Rett Syndrome: Investigation of Nine Patients, including PET Scan

Henry G. Dunn, A. Jon Stoessl, Helena H. Ho, Patrick M. MacLeod, Kenneth J. Poskitt, Doris J. Doudet, Michael Schulzer, Derek Blackstock, Teresa Dobko, Ben Koop, Giovana V. de Amorim

ABSTRACT: Background: We describe nine females with Rett Syndrome (RS), aged 14 to 26 years. All had had developmental delay before the end of their first year and had subsequently regressed to profound dementia with apraxia, ataxia, irregular respirations and often also seizures. Methods: The Revised Gesell developmental assessment and Alpern-Boll Developmental Profile were used in modified form. Volumetric measurements of basal ganglia using MRI were compared with the findings in nine age-matched volunteer females. Positron emission scans with [18F]-6-fluorodopa and [11C]-raclopride were performed under light anesthesia with intravenous Propofol, and the findings were compared with those in healthy control girls. Bidirectional sequencing of the coding regions of the MECP2 gene was investigated in blood samples for mutational analyses. Results: The RS females functioned at a mental age level ranging from about 4 to 15 months. The scores correlated with height, weight and head circumference. Magnetic resonance scans of basal ganglia showed a significant reduction in the size of the caudate heads and thalami in the Rett cases. Positron emission scans demonstrated that the mean uptake of fluorodopa in RS was reduced by 13.1% in caudate and by 12.5% in putamen as compared to the controls, while dopamine D2 receptor binding was increased significantly by 9.7% in caudate and 9.6% in putamen. Mutations in the coding regions of the MECP2 gene were present in all nine patients. No significant correlation between type and location of mutation and volumetric changes or isotope uptake was demonstrable. Conclusions: Our findings suggest a mild presynaptic deficit of nigrostriatal activity in Rett syndrome.

RÉSUMÉ: Syndrome de Rett: investigation de neuf patientes, incluant la tomodensitométrie. Introduction: Nous décrivons les cas de neuf femmes, âgées de 14 à 26 ans, atteintes du syndrome de Rett (SR). Un retard de développement avait été observé chez toutes avant la fin de leur première année de vie. Toutes avaient régressé par la suite et présentaient une démence profonde avec apraxie, ataxie, respiration irrégulière et, dans plusieurs cas, des crises convulsives. Méthodes: Des versions modifiées de l’échelle révisée du développement de Gesell et du profil du développement d’Alpern-Boll ont été utilisées. Des mesures volumétriques des noyaux gris centraux par résonance magnétique ont été comparées à celles faites chez neuf femmes volontaires, appariées pour l’âge. La tomodensitométrie par émission de positons (PET scan) au [18F]-6-fluorodopa et au [11C]-raclopride a été effectuée sous anesthésie légère par le Propofol intraveineux et les observations ont été comparées à celles de femmes témoins normales. On a procédé à une analyse mutationnelle par séquençage bidirectionnel des régions codantes du gène MECP2. Résultats: Les femmes atteintes du SR fonctionnaient à un niveau d’âge mental de 4 à 15 mois. Les scores étaient corrélés à la taille, au poids et à la circonférence de la tête. La résonance magnétique des noyaux gris centraux a montré une réduction significative de la taille de la tête du noyau caudé et du thalamus chez les cas de SR. Le PET scan a montré que la captation moyenne de fluorodopa dans le SR était réduite de 13,1% dans le noyau caudé et de 12,4% dans le putamen par rapport aux contrôles et que la liaison aux récepteurs dopaminergiques D2 était augmentée significativement de 9,7% dans le noyau caudé et de 9,6% dans le putamen. Des mutations dans les régions codantes du gène MECP2 étaient présentes chez les neuf patientes. Aucune corrélation significative entre le type et le site des mutation et les changements volumétriques ou la captation isotopique n’a pu être démontrée. Conclusions: Nos observations suggèrent qu’il existe un léger déficit présynaptique de l’activité nigro-striée dans le SR.


Since 1983, when Hagberg et al. published an extensive report on the syndrome of brain atrophy in girls first described by Andreas Rett in Austria, it has become customary to divide the classical course of the disease into four stages. After the early onset stagnation stage at ½ to 1½ years, there follows the rapid destructive stage lasting weeks to months at one to three years, with stereotypic and autistic features, loss of speech and hand skills progressing to severe dementia, frequently with...
seizures and irregular breathing. Third, there is the pseudo-
stationary stage, lasting from preschool to school years, in which
profound mental retardation and motor dysfunctions persist and
seizures are common but there is some emotional contact.
Osteopenia\(^a\) and scoliosis are common, and difficulties with
swallowing and gastroesophageal motility\(^a\) as well as
constipation may also require attention. Fourth, there follows the
late motor deterioration stage which may last decades at 15 to
50 or more years. With decreasing mobility, some Parkinsonian
features, spastic para- or tetraparesis, tense heel cords and trophic
cyamotic feet, the girls may become wheelchair-bound. On the
other hand, emotional contact may improve further and epilepsy
is then less common.

The neuropathology has been discussed elsewhere.\(^b\) Head
and brain growth are reduced after the age of about three months. A
global decrease in the size of individual neurons is associated
with increased packing density and with focal thinning of
dendrites in some layers of the cortex.\(^c\)\(^d\) Particular neuronal
abnormalities have been described in the pars compacta of the
substantia nigra.\(^e\)

Biochemical studies have shown normal\(^f\)\(^g\) or reduced\(^h\)
monoamine metabolites in the spinal fluid of Rett patients.
Distinctly reduced levels of dopamine, serotonin and
noradrenaline have been found in various brain regions at
autopsy.\(^i\)\(^j\)\(^k\) In 1995, Wenk\(^l\) first showed that dopamine
reuptake sites in postmortem Rett syndrome (RS) material had a
normal density in cingulate and mid-frontal gyri as compared to
normal female controls, but were decreased within the caudate
nucleus and putamen. However, in the following year the same
author\(^m\) reported that endogenous levels of dopamine, its
metabolite homovanillic acid, dopamine reuptake sites and
dopamine type 2 receptors did not differ significantly between
RS and control girls in any brain region examined. Recently
Naidu and her associates\(^n\) studied 12 adult RS patients (aged 15-
39 years) by PET scan using \([^{11}C]\) N-methyl-spiperone and found
low normal levels of post-synaptic D2-like dopamine receptors
in caudate. This was in contrast to the observations of Chiron et
al\(^o\) who had reported markedly increased specific binding of
\([^{125}I]\) iodolisuride for the D2-like dopamine receptor in 11
children with RS, aged 4-15 years. It raises the question whether
there are age-specific changes in D2 receptor activity in RS with
reduction in the older patients. To try and clarify this issue of
dopaminergic function we have performed PET studies of both
pre- and post-synaptic activity in RS.

As to genetics, the almost exclusive occurrence of RS in girls
suggested X-linked dominant inheritance, with possible lethality
in males in utero. Linkage studies in cases of other family
members being affected indicated a critical region at Xq28, the
telomeric part of the long arm of the X chromosome.\(^p\)\(^q\)\(^r\) In
1999, Amir and other investigators\(^s\) found several
mutations in the gene \(MECP2\) in a proportion of RS patients.
This gene encodes the X-linked methyl cytosine binding protein
\(MeCP2\) which is thought to function as a transcriptional
repressor in methylated regions of DNA via the methyl-binding
domain (MBD)\(^t\) and a transcriptional repression domain. These
domains are thought to interact with several other proteins to
promote gene silencing during development.\(^u\)\(^v\)\(^w\)\(^x\) In fact, \(MeCP2\)
has been shown to become more abundant once neurons have
reached a certain maturity, both in mouse and human brain, but
is absent from glia.\(^y\)\(^z\) A possible third functional domain is the C-
terminus region which is shortened in truncating mutations of
DNA; deletion mutations appear most common in this area.\(^a\)
The region was also recognized recently\(^l\) as being reduced in
rare male cases of a disorder with \(MECP2\) mutation, X-linked
mental retardation and progressive spasticity. However, it
appears that \(MECP2\) mutations may also be incidental in this
domain in mentally handicapped boys.\(^o\)

Milder phenotypes may occur if X chromosome inactivation
is nonrandom, thus favouring the expression of the normal
\(MECP2\) allele.\(^m\)\(^n\) Milder phenotypes have also been found in
males with more than one X chromosome, particularly
Klinefelter syndrome\(^o\)\(^p\)\(^q\) and with mosaicism for truncating
\(MeCP2\) mutation R270X.\(^r\) Nonspecific X-linked mental
retardation may also occur with the A140V mutation of the
\(MECP2\) gene in either sex, but more severely in males.\(^e\) The
A140V mutation has also been found to be the genetic cause of
the X-linked syndrome of psychosis, pyramidal signs and macro-
orchidism (PPM-X) in a three-generation family with the
\(MECP2\) gene in Xq28.\(^s\)\(^t\) A simple polymerase chain reaction
(PCR) approach has been developed for detection of the hotspot
for A140V mutation.

At least 150 different mutations of \(MECP2\) have now been
characterized in RS.\(^u\)\(^v\)\(^w\)\(^x\) About 75%-80% of classical RS patients
have characteristic mutations in \(MECP2\)\(^y\)\(^z\)\(^a\)\(^b\)\(^c\)\(^d\) On the other
hand, in familial and atypical RS patients only 20%-40% have
the characteristic abnormality of the \(MECP2\) gene.\(^e\)\(^f\)\(^g\)\(^h\) Studies
of \(MECP2\) gene and mutants now facilitate the diagnosis of RS,
particularly in infants and in clinically doubtful cases.

**STUDY DESIGN AND METHODS**

In view of our particular interest in nigrostriatal dopaminergic
activity, the function of dopamine receptors, and \(MeCP2\) mutation in RS we decided to study girls aged at least 12 years
who were likely to be in Stage III to IV and thus to exhibit
movement disorders and Parkinsonian features. All patients
underwent:

1. a detailed clinical examination in the out-patient clinic,
   including the use of the Modified Columbia Scale in the
   assessment of extra-pyramidal features\(^y\)\(^z\) and Fahn and
   Marsden’s Functional Disability Scale for Dystonia.\(^a\)

2. an assessment of the girl’s behavioral development by the
   Revised Gesell technique\(^a\)\(^b\)\(^c\)\(^d\) and Alpern Boll
   Developmental Profile.\(^e\)

3. a videotape recording by a separate observer, with a defined
   sequence of motor tasks.

4. magnetic resonance imaging (MRI) at B.C.’s Children’s
   Hospital with anesthetist available. These scans were
   supplemented with volumetric controlled studies.

5. PET scan at the University Hospital site, utilizing first \([^{11}C]\)-
raclopride to assess D2 receptors and then \([^{18}F]\) fluorodopa,
   again with controls.

6. mutational analysis of \(MECP2\) gene using peripheral blood
   lymphocytes.

Permission for this research project was obtained from the
Clinical Screening Committee for Research involving Human
Subjects at the University of British Columbia and from B.C.’s
Children’s Hospital Research Review Committee. In nine cases
the parents gave written permission for their daughter to be enrolled in the present study. As controls for the MR studies we had nine age-matched young women who volunteered for functional MRI scans, with normal results. As controls in the PET scans we utilized the findings in healthy young women aged 20 to 29 years, who had agreed to receive these isotopes in previous studies, but they had not required light anesthesia with Propofol like the Rett girls.

The nine Rett patients had such profound mental subnormality that they had limited ability to cooperate on testing. They also had hardly any speech and they exhibited apraxia and dystonia as well as wringing of hands, tremor and other movement disorders. Nonetheless, we used the Gesell Developmental assessment, as modified by Knobloch, Stevens and Malone. It indicates adaptive, gross motor, fine motor, language and personal-social skills expected at intervals of four weeks in the first year of life, and at 13, 15, 18, 21, 24, 30 and 36 months. This gave us an approximate level of overall ability in the Rett girls. The developmental quotient (DQ) could then be calculated according to the formula:

\[
DQ = \frac{\text{maturity age}}{\text{chronologic age}} \times 100
\]

but only to a maximum chronologic age of 16 years (192 months) for the older patients. In addition we used the Alpern-Boll Developmental Profile, and there expressed overall ability according to the Academic Scale (see Table 1).

With respect to the movement disorders, the extrapyramidal features were recorded in detail according to the Modified Columbia Scale, but this had to be changed further, omitting disorders of articulation and also finger dexterity, successive movements and foot tapping, as the patients could not cooperate in these tasks. The remaining features like facial expression, sialorrhea, tremor, rigidity and bradykinesia were scored. In Fahn and Marsden’s Disability Scale for Dystonia speech and sialorrhea, tremor, rigidity and bradykinesia were scored. In these tasks. The remaining features like facial expression, disorders of articulation and also finger dexterity, successive movements included a three-dimensional T1-weighted field echo sequences with brief administration of intravenous Propofol. The MR acquisition TR 24/TE 4.4/FLIP 30° performed with a 25.6 cm field of view and 256 x 256 matrix to yield 1x1x2 mm voxels of the entire brain for volumetric evaluation of the central nuclei.

The MRI scans were very detailed and were usually recorded with brief administration of intravenous Propofol. The MR sequences included a three-dimensional T1-weighted field echo acquisition TR 24/TE 4.4/FLIP 30° performed with a 25.6 cm field of view and 256 x 256 matrix to yield 1x1x2 mm voxels of the entire brain for volumetric evaluation of the central nuclei. This sequence was designed to permit direct overlap with the functional PET data. A standard T2-weighted fast spin echo sequence of the entire brain was also acquired as 5 mm axial slices (TR 3555/TE 16,80/AV 1). The three-dimensional T1 volume acquisition was also used as a reference map for regions of interest (ROI) on PET scans. Since there was considerable overlap between globus pallidus and putamen, it was decided to measure their combined size in the lentiform nuclei.

Paired t-tests were used to test the significance of the difference between controls and cases in each region.

The PET scans were performed at the University Hospital using a Siemens/ECAT 953B scanner which permitted the simultaneous acquisition of 31 slices with an in-plane resolution of 5.6 mm FWHM and a slice thickness of 4.5 mm.

The patients were fasted for eight hours prior to scanning. About one hour before the injection of isotope, a sample of EMLA cream was applied at the site of the venipuncture to provide local anesthesia. Since the patients had to be immobile for a total of about five hours in the scanner with a thermoplastic face mask over their heads, we considered it most humane to give light intravenous anesthesia with Propofol (2.6 disproporpholphenol) during that time.

The nine patients had a median weight of 39.6 kg (range 24-61.5 kg). An infusion of Propofol was begun at 200-350 µg·kg⁻¹·min⁻¹. Sleep was maintained with Propofol infusion, range 57.6-189.5 µg·kg⁻¹·min⁻¹ (median 107.58), as clinically indicated by observing the depth and pattern of respiration, and guided by the end tidal carbon dioxide (CO₂).

The plasma concentration of Propofol was measured in non-arterialized venous blood, following induction of anesthesia (IS) and again just before discontinuation of the Propofol infusion (DS). Mean plasma concentrations of Propofol ± 1SD (standard deviations) were, respectively, IS 2.61 ± 1.19 µg·ml⁻¹ and DS 2.56 ± 1.4 µg·ml⁻¹, as measured by the method described by Plummer. One RS patient, aged 18 years, was noted to hyperventilate in the waiting area and then became deeply cyanosed. When placed in the scanner later, she was found to have an oxygen haemoglobin saturation (SpO₂) dropping to 53 and 60 (Nellcor Inc. USA) on two occasions. When she awoke from Propofol, the end tidal CO₂, monitored at the nares was observed to decrease from 30 to 8 mm Hg after a period of tachyypnoea (RR 39) followed by severe hypoxia (SpO₂ 43) when breath-holding had lasted for 65 seconds, thus illustrating the labile respiratory state.

The patients were placed in the scanner with the image plane parallel to the orbito-mental line and a moulded thermoplastic face mask was applied to restrict head movement. A 10 minute transmission scan using rotating 68Ga/Ge rods was performed for attenuation correction. The first isotope, [11C]-raclopride, was injected intravenously. The injected dose was 1.96 to 4.33 MBq/kg (mean 2.79) depending on the patient’s age and weight. Six scans of 300 s duration were performed in 3D mode between 30 and 60 minutes following the tracer administration. Approximately 15 minutes after the end of the raclopride scan the patients received an intravenous injection of 6-fluoro-[18F]-L-dopa (6FD). The injected dose of this ranged from 1.96 to 4.37 MBq/kg (mean 2.77).

In scan analysis, activity was summed over 30-60 minutes for [11C]-raclopride and 60-120 minutes for 6FD, following tracer administration. T1-weighted axial MR images were co-registered to integrated PET images using the automated registration program described by Woods et al. Circular ROIs, 8.8 mm in diameter, were placed over the head of the caudate nucleus and contiguously without overlap along the long axis of the putamen on each side, using the registered MR images. These regions were then transposed to the corresponding PET images and the position was optimized to obtain the greatest activity. Three circular ROIs with a diameter of 19.4 mm were placed...
Table 1: Analysis of Nine Females With Rett Syndrome Above Age 12 Years

<table>
<thead>
<tr>
<th>Stage</th>
<th>Age (yrs)</th>
<th>Neonatal history</th>
<th>Walked freely (age)</th>
<th>Speech</th>
<th>Nutrition</th>
<th>Orthopedic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>III</td>
<td>24</td>
<td>Mild problems</td>
<td>15-1/2 mos.</td>
<td>Had 1 word (up)</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sucking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td>III</td>
<td>16</td>
<td>Skin disorder</td>
<td>2 years</td>
<td>None</td>
<td>Slow to gain, eats well</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>? eczema; short right leg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 3</td>
<td>III/IVB</td>
<td>14</td>
<td>Vomiting, hypotonia</td>
<td>never</td>
<td>Had 2-3 words together in 3rd year</td>
<td>Feeding problems</td>
</tr>
<tr>
<td>Case 4</td>
<td>IVB</td>
<td>19</td>
<td>Limpness, fever; then little interest at 3 months</td>
<td>never</td>
<td>None</td>
<td>Feeding and chewing problems, has button gastrosomy</td>
</tr>
<tr>
<td>Case 5</td>
<td>III</td>
<td>26</td>
<td>Mucus and difficulty breathing, incubator overnight</td>
<td>2 years</td>
<td>Single words at 8-9 mos, and until 9th year</td>
<td>N</td>
</tr>
<tr>
<td>Case 6</td>
<td>III</td>
<td>16</td>
<td>Problems with sucking</td>
<td>2½ years</td>
<td>Few words at 2 years</td>
<td>N</td>
</tr>
<tr>
<td>Case 7</td>
<td>III</td>
<td>18</td>
<td>Sleepy; incubator</td>
<td>22 mos.</td>
<td>Many words from 8 mos and later some sentences, though with echolalia. Lost speech at 5-8 years, but still understands it moderately</td>
<td>Now N but slow feeding in infancy</td>
</tr>
<tr>
<td>Case 8</td>
<td>III</td>
<td>17</td>
<td>Occasional apnoea</td>
<td>18 mos.</td>
<td>Babbled at 1 year, said &quot;papa&quot;</td>
<td>N</td>
</tr>
<tr>
<td>Case 9</td>
<td>III</td>
<td>16</td>
<td>Cyanosis, dyspnoca, colics</td>
<td>18 mos.</td>
<td>Single words and “Hi, Mum” at 1 year</td>
<td>Feeding problems first 8 years</td>
</tr>
</tbody>
</table>

N = normal
Stage IVB = late motor deterioration, never walked freely

over the parieto-occipital cortex on each side and the mean activity was used as a reference. Data are reported as the mean ratio of integrated activity in caudate or putamen to that in parieto-occipital cortex. Such values have been shown to correlate well with the influx constant (kᵢ) for 6FDG and the binding potential (Bₘₐₓ/Kᵤ) for [¹¹C]-raclopride.

Mutational analysis

Peripheral blood samples were drawn from all patients and spotted onto FTA paper (Gibco BRL), processed according to manufacturer’s instructions and used in PCR for MECP2 amplification. Exons 2 and 3 were amplified using three pairs of primers in standard touchdown protocol (primers and protocol are available upon request). The MECP2 gene fragments were purified using the QIAquick PCR kit (QIagen) and sequenced bidirectionally using the ABI Prism BigDye Terminator Cycle Sequencing Reaction kit (PE Biosystems) analyzed on an ABI 377 DNA sequencer (PE Biosystems). Data analysis was performed with visual inspection of chromatograms.

RESULTS

Clinical analysis

The clinical details of the nine patients are summarized in Table 1. It will be seen that their ages ranged from 14 to 26 years (mean 18.4 years). None of the patients had been born prematurely or with low birth weight but neonatal problems (in first four weeks) like difficulties with breathing or sucking, hypotonia or drowsiness had been observed in all nine, and two had been placed in incubators. Despite this, all nine had been considered as essentially healthy during the first six months. Later, two had never learned to walk freely; the other seven learned to walk at a mean age of 21.6 months. Two had never learned to form any words; four had not put two or three words together; the other three had done so temporarily from the end of the first to the third year onwards. Four of the nine girls had been slow to gain weight in the first few years. All nine had orthopedic problems and seven had undergone remedial surgery, particularly heelcord lengthening; eight had at least mild
scoliosis (four with kyphosis), and contractures were common.
At the recent examination two girls were toe walking, although
the heel cords had been lengthened. Two had fractured long
bones and osteopenia was common. Seven of the nine had had
seizures, ranging from tonic-clonic (4) to simple partial (4),
atonic (4), absence (3), and myoclonic (3). Infantile spasms had
not been observed.

On examination all the nine girls were significantly small
 (>2SD below the mean for age) and three had a significantly
small head circumference, while two others had a borderline
small circumference (-2SD). The evolution of head
circumferences in these nine girls is shown in Figure 1 in
comparison to the normal range.63,64 Stenbom et al65 have noted
a correlation between the severity of motor disability at 12 years
and the rate of deceleration of head growth in RS. This would
apply in our cases 3 and 4 who had never been able to walk and
are also the most handicapped mentally. Hagberg66 and her
associates have recently added to the correlations of head growth
with height and motor functions in RS.

Hand skills in our patients were usually poor, and the frequent
hand wringing tended to interfere with purposive motion. The
dystonia and apraxia also made it difficult for the girls to get into
the standing position but, with some assistance they were often
able to walk a few steps and their skills were usually highest in
the gross motor sphere, but averaged at less than one year. As
will be seen from Table 1, the Revised Gesell DQ scores ranged
from only 2.3 to 8.1 (mean 5.32). The Gesell DQ and the Alpern-
Boll Academic scores show a Pearson correlation coefficient of
0.65 in the nine patients, which is significant at the p=0.05 level.

Thus the girls were all profoundly handicapped mentally.
However, all the mothers had found isolated higher abilities, e.g.
in “eye pointing”, in comprehension of gestures and even of
spoken orders, in recognizing musical tunes and in indicating
toilet needs. Intermittent hyperventilation was noted, particularly
with emotional tension, but no respiratory abnormalities were
seen in non-REM sleep.67

With respect to the scores of behavioral development there
was a significant correlation with the height (p=0.05), weight

<table>
<thead>
<tr>
<th>Seizures</th>
<th>Present</th>
<th>Weight for</th>
<th>Head</th>
<th>Hand skills</th>
<th>Gesell Developmental Level</th>
<th>Alpern-Boll Academic Scale</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>height</td>
<td>height</td>
<td>circm.</td>
<td></td>
<td>DQ</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>None</td>
<td>150 cm</td>
<td>N</td>
<td>51.9</td>
<td>Feeds self, right handed</td>
<td>5.2</td>
<td>10 mos.</td>
</tr>
<tr>
<td>Case 2</td>
<td>From 3 yrs</td>
<td>143 cm</td>
<td>N</td>
<td>51.9</td>
<td>Feeds self with Velcro mitten, left limbs more mobile</td>
<td>4.4</td>
<td>8-9 mos.</td>
</tr>
<tr>
<td>Case 3</td>
<td>From 4-5 yrs</td>
<td>127 cm</td>
<td>N</td>
<td>49.1</td>
<td>Helpless but grasps toy, tremor</td>
<td>2.9</td>
<td>5 mos.</td>
</tr>
<tr>
<td>Case 4</td>
<td>From 6 years</td>
<td>144 cm</td>
<td>N</td>
<td>51.8</td>
<td>Slight tremor, rigid, wrings hands +</td>
<td>2.3</td>
<td>4-5 mos.</td>
</tr>
<tr>
<td>Case 5</td>
<td>Yes, partial, ? onset at 4 years-2 yrs</td>
<td>152.5 cm</td>
<td>N</td>
<td>54.4</td>
<td>Turns pages, picks up crumbs, left handed</td>
<td>8.1</td>
<td>15-16 mos.</td>
</tr>
<tr>
<td>Case 6</td>
<td>From 4 yrs</td>
<td>149 cm</td>
<td>N</td>
<td>52</td>
<td>Feeds self, may bite left hand but prefers using it</td>
<td>7.1</td>
<td>13-14 mos.</td>
</tr>
<tr>
<td>Case 7</td>
<td>From 4 yrs</td>
<td>145 cm</td>
<td>N</td>
<td>52.5</td>
<td>Can feed self, not cut, uses spoon</td>
<td>6.5</td>
<td>11-12 mos.</td>
</tr>
<tr>
<td>Case 8</td>
<td>None</td>
<td>144 cm</td>
<td>N</td>
<td>51.7</td>
<td>Left handed, cannot feed self since age 2 yrs 3 mos.</td>
<td>5.9</td>
<td>11-12 mos.</td>
</tr>
<tr>
<td>Case 9</td>
<td>From 11 years</td>
<td>150.7 cm</td>
<td>N</td>
<td>51.5</td>
<td>Poor hand skills but finger feeds, pronation in mutual grasping right &gt; left</td>
<td>5.5</td>
<td>10-11 mos.</td>
</tr>
</tbody>
</table>

N = normal
Gesell D.Q. = Revised Gesell Developmental Quotient
Observations on Movements

Of the nine patients, four were left-handed; the relatively high proportion of left-handers appears well-established, and Witt-Engerström even found nine out of 11 girls with RS left-handed at the age of 20-51 months (mean 2½ years). In a British survey of 201 families with classical Rett girls, 44% reported right-hand preference, 27% left preference, and 29% no preference. Rigidity of limbs was noted in three of the present girls, while the remaining six were considered to have slight to moderate dystonia on the Fahn-Marsden assessment, often somewhat asymmetrical. Five of the nine had sufficient use of the better hand to assist in feeding themselves despite apraxia. Six also had some difficulty in chewing or swallowing, with occasional choking. Bruxism was noted in five of nine RS girls. Reduced facial expression (hypomimia) was observed in eight of the nine girls, drooling in four.

