Study of MECP2 Gene in Rett Syndrome Variants and Autistic Girls

Michele Zappella,1 Ilaria Meloni,2 Ilaria Longo,2 Roberto Canitano,1 Giuseppe Hayek,1 Lucia Rosaia,3 Francesca Mari,2 and Alessandra Renieri2*

1Department of Child Neuropsychiatry, Azienda Ospedaliera Senese, Siena, Italy
2Medical Genetics, Department of Molecular Biology, University of Siena, Italy
3Molecular Genetics, Istituto G.Gaslini, Genova, Italy

Mutations in MECP2 gene account for approximately 80% of cases of Rett syndrome (RTT), an X-linked severe developmental disorder affecting young girls, as well as for most cases of Preserved Speech Variant (PSV), a mild RTT variant in which autistic behavior is common. The aim of this study is to determine whether MECP2 mutations are responsible for PSV only or may cause other forms of autistic disorders. We screened for mutations by SSCP 19 girls with a clinical diagnosis of autism, two of them fulfilling the PSV criteria. A pathogenic mutation was found only in the latter two cases (R133C and R453X). A long follow-up of these two girls revealed a unique clinical course. They initially developed the first three stages of RTT, they were severely retarded and had autistic behavior. Over the years their abilities increased progressively and by early adolescence they lost autistic behavior, becoming adequately accustomed to people and reaching an IQ close to 45. These results confirm previous clinical studies suggesting that a wide spectrum of RTT exists including girls with mental abilities considerably higher than in classic RTT. We conclude that MECP2 mutations (missense or late truncating) can be found in girls with an IQ close to 45 and a clinical history of PSV of Rett syndrome. Furthermore, MECP2 mutations are not found in patients in which autism remains stable over the years. © 2003 Wiley-Liss, Inc.

KEY WORDS: RTT; PSV; preserved speech variant; mild mental retardation

INTRODUCTION

In the study of Autism, considerable attention has long been given to syndromes where a strong association exists between a given syndrome and autistic behavior. In a limited number of cases, autism can be a feature of chromosomal, metabolic and dysmorphic disorders, usually with an IQ below 50 [Coleman, 1976; Gillberg and Coleman, 2000]. In most cases, however, Autism does not show such specific associations and appears as a disorder whose main features are relatively stable in time. Within this context, Rett syndrome (RTT, OMIM # 312750) shows a limited period of autistic behavior. RTT is a neurological disorder predominantly affecting females and showing a peculiar course structured in stages. After a few months of almost normal development, patients display a developmental arrest (stage 1) followed by a regression with loss of speech and purposeful hand use and appearance of postnatal microcephaly, stereotypic ‘hand-washing’ activities, ataxia, hand-apraxia, and abnormal breathing (stage 2). At this stage, similarities with autistic behavior are present. At stage 3 there is a limited amelioration followed in older girls by a final somatic and neurologic deterioration (stage 4). For a long time the relation between Autism and RTT was controversial. RTT was initially described in 1966 [Rett, 1966] but went almost unnoticed for many years and these girls were often inappropriately diagnosed as autistic [Witt-Engerstrom and Gillberg, 1987]. In 1983, when RTT was introduced into the mainstream literature, Autism was described as one of its main features [Hagberg et al., 1983]. Subsequently however, a distinction between Autism and RTT was made [Gillberg, 1986; The Rett Syndrome Diagnostic Criteria Work Group, 1988]. DSM IVR notes that the diagnosis of Autism requires the absence of a diagnosis of RTT, which is, however, included among Pervasive Developmental Disorders (PDD). In fact, some similarities with autistic behavior are accepted for stage 2.

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*Correspondence to: Dr. Alessandra Renieri, Medical Genetics, Department of Molecular Biology, University of Siena, Viale Bracci 2, 53100 Siena, Italy. E-mail: renieri@unisi.it
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A number of variants of RTT have been described. Among these, the Preserved Speech Variant (PSV) is characterized by a relatively benign course [Zappella, 1992]. It shows the same stage system and a number of symptoms (initial absence of speech, hand-washing stereotypic activities, etc.) common to classic RTT. However, during stage 3, these girls slowly improve the use of their hands and start to talk in short or longer phrases. Their mental abilities usually increase to levels between 2 and 4 years of mental age but their behavior and spoken language is autistic according to DSM IV criteria. Moreover, they differ from RTT in having normal head circumference, normal or even exceeding weight, slight kyphoscoliosis, and hardly ever epilepsy [Zappella et al., 2001]. The diagnosis of PSV, which has been considered as part of a ‘complex’ of disorders including classic RTT and other RTT variants [Zappella et al., 1998], rests on the inclusion criteria for RTT variants [Hagberg and Skjeldal, 1994] in addition to the course and clinical features described above. In summary, both RTT and its variants are correctly diagnosed as PDD: most PSV show an autistic-like behavior often classic RTT girls have a transient autistic phase in stage 2 and clinical features described above. In summary, both RTT and other RTT variants [Zappella et al., 1992]. It shows the same stage system and a number of symptoms (initial absence of speech, hand-washing stereotypic activities, etc.) common to classic RTT. How-ever, during stage 3, these girls slowly improve the use of their hands and start to talk in short or longer phrases. Their mental abilities usually increase to levels between 2 and 4 years of mental age but their behavior and spoken language is autistic according to DSM IV criteria. Moreover, they differ from RTT in having normal head circumference, normal or even exceeding weight, slight kyphoscoliosis, and hardly ever epilepsy [Zappella et al., 2001]. The diagnosis of PSV, which has been considered as part of a ‘complex’ of disorders including classic RTT and other RTT variants [Zappella et al., 1998], rests on the inclusion criteria for RTT variants [Hagberg and Skjeldal, 1994] in addition to the course and clinical features described above. In summary, both RