ORIGINAL ARTICLE

Rett Syndrome: Investigation of Nine Patients, including PET Scan

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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 [^{18}F]-6-fluorodopa and [^{11}C]-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 tomographie par émission de positons. ***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 tomographie par émission de positons (PET scan) au [^{18}F]-6-fluorodopa et au [^{11}C]-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.

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Since 1983, when Hagberg et al[1] published an extensive report on the syndrome of brain atrophy in girls first described by Andreas Rett[2] in Austria, it has become customary to divide the classical course of the disease into four stages.[3,4] 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

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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[5,6] and scoliosis are common, and difficulties with swallowing and gastroesophageal motility[7] 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 heelcords and trophic cyanotic 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.[8] 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.[9,10] Particular neuronal abnormalities have been described in the pars compacta of the substantia nigra.[11]

Biochemical studies have shown normal[12,13] or reduced[14] 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.[15-17] In 1995, Wenk[18] 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[19] 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[20] 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[21] who had reported markedly *increased* specific binding of [^{123}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.[22-24] In 1999, Amir and other investigators[25] then 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)[26] and a transcriptional repression domain. These domains are thought to interact with several other proteins to promote gene silencing during development.[27-29] 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.[30] 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.[28] The region was also recognized recently[31] 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.[32]

Milder phenotypes may occur if X chromosome inactivation is nonrandom, thus favouring the expression of the normal *MECP2* allele.[33,34] Milder phenotypes have also been found in males with more than one X chromosome, particularly Klinefelter syndrome[35-37] and with mosaicism for truncating MeCP2 mutation R270X.[38] Nonspecific X-linked mental retardation may also occur with the A140V mutation of the *MECP2* gene in either sex, but more severely in males.[39] 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.[40] 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.[41-51] About 75%-80% of classical RS patients have characteristic mutations in *MECP2*.[33,44-46,48,51] On the other hand, in familial and atypical RS patients only 20%-40% have the characteristic abnormality of the *MECP2* gene.[45,46,52] 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[53] and Fahn and Marsden's Functional Disability Scale for Dystonia.[54]
2. an assessment of the girl's behavioral development by the Revised Gesell technique[55,56] and Alpern Boll Developmental Profile.[57]
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

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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.[56] 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,[53] 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[54] speech and writing were insufficient for scoring, and dressing could not be considered in practice, but feeding, hygiene and walking were assessed. The motor skills of the RS patients were also documented on videotape. Provoking and severity factors were calculated for each body region, and their weighted products were summed for the movement scale, with Pearson or Spearman correlation.

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.

In each of the nine cases and in their corresponding age-matched controls the volumetric measurements of the two sides were averaged in the caudate, lentiform nuclei and thalami. 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 diisopropylphenol) 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 $\mu g \cdot kg^{-1} \cdot min^{-1}$. Sleep was maintained with Propofol infusion, range 57.6-189.5 $\mu g \cdot kg^{-1} \cdot min^{-1}$ (median 107.58), as clinically indicated by observing the depth and pattern of respiration, and guided by the end tidal carbon dioxide (CO_2).

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 $\mu g \cdot ml^{-1}$ and DS 2.56 ± 1.4 $\mu g \cdot ml^{-1}$, as measured by the method described by Plummer.[58] 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 (SpO_2) dropping to 53 and 60 (Nellcor Inc. USA) on two occasions. When she awoke from Propofol, the end tidal CO_2 monitored at the nares was observed to decrease from 30 to 8 mm Hg after a period of tachypnoea (RR 39) followed by severe hypoxia (SpO_2 43) when breath-holding had lasted for 65 seconds, thus illustrating the labile respiratory state.[59]

The patients were placed in the scanner with the image plane parallel to the orbito-meatal line and a moulded thermoplastic face mask was applied to restrict head movement. A 10 minute transmission scan using rotating $^{68}Ga/Ge$ rods was performed for attenuation correction. The first isotope, [^{11}C]-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-[^{18}F]-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 [^{11}C]-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.[60] 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

	Stage	Age (yrs)	Neonatal history	Walked freely (age)	Speech	Nutrition	Orthopedic
Case 1	III	24	Mild problems sucking	15-1/2 mos.	Had 1 word (up)	N	Mild kypho-scoliosis, tense heelcords, elbow contractures
Case 2	III	16	Skin disorder ? eczema; short right leg	2 years	None	Slow to gain, eats well	Scoliosis, contractures of elbows and ankles
Case 3	III/IVB	14	Vomiting, hypotonia	never	Had 2-3 words together in 3rd year	Feeding problems	Scoliosis, tight heelcords, flexion contracture L. knee
Case 4	IVB	19	Limpness, fever; then little interest at 3 months	never	None	Feeding and chewing problems, has button gastrostomy	Kypho-scoliosis, knee contractures, tight heelcords
Case 5	III	26	Mucus and difficulty breathing, incubator overnight	2 years	Single words at 8-9 mos. and until 9th year	N	Genu valgum, heelcords lengthened at 16 years, has orthoses, slight scoliosis
Case 6	III	16	Problems with sucking	2½ years	Few words at 2 years	N	Mild scoliosis, orthoses for ankles, pes plano-valgus
Case 7	III	18	Sleepy; incubator 5 days, poor sucking	22 mos.	Many words from 8 mos and later some sentences, though with echolalia. Lost speech at 5-8 years, but still understands it moderately	Now N but slow feeding in infancy	Mild kyphosis, tight heelcords, L. foot turns in, elbow contractures
Case 8	III	17	Occasional apnoea	18 mos.	Babbled at 1 year, said "papa"	N	Kypho-scoliosis; fractured L. femur at 3 years
Case 9	III	16	Cyanosis, dyspnoea, colics	18 mos.	Single words and "Hi, Mum" at 1 year	Feeding problems first 8 years	Toe walking (despite orthoses) from 11 years, slight scoliosis; heelcords lengthened

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 (k_i) for 6FD[61] and the binding potential (B_{max}/K_D) for [^{11}C]-raclopride.[62]

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

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Table 1: Analysis of Nine Females With Rett Syndrome Above Age 12 Years ... continued

	Seizures	Present height	Weight for height	Head circumf. (cm)	Hand skills	Gesell Developmental Level **DQ**		Alpern-Boll Academic Scale	Notes
Case 1	None	150 cm -3 SD	N	51.9 -<2 SD	Feeds self, right handed	5.2	10 mos.	9 mos.	Allergies, strabismus
Case 2	From 3 yrs	143 cm -4 SD	-<2 SD	51.9 -2 SD	Feeds self with Velcro mitten, left limbs more mobile	4.4	8-9 mos.	12 mos.	Menarche delayed, leads way to washroom
Case 3	From 4-5 yrs	127 cm -6 SD	N	49.1 -3 SD	Helpless but grasps toy, tremor	2.9	5 mos.	4 mos.	No menarche yet. Myopia. Severely abnormal EEG.
Case 4	From 6 years	144 cm -4 SD	-1 SD now	51.8 -<2 SD	Slight tremor, rigid, wrings hands +	2.3	4-5 mos.	4-5 mos.	Rigidity, gastro-intestinal problems, esophagitis, hyperventilation, variable esotropia
Case 5	Yes, partial, ? onset at 4 years	152.5 cm ->2 SD	+2 SD	54.4 N	Turns pages, picks up crumbs, left handed	8.1	15-16 mos.	15 mos.	Acne rosacea, activation tremor
Case 6	From 4 years	149 cm ->2 SD	+<2 SD	52 -2 SD	Feeds self, may bite left hand but prefers using it	7.1	13-14 mos.	12 mos.	Irregular breathing, severely abnormal EEG, wrings hands
Case 7	From 4 years	145 cm -4 SD	N	52.5 -<2 SD	Can feed self, not cut, uses spoon	6.5	11-12 mos.	9 mos.	Drooling reduced by surgery, hyperventilates
Case 8	None	144 cm -4 SD	N	51.7 ->2 SD	Left handed, cannot feed self since age 2 yrs 3 mos.	5.9	11-12 mos.	8 mos.	Shoulders hunched
Case 9	From 11 years	150.7 cm - nearly 3 SD	-1 SD now	51.5 ->2 SD	Poor hand skills but finger feeds, pronation in mutual grasping right > left	5.5	10-11 mos.	10 mos.	Mild acne, understands speech, recognizes songs

N = normal
Gesell D.Q. = Revised Gesell Developmental Quotient

scoliosis (four with kyphosis), and contractures were common. At the recent examination two girls were toe walking, although the heelcords 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 al[65] 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. Hagberg[66] 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

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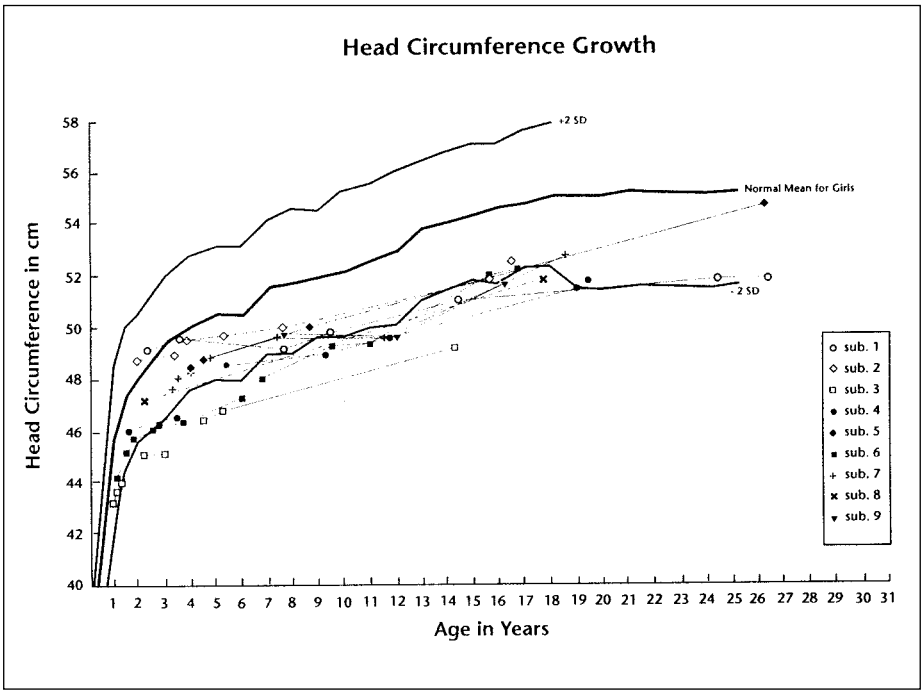

Figure 1: *Head circumference growth in the nine RS patients is superimposed on mean head growth curve of normal females ± 2SD.[63, 64] Note that the head circumference in RS was always below the normal mean after the age of three years.*

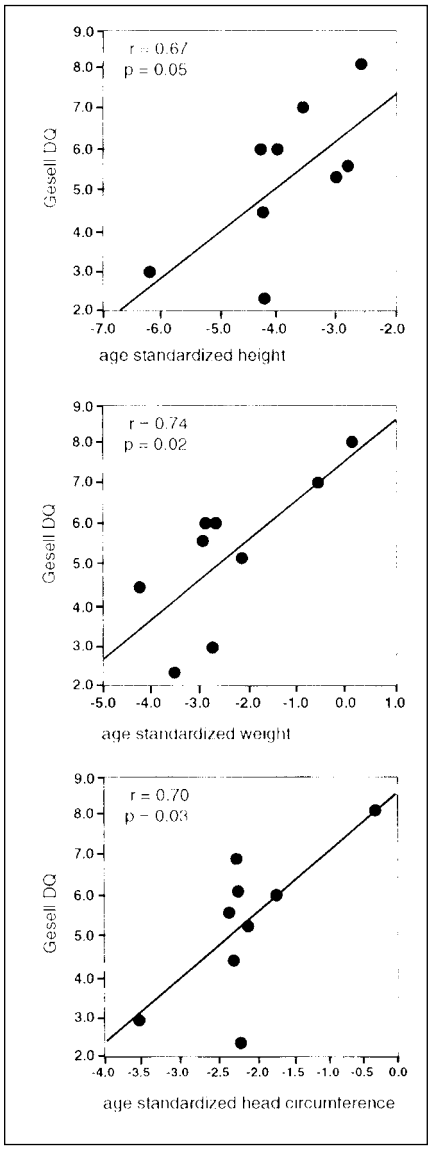

Figure 2: *Standardized height, weight and head circumference in nine RS patients, as related to Revised Gesell DQ score (modified from Knobloch, Stevens and Malone[56]). Significant relationships are demonstrated*

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

Observations on Movements

Of the nine patients, four were left-handed; the relatively high proportion of left-handers appears well-established, and Witt-Engerström[68] 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.[69] 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.[70] 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,[53] and the degree of dystonia as indicated by the Dystonia Product[54] 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.

