL is characterised by increasing visual failure (with an onset at ages between four and eight years), epilepsy, progressive dementia, dysarthric speech, and development of slight to moderate ataxia. In addition, extrapyramidal symptoms occur with increasing frequency during the course of the disease. In a series of 53 patients with JNCL³ the mean age of the

patients when a parkinsonian type walk was first seen was 13.7 years. The patients lost their ability to walk at a mean age of 17.3 years.³

The gene responsible for JNCL has recently been identified at chromosome 16p12 and shows no homology with previously known proteins.⁴ One founder mutation, a 1.02 kb deletion, causes the disease in about 80% of affected chromosomes worldwide.⁴ Because of the unknown biochemical defect, there is no specific treatment, and available therapies remain largely only symptomatic.^{3, 5}

The effect of antiparkinsonian drugs has been variable.⁶⁻⁸ Despite the importance of extrapyramidal symptoms in patients with JNCL, little attention has been paid to them. The motor dysfunction, which is added to the clinical picture as the disease progresses, itself sets a major therapeutic problem: extrapyramidal signs associated with amblyopia lead to severe disturbance of locomotion. However, the pathophysiological mechanisms responsible for their appearance in JNCL are not known. Therefore, to assess whether a striatal dopaminergic dysfunction is present in some patients with JNCL is particularly relevant as such dysfunction might explain their extrapyramidal symptoms. It is also necessary before considering symptomatic treatment with substitutive dopaminergic drugs.

The purpose of the present study was to find out whether patients with JNCL show evidence of presynaptic dopaminergic hypofunction, evaluated with [¹⁸F]fluorodopa PET, and whether that is related to the extrapyramidal symptoms of the patients.

Patients and methods

PATIENTS

We studied nine patients with JNCL (one female, eight male). The age of the patients was 19.1 (SD 4.7) years, ranging from 15 to 27 years. Table 1 presents their clinical characteristics. All patients had a normal early development and were able to walk and run well until gait problems developed and first signs of a parkinsonian walk (slightly flexed posture, slow steps with flexion in hips and knees, and failure of arm swing) were noticed. Unlike idiopathic Parkinson's disease, tremor was an uncommon sign. All the patients were initially investigated because they had severe visual failure. They all showed tapetoretinal degeneration with abolished electroretinograms. Vacuolated lymphocytes in peripheral blood were found on repeated examinations. The diagnosis was confirmed from a rectal biopsy specimen by elec-

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Table 1 Clinical characteristics of the patients with juvenile neuronal ceroid lipofuscinosis (JNCL)

Patient No	Age (y)	Age at onset of JNCL (y)	Motor UPDRS score	Total score	Total minus epilepsy score
1	19	4	28	9	7
2	27	6	21	8	8
3*	27	6	0	3	3
4	15	5	28	11	9
5*	16	4	23	5	3
6	18	4	35	9	8
7	15	5	12	6	5
8	19	4	16	6	6
9	16	4	19	6	5
Total	19.1 (4.7)	4.7 (0.9)	20.2 (10.3)	7.0 (2.4)	6.0 (2.2)

*Heterozygous; all the others are homozygous.
Total results are mean (SD).

tron microscopy, which showed characteristic curvilinear and fingerprint profiles.⁹ The mutation analysis showed that seven of the patients with JNCL were homozygous and two were heterozygous for the major mutation, a 1.02 kb deletion of the CLN3 gene.¹⁰

Extrapyramidal symptoms (rigidity, hypokinesia, slightly flexed posture, and impaired balance) were noted in all but one of the patients, without any clinically relevant asymmetries in symptoms. The severity of the extrapyramidal symptoms was evaluated using the motor part of the unified Parkinson's disease rating scale (UPDRS).¹¹ All the patients were on antioxidant medication and eight patients received antiepileptic drugs, but none of them was receiving antiparkinsonian treatment. The overall severity of the disease was evaluated using a scale taking into account motor performance, balance, coordination, speech, and the frequency of epileptic seizures.⁶ Each item was scored from zero to four and thus the scale ranged from zero to 20: the higher the score the greater the severity.

The controls (one woman, six men) were healthy volunteers aged 34.6 (SD 12.9) years, ranging from 23 to 50 years. They had no neurological or psychiatric diseases and were not under regular medication. Informed consent for all subjects was obtained.

METHODS

All the subjects underwent an [¹⁸F]fluorodopa PET investigation according to a protocol described previously by Ruottinen *et al*¹² with two modifications: arterial blood sampling was not performed and the dose of carbidopa was 100 mg instead of 150 mg. The PET investigations were performed using an eight ring whole body ECAT 931/08 tomograph (Siemens/CTI Corp, Knoxville, TN, USA), described earlier.¹² The scanner gives 15 simultaneous transaxial slices with final in plane resolution of 8 mm after reconstruction. The subjects were positioned in the tomograph in an individually prepared head holder with three dimensional laser alignment with reference to the orbito-meatal line.

The synthesis of [¹⁸F]F₂ gas for labelling¹³ and [¹⁸F]fluorodopa production were carried out according to the methods of Namavari *et al*¹⁴ and Bergman *et al*.¹⁵ At the end of the synthesis the mean batch size was 1190 (SD 610) MBq with a specific radioactivity of 2510 (SD

970) MBq/μmol. The radiochemical purity exceeded 98% in every case. On average 172 (SD 31) MBq [¹⁸F]fluorodopa (range 107–204 MBq) was injected intravenously into patients and controls. The specific radioactivity of the [¹⁸F]fluorodopa was 830 (SD 500) MBq/μmol at the time of injection.

A dynamic study of 37 time frames (12 × 15 s, 4 × 30 s, 5 × 60 s, and 16 × 300 s) with a total duration of 90 minutes¹² was performed for each subject. The trace regions of interest (ROIs) were drawn bilaterally on the head of the caudate nucleus (with an average of 140 mm²), putamen (330 mm²), and lateral occipital region containing both grey and white matter. The ROIs were identified by visual inspection, with reference to the neuroanatomical atlas of Aquilonius and Eckernäs,¹⁶ on the integrated PET images representing activity concentration, from 20–90 minutes after [¹⁸F]fluorodopa injection. The ROIs were placed on two adjacent transaxial slices and the average radioactivity concentration was computed for each ROI. The values of the corresponding ROIs for left and right structures were averaged. To ensure that there was no difference between patients and controls in the occipital region, the area under the time activity curve from 0 to 90 minutes (AUC_{0–90}) of the occipital reference area was calculated. The AUC_{0–90} was corrected for the subject's weight and injected [¹⁸F]fluorodopa dose.

The graphical analysis method was used to calculate the metabolic rate of [¹⁸F]fluorodopa (K_i^{occ}),^{17–19} which reflects the decarboxylation rate of [¹⁸F]fluorodopa to [¹⁸F]fluorodopamine. In this analysis the time course of occipital radioactivity was used as input function; the time course of striatal/occipital activity was plotted against the ratio of integrated occipital to occipital activity. The K_i^{occ}, defined in the time range from 10 to 90 minutes after the [¹⁸F]fluorodopa injection, was calculated from the slope of the graphical plot.^{17–19} All the subjects underwent a brain MRI (1.5 tesla) and one patient had a brain CT.

The difference in the [¹⁸F]fluorodopa uptake values between patients and controls was compared using Student's unpaired *t* test. Correlations were calculated using Pearson's correlation coefficients. Statistical significance was defined as *P* < 0.05.

Results

Table 2 presents the [¹⁸F]fluorodopa uptake values (K_i^{occ}) in patients with JNCL and controls. The putamenal K_i^{occ} was 7.1 (SD 1.9) × 10^{–3} min^{–1} for the patients with JNCL, which

Table 2 [¹⁸F] fluorodopa uptake values (K_i^{occ}) in patients with juvenile neuronal ceroid lipofuscinosis (JNCL) and healthy controls

Group	K _i ^{occ} (× 10 ^{–3} min ^{–1})	
	Putamen	Caudate nucleus
JNCL	7.1 (1.9)***	9.2 (1.6)**
Control	11.7 (0.7)	11.6 (0.9)

Results are means (SD).

****P* < 0.01; ***P* < 0.001 *v* controls.

Figure 1 Comparison of individual [^{18}F]fluorodopa K_i^{occ} values ($\times 10^{-3} \text{ min}^{-1}$) in patients with juvenile neuronal ceroid lipofuscinosis (JNCL) and healthy controls. EP- = the patient without extrapyramidal symptoms. Bar = mean.

was significantly lower than the corresponding value for the controls ($11.7 \text{ (SD } 0.7) \times 10^{-3} \text{ min}^{-1}$, $P < 0.01$). The mean putamenal K_i^{occ} was 60% of the control mean. Figure 1 shows the individual K_i^{occ} values. The patient without extrapyramidal symptoms had the highest value among the patients with JNCL, which was at the lower border of the control range. In the other patients with extrapyramidal symptoms the putamenal K_i^{occ} was below 3 SD of the control mean.

In the caudate nucleus the average K_i^{occ} for the patients with JNCL was reduced to 79% of the control mean. The absolute values were $9.2 \text{ (SD } 1.6) \times 10^{-3} \text{ min}^{-1}$ for the patients with JNCL and $11.6 \text{ (SD } 0.9) \times 10^{-3} \text{ min}^{-1}$ for the controls ($P < 0.01$). The K_i^{occ} value found in the caudate nucleus for the patient without extrapyramidal symptoms (EP-) was in the upper patient range. However, this value did not differentiate this patient from other patients with JNCL as clearly as did the putamen K_i^{occ} value. Figure 2 shows the reduced striatal [^{18}F]fluorodopa accumulation in one patient with JNCL compared with a healthy control.