Hyperventilation, often followed by breath holding, was noted in six of the nine girls and was associated with screaming or laughing in three. The associated hand movements were often asymmetrical and used for communication. Tremor was seen intermittently in six of the nine girls; it was usually mild and slow (3-6/sec) and associated with activation of movement or with apprehension. Bradykinesia was noted in six of the nine patients, usually with dystonia in the affected limbs; as defined in the Modified Columbia Scale it combined slowness of movement with poverty of movement in general.

Dystonia was most marked in the limbs and particularly in the legs, where heelcord contractures were almost constant but often asymmetrical. However, it was clearly dependent on the girls’ emotions, and provoking factors were noted. There was a significantly positive nonlinear association between the score of Parkinsonian features as measured in the Modified Columbia Scale, and the degree of dystonia as indicated by the Dystonia Product in the individual patients (see Figure 3). On the other hand, there was no significant association between standardized head circumference and measurements of either Parkinsonism or dystonia.

(p=0.02) and head circumference (p=0.03) of the Rett girls, as shown for the Gesell DQ in Figure 2.

Figure 1: Head circumference growth in the nine RS patients is superimposed on mean head growth curve of normal females ± 2SD. Note that the head circumference in RS was always below the normal mean after the age of three years.

Figure 2: Standardized height, weight and head circumference in nine RS patients, as related to Revised Gesell DQ score (modified from Knobloch, Stevens and Malone). Significant relationships are demonstrated.
Comparison to controls. All these differences between RS patients and control females are significant as shown by the p values of <0.05.

The extrapyramidal features in the Modified Columbia Scale correlated quite well with the Fahn and Marsden Functional Disability Scale products for dystonia (see Figure 3). However, there was no significant correlation between scores of motor function and the uptake of fluorodopa or binding of raclopride.

Results of mutational analysis

Analysis of MECP2 mutations in our nine patients (Figure 4) reveals that with careful technique a mutation in the MECP2 gene was found in all nine cases, five being nonsense mutations and four being missense mutations. The missense mutation P127L in Case 6 was located in the MBD; it was novel but was recently also noted in a Finnish girl with RS (see Discussion).71,72

Statistical analysis (t-test) showed no significant correlation between the type of mutation and the findings in volumetric difference in the caudate nuclei and thalami. [18F]-6-fluoro-L-dopa uptake and [11C]-raclopride binding in caudate and putamen also were not statistically correlated with the type and location of mutation.

Discussion

It has long been recognized that reduction of striatal dopaminergic activity in Parkinson’s disease is demonstrable by PET with use of the tracer [18F]-fluoro-L-dopa61,73 and this can be shown even at a presymptomatic stage.74,75 Vingerhoets et al76 demonstrated that PET scans using fluorodopa in normal subjects are reproducible and discriminating to a satisfactory extent. The further use of [11C]-raclopride in studying postsynaptic dopamine D2 receptor activity in Parkinson’s disease and other movement disorders has shown either increased or normal values.77-80 Antonini and his colleagues81 found that patients with Parkinson’s disease at Stages I to II exhibited reduced F-Dopa metabolism, particularly in the putamen, and this declined further in the course of the disease. Raclopride binding to dopamine D2 receptors in the putamen appeared to be up-regulated in patients with early Parkinson’s disease but subsequently declined towards control values in the later stages.

Our findings in RS may suggest a similar process but with less difference between putamen and caudate than in Parkinson’s disease. Also, while the caudate structures have been reported to have the most marked reduction on volumetric MRI studies in the upper half of Table 2. Group means and SD are indicated below the individual results. In the lower half of Table 2 the corresponding results in young healthy control women aged 21 to 29 years are also listed. However, they did not have Propofol anesthesia and were given carbidopa 200 mg orally one hour before fluorodopa. The difference in the mean uptake of fluorodopa in the caudate nuclei of the Rett patients compared to the controls may be calculated as a reduction of 0.302, i.e. 13.1% of the mean control value, while the corresponding reduction of mean fluorodopa uptake in putamen is 0.283, or 12.5%. With respect to the binding of raclopride the mean value in Rett patients is about 4.008 in caudate, and thus 0.354 higher than the mean in the controls, representing an increase of 9.7%. The corresponding difference in the raclopride binding in putamen is a mean increase of 0.359 in the Rett patients, which represents 9.6% in comparison to controls. All these differences between RS patients and control females are significant as shown by the p values of <0.05.

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Our findings in RS may suggest a similar process but with less difference between putamen and caudate than in Parkinson’s disease. Also, while the caudate structures have been reported to have the most marked reduction on volumetric MRI studies in RS, the up-regulation of D2 dopamine receptor activity evidently continues in Stages III and IV.

With respect to the movement disorder of RS patients the pathogenesis of the apraxia, synkinesias and dystonia remains obscure. It is recognized in humans that delayed onset dystonia may also follow perinatal or early childhood asphyxia82 and particularly anoxic damage to putamen,83,84 Segawa85 has recently suggested that in RS, failure of the supplementary motor area may be responsible for the impairment of purposeful hand use, while the dystonia and stereotyped hand movements may be caused by dysfunction of the nigrostriatal dopamine neurons and basal ganglia.