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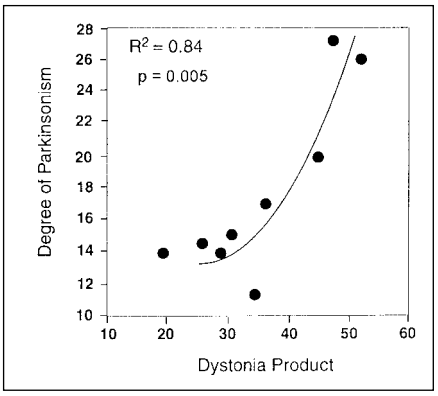

Figure 3: *Degree of Parkinsonian features[53] shows significantly positive non-linear association with Dystonia Product[54] in the nine RS patients. Significant relationship best shown by quadratic curve: Parkinsonian features = 25.9 - 0.99 x dystonia + 0.02 x dystonia$^{(2)}$.*

Results of MRI scans

Visual inspection of MRI imaging data showed four RS subjects with cerebral tissue loss, three of which were frontal-temporal in distribution. Two subjects had mild cerebellar tissue loss. Selective volume loss of the basal ganglia was only evident on volumetric studies. In this respect the values were taken from axial 1 mm slices oriented to the axis of the corpus callosum, extending from the anterior commissure to 1 mm above the thalami. The calculated volumes of the relevant part of the brain in each case were compared with those in the respective control girl. The mean difference (controls minus cases) in the caudate volume was 8.78, with an SD of 4.88 (p=0.006). In the lentiform nuclei the mean difference (4.42, SD 9.43) was not significant (p=0.197). In the thalami, a significant mean difference of 9.53 (SD 6.93) was observed (p=0.0033). Thus, in the caudate and in the thalami the volumetric measurements in the controls were significantly larger than in the cases.

Results of PET scans

The findings concerning the integrated activity of [^{18}F]-6-fluoro-L-dopa and [^{11}C]-raclopride in caudate and putamen as a ratio of that in parieto-occipital cortex are listed 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.

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. [^{18}F]-6-fluoro-L-dopa uptake and [^{11}C]-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 [^{18}F]-fluoro-L-dopa[61,73] and this can be shown even at a presymptomatic stage.[74,75] Vingerhoets et al[76] demonstrated that PET scans using fluorodopa in normal subjects are reproducible and discriminating to a satisfactory extent. The further use of [^{11}C]-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 colleagues[81] 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 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 asphyxia[82] and particularly anoxic damage to putamen.[83,84] Segawa[85] 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

Table 2: Integrated Activity of Isotopes in PET Scans

Rett Syndrome					
	FD		RAC		
Age (years)	CD	PUT	CD	PUT	
24	2.18	2.095	4	3.87	
16	2.035	1.925	4.095	3.835	
14	1.695	1.77	4.095	4.195	
19	2.11	2.04	3.885	4.09	
26	1.825	1.9	3.15	3.55	
16	1.9	1.795	4.13	3.985	
18	2.345	2.32	4.06	4.3	
17	2	2.005	4.41	4.495	
16	1.99	2.05	4.25	4.545	
18.4	**2.009**	**1.988**	**4.008**	**4.096**	**MEAN**
	0.193	**0.168**	**0.354**	**0.324**	**SD**
Control Females					
	FD		RAC		
Age (years)	CD	PUT	CD	PUT	
23	2.435	2.36	3.41	3.455	
26	2.52	2.545	3.56	4.045	
20	2.44	2.49	3.56	3.635	
29	2.16	2.1			
21	2.32	2.32			
26	2.17	2.11			
22	2.13	1.97			
21			3.97	3.8	
21			3.77	3.75	
23.2	**2.311**	**2.271**	**3.654**	**3.737**	**MEAN**
	0.159	**0.216**	**0.218**	**0.217**	**SD**
	0.005	**0.011**	**0.040**	**0.031**	**p-values**

CD = caudate
FD = [^{18}F]-6-fluoro-L-dopa (mean right and left uptake)
PUT = putamen
RAC = [^{11}C]-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 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 less than 0.05.