There was a significant correlation between the putamen and caudate K_i^{occ} values of the

patients with JNCL ($r = +0.79$, $P < 0.05$). A weak inverse correlation was noted between the putamen K_i^{occ} values and the UPDRS motor score ($r = -0.57$, $P < 0.05$). The overall severity of the disease had a weak negative correlation with the K_i^{occ} values in the putamen ($r = -0.64$, $P = 0.06$), and when the score "total minus epilepsy" was used, the correlation was more obvious ($r = -0.71$, $P < 0.05$). The mean AUC_{0-90} of the occipital time activity curves was not significantly different between patients and controls ($P > 0.1$).

Discussion

Our results show that there is reduced uptake of [^{18}F]fluorodopa in the putamen and, to a lesser degree, also in the caudate nucleus, in patients with JNCL. This most probably indicates hypofunction of nigrostriatal dopaminergic projections. We emphasise that this study concerns the juvenile type of NCL disease, and that the applicability of the results to the other subtypes of NCL needs further study.

There was a slight correlation between the extrapyramidal symptoms assessed by the motor part of the UPDRS and the putamenal [^{18}F]fluorodopa uptake. This association is less striking than that reported for patients with Parkinson's disease or multiple system atrophy.¹⁸ Most probably degeneration of striatal and other brain areas and their connections also contribute to the extrapyramidal symptoms of the patients with JNCL. This may also explain why the patients' response to levodopa is variable, usually fair to moderate.^{6-8, 20}

Philippart *et al*²¹ investigated brain metabolic activity in seven patients with Spielmeyer-Vogt disease, using fluorodeoxyglucose (FDG) PET, and found reduced metabolism in the calcarine area, cingulate cortex, and thalamus, but basal ganglia and

Figure 2 A demonstration of [^{18}F]fluorodopa PET image in a healthy control and in a patient with juvenile neuronal ceroid lipofuscinosis (JNCL). Note the reduced tracer uptake both in the putamen and caudate nucleus of the patient with JNCL. The PET images are scaled to identical occipital counts. The activity concentration declines along the colour scale red-yellow-green-blue.

brainstem uptake remained normal. In four siblings with neuronal ceroid lipofuscinosis the greatest decrease in glucose utilisation studied with FDG was seen in the thalamus and posterior association cortex.⁷ In a case of the late infantile form a widespread reduction of cortical and thalamic FDG uptake was reported.²²

In our study we used [¹⁸F]fluorodopa, the uptake of which reflects its decarboxylation and storage to [¹⁸F]fluorodopamine. Thus it is a marker of the functional activity of dopaminergic nerve endings. The use of radioactivity concentration in the occipital cortex as input function in [¹⁸F]fluorodopa PET instead of metabolite corrected arterial plasma has been validated.^{18,19} The reference area used in this study was in the lateral occipital cortex, which shows less atrophy and FDG uptake impairment than the primary visual cortex.²¹ Possible atrophic or other changes in the reference area did not bias the results, as the AUC₀₋₉₀ of [¹⁸F]fluorodopa in the occipital region did not differ significantly between the patients and the controls. Potential atrophy in the cortical or subcortical region would have increased the striatal to occipital ratios, and thus it would have rather underestimated the difference in [¹⁸F]fluorodopa uptake between patients with JNCL and control subjects. Our controls were older than the patients, as we did not consider it ethical to study controls below the age of 20; but this would, if anything, underestimate the decline in [¹⁸F]fluorodopa uptake, as in healthy persons the [¹⁸F]fluorodopa uptake has been reported to decline slightly²³ or to remain virtually unchanged with age.^{24,25} The medication used by the patients with JNCL is presumed not to affect the presynaptic dopaminergic system nor the K_i^{occ} values.

Autti *et al.*²⁶ found only a slight signal intensity change in basal ganglia studied with MRI (1.0 tesla). However, there was a correlation between the overall motor impairment and a reduced signal intensity in the putamen and thalamus. A similar association was seen with motor performance and increased signal intensity of the white matter and atrophic changes in the pons, mesencephalon, and medulla oblongata.²⁶ This may reflect overall severity of the disease process without a causal relation, but in any case factors other than degeneration of the nigrostriatal dopamine system most probably contribute to the extrapyramidal symptoms of patients with JNCL. Similarly, in our study the overall severity of the disease had a significant correlation with [¹⁸F]fluorodopa uptake in the putamen, especially when a score including motor performance, balance, coordination, and speech was used.

In conclusion, patients with JNCL have a reduced [¹⁸F]fluorodopa uptake in the putamen and to a lesser degree in the caudate nucleus. This decline showed a modest correlation with extrapyramidal symptoms of the patients. Therefore, most probably dysfunction of nigrostriatal dopaminergic neurons is not the only cause of the patients' extrapyramidal symptoms, but degenerative changes in other brain areas are also contributory.

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