It may be asked whether the low uptake of fluorodopa on the PET scan of the basal ganglia in our RS girls could be explained...
by the smaller size of the corpus striatum in comparison to the control subjects. Rousset et al.86 investigated the effect of partial volume correction on estimates of the influx and cerebral metabolism of 6-[18F] fluoro-L-dopa studied with PET in normal control and in Parkinson’s disease subjects. After partial volume correction the apparent net blood-brain clearance of FDOPA (K_i) was greatly increased in caudate and putamen of normal subjects and in caudate of Parkinson’s disease patients. However, the careful co-registration with MRI in our RS patients and controls argues against the likelihood of such a major effect. Partial volume averaging effect due to striatal atrophy would also be expected to result in reduced [11C]-raclopride binding whereas, in contrast, an increase was seen. Accordingly, reduced dopaminergic activity in this area would seem to be a more likely cause.

With respect to another problem, the lack of carbidopa given to Rett patients could not account for the decreased fluorodopa uptake.

### Table 2: Integrated Activity of Isotopes in PET Scans

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<tr>
<th>Rett Syndrome</th>
<th>FD</th>
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</table>

CD = caudate  
FD = [18F]-6-fluoro-L-dopa (mean right and left uptake)  
PUT = putamen  
RAC = [11C]-raclopride (mean right and left binding)

The difference in the mean uptake of fluorodopa in the caudate nuclei of the Rett patients compared to the controls may be calculated as a reduction of 0.302, i.e. 13.1% of the mean control value, while the corresponding reduction of mean fluorodopa uptake in putamen is 0.283, or 12.5%. With respect to the binding of raclopride the mean value in Rett patients is about 0.408 in caudate, and thus 0.354 higher than the mean in the controls, representing an increase of 9.7%. The corresponding difference in the raclopride binding in putamen is a mean increase of 0.359 in the Rett patients, which represents 9.6% in comparison to controls. All these differences between RS patients and control females are significant as shown by the p values of less than 0.05.

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uptake, as neither this uptake rate nor the striatal/cortical ratio are dependent on the administration of carbidopa.\(^87\)

Another factor that may affect our interpretation is the slight age difference between the nine control females in the PET scans (mean 23.2 years), as compared to the RS patients (mean age 18.4 years). It is still controversial whether there is an age-dependent decline of nigrostriatal dopaminergic function as measured by striatal \(^{18}\)F-dopa uptake.\(^88,89\) Although there is a consensus that raclopride binding is reduced with age, this effect, resulting in a decline of approximately 0.16 per decade in the striatal:occipital ratios (unpublished results) would be insufficient to account for the difference seen here. In any case, this age difference should be of little significance as it is small and, if there were an age effect, it should be associated with a lower uptake of fluorodopa and declining nigrostriatal dopaminergic function in the control females, whereas the opposite was found.

**Figure 4:** Analysis of MeCP2 mutations in our nine patients reveals that with careful technique a mutation in the MECP2 gene was found in all nine cases, five being nonsense mutations (■) and four being missense mutations (●).

### Use of Propofol

The question whether the reduced uptake of fluorodopa and also the increased binding of raclopride in the girls with RS might be at least partly attributable to their Propofol anesthesia requires more detailed discussion.

Propofol (2,6-diisopropylphenol) is available as an induction agent and sedative for anesthesia and is also useful by its direct antiemetic properties. Borgeat et al\(^90\) summarized its therapeutic applications but noted that it was vagotonic and potentiated GABA-mediated effects at both spinal and supraspinal levels.

In an overview article in 1995, Fulton and Sorkin\(^91\) described this medication as controlling stress responses and having anticonvulsant and amnesic properties. It does have cardiac depressant effects, usually with modest decrease in heart rate and blood pressure but is generally associated with adequate hemodynamic stability in patients requiring sedation for short periods. A more serious Propofol infusion syndrome, which may
be fatal in children, has been described\(^9^2\) but does not occur with infusions for less than 48 hours.

Alkire et al\(^9^3\) studied cerebral metabolism during Propofol anesthesia in humans with PET. Using \(^{18}\)F fluoro-dextrose-glucose they found in six volunteers that in the awake state whole brain glucose metabolic rate (GMR) averaged 29 ± 8 \(\mu\)moles \(\cdot\) 100 g\(^{-1}\) \cdot min\(^{-1}\) (mean ± SD), whereas anesthetized whole brain GMR averaged only 13 ± 4 \(\mu\)moles \(\cdot\) 100 g\(^{-1}\) \cdot min\(^{-1}\) (paired t-test \(P=0.007\)). Glucose metabolic rate decreased in all measured areas during anesthesia but not uniformly. In the mid-brain and basal ganglia GMR was preserved slightly better during anesthesia than in other parts of the brain.

One recent report\(^9^4\) documents reduced electrically stimulated dopamine release in rat nucleus accumbens slices in the presence of Propofol. However, Shyr et al\(^9^5\) investigated the activities of dopamine and serotonin in rats receiving Propofol anesthesia. \textit{In vivo} microdialysis was used to measure the major metabolites of dopamine and serotonin, i.e. 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) in the somatosensory cortex. The authors found that IV infusion of Propofol at the high rate of 60 mg \(\cdot\) kg\(^{-1}\) \cdot h\(^{-1}\) significantly increased the amount of all three metabolites. These changes correlated well with Propofol levels in brain and blood. It was concluded that anaesthetic doses of Propofol increased the functional activities of dopamine and serotonin in the cortex. Thus, at least in cortex, dopaminergic activity is \textit{increased} rather than diminished by Propofol. Also, if Propofol were to increase striatal dopamine release one might expect to see a concurrent reduction in the binding of \(^{11}\)C-raclopride, reflecting increased occupancy of D2 receptors by dopamine. However, the converse was true.