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-[^{18}F] 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 [^{11}C]-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

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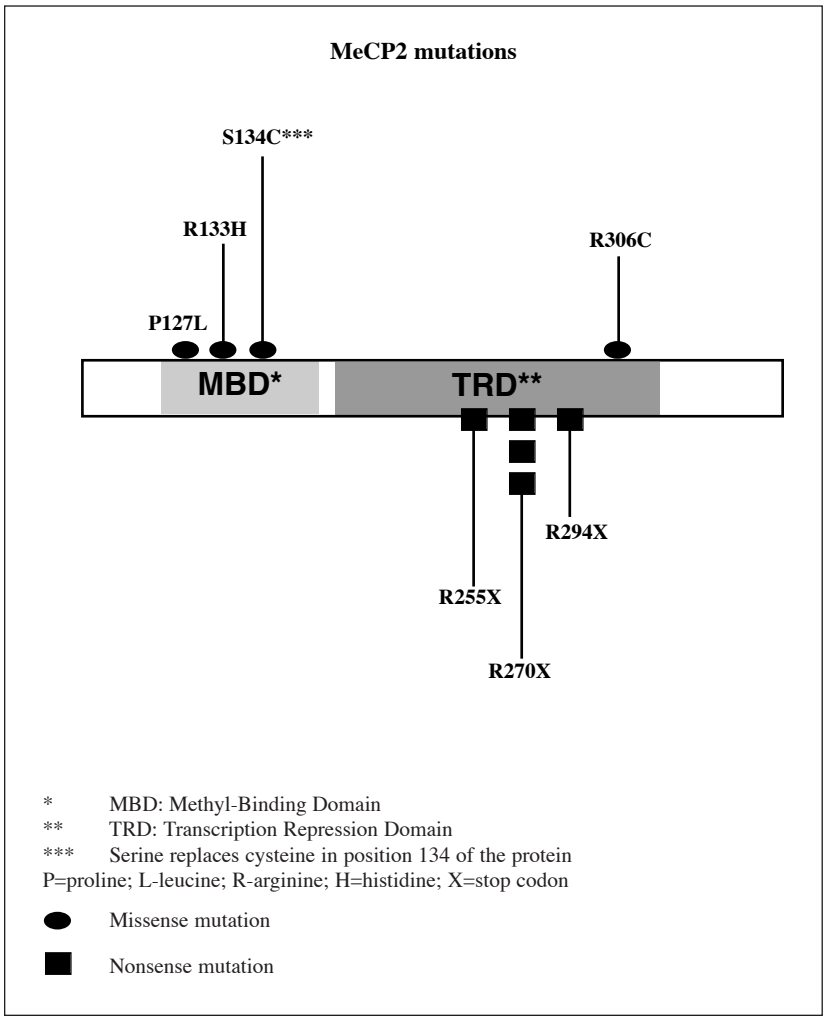

Figure 4: *Analysis of* MeCP2 *mutations in our nine patients reveals that with careful technique a mutation in the MECP2 gene was found in all nine cases, five being nonsense mutations (■) and four being missense mutations (●).*

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.

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 anti-convulsant 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[92] but does not occur with infusions for less than 48 hours.

Alkire et al[93] studied cerebral metabolism during Propofol anesthesia in humans with PET. Using [^{18}F] fluorodeoxyglucose they found in six volunteers that in the awake state whole brain glucose metabolic rate (GMR) averaged 29 ± 8 μmoles · 100 g^{-1} · min^{-1} (mean ± SD), whereas anesthetized whole brain GMR averaged only 13 ± 4 μmoles · 100 · g^{-1} · 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[94] documents reduced electrically stimulated dopamine release in rat nucleus accumbens slices in the presence of Propofol. However, Shyr et al[95] investigated the activities of dopamine and serotonin in rats receiving Propofol anesthesia. *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 · kg^{-1} · 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 *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[96] 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.[97] 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 *genetics*, there has been growing interest in determining the significance of MeCP2 mutations in the phenotype of patients with RS. Cheadle et al[46] 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.[98-100] Early truncating mutations seem to produce a more severe phenotype which may not be compatible with survival unless there is nonrandom X-chromosome inactivation.[45]

The patients in our study had classical RS. With careful technique, a mutation in the *MECP2* gene was found in all nine cases. However, a recent article[71] described 39 Finnish patients with classical RS who also *all* exhibited the *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.[101] 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[72] in conjunction with MRI and PET scans and even with cerebral proton magnetic resonance spectroscopy. Detailed assessment of changes in the gene *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 *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.[102] On the other hand, a recent Swedish article[103] describes four families with two clinical Rett patients in each family, who were found completely negative for *MECP2* mutations. Thus the question of genetic mechanisms in non-classical cases of RS remains wide open.

Addendum

Subsequent to submission of this paper, the publication of three new articles examining the function of *MECP2* has confirmed widespread and complex phenotypic consequences of mutations within this gene. Couvert, Bienvenu, Aquaviva et al[104]

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[105] have confirmed the results of Cheadle et al[46] 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[106] 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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