The question arises whether Propofol can interact directly with D2 dopamine receptors. Appadu and colleagues\(^9^6\) investigated this question in Chinese hamster ovary cells. The binding of Propofol, spiperone and metoclopramide to D2 receptors was measured. Both spiperone and metoclopramide displaced the binding of \(^{3}\)H spiperone dose-dependently. Although Propofol did cause a modest dose-dependent displacement of \(^{3}\)H spiperone binding, 50% displacement was not achieved, even at the highest concentration. Like dopamine D2 receptor antagonists, Propofol in subhypnotic doses is an antiemetic but these studies showed that it does not interact strongly with D2 dopamine receptors. Propofol could neither prevent apomorphine-induced vomiting, which is mediated by dopaminergic receptors, nor could a continuous infusion of subhypnotic doses of Propofol increase the plasma levels of prolactin, a very sensitive marker of dopaminergic receptor blockade.\(^9^7\) We may conclude that Propofol is unlikely to have had any marked effect on D2 dopamine receptors in the RS patients during the PET scans.

With respect to \textit{genetics}, there has been growing interest in determining the significance of MeCP2 mutations in the phenotype of patients with RS. Cheadle et al\(^4^6\) suggested that patients with missense mutations tend to have milder forms of the disease as compared with those carrying truncating mutations. However, this correlation is still under debate.\(^9^8\)-\(^1^0^0\) Early truncating mutations seem to produce a more severe phenotype which may not be compatible with survival unless there is nonrandom X-chromosome inactivation.\(^4^5\)

The patients in our study had classical RS. With careful technique, a mutation in the \textit{MECP2} gene was found in all nine cases. However, a recent article\(^7^1\) described 39 Finnish patients with classical RS who also \textit{all} exhibited the \textit{MECP2} mutation. Interestingly, in our previous paper\(^8\) we also noted that one of our classical RS patients (the present Case 6) was classified as P127L, a newly recognized mutation in the MBD. The same mutation P127L was found in one of these Finnish girls, but she was noted to have the preserved speech variant, which was not present in our patient. Further studies are warranted to clarify the role of this mutation. No correlation was found between the type and location of mutation and the \(^{18}\)F-6-fluoro-L-dopa uptake and \(^{11}\)C-raclopride binding in caudate and putamen.

**Conclusion**

In our investigations the demonstration of significantly reduced volume of the caudate nuclei and thalami in the Rett patients on volumetric MR scanning confirms previous findings. The PET scans show a notable reduction in the mean uptake of 6-fluoro-\(^{18}\)F-dopa as compared to healthy control girls of similar age, namely 12 to 13% in caudate nuclei and putamen (see Table 2). This raises the question of partial volume averaging effects. However, the binding of raclopride was increased in comparison to the control girls by nearly 10% in the same regions. All these differences are statistically significant and suggest that dopaminergic activity is reduced and there is a compensatory increase in D2 receptor activity. Whether the increase in \(^{11}\)C-raclopride binding reflects an increase in receptor density, or reduced receptor occupancy secondary to loss of synaptic dopamine cannot be determined on the basis of our results. It is known that increased D2 binding as measured by PET in early parkinsonism probably reflects increased receptor density in putamen.\(^1^0^1\) While this might also be the case in RS, the findings would also be compatible with reduced receptor occupancy by endogenous dopamine. If reduced dopaminergic activity in RS can be confirmed, controlled dopaminergic therapy might be attempted. However, it is evident that careful neurological analysis of the patients will continue to be required\(^7^2\) in conjunction with MRI and PET scans and even with cerebral proton magnetic resonance spectroscopy. Detailed assessment of changes in the gene \textit{MECP2}, its distribution and mutations of encoded proteins as well as investigation of transgenic models in animal brains will also have to be continued. It will be of interest to screen \textit{MECP2} mutations in any unaffected female siblings, always in conjunction with the karyotype and pattern of X chromosome inactivation, in order to diagnose a nonmanifesting carrier who could transmit the defect to future offspring.\(^1^0^2\) On the other hand, a recent Swedish article\(^1^0^3\) describes four families with two clinical Rett patients in each family, who were found completely negative for \textit{MECP2} mutations. Thus the question of genetic mechanisms in nonclassical cases of RS remains wide open.

**Addendum**

Subsequent to submission of this paper, the publication of three new articles examining the function of \textit{MECP2} has confirmed widespread and complex phenotypic consequences of mutations within this gene. Couvert, Bienvenu, Aquaviva et al\(^1^0^4\)
have identified four of 185 patients (2%) with X-linked mental retardation who have mutations in MECP2, and conclude that this is an important gene in retarded persons. Huppke, Held, Hanefeld et al. have confirmed the results of Cheadle et al. indicating a greater phenotypic severity associated with truncating mutations and have further shown that mutations associated with truncation of the region coding nuclear localization signals have particularly severe phenotypic abnormalities. Huppke, Bohlander, Krämer et al. found weak correlation between mutations in MeCP2 and altered methylation patterns in X-linked genes (G6PD and SYBL1) and suggest widespread dysregulation of X chromosomal genes in Rett syndrome.

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We thank all the RS patients and their families and the control volunteers for their participation in these studies. The following persons have supported our investigations and we thank: Dr. Sarojini Budden, Dr. Donald Calne, Dr. Barry Snow, Dr. Tom Ruth and the UBC-TRIUMF PET team, Dr. Ruth Grunau, Dr. David B. Levin, Ms. Laurie Ainsworth, Ms. Susan Rybak, and Ms. D. Susan Kube. The genetic studies were supported by the International Rett Syndrome Association and the rest of the study was aided by the Vancouver Foundation.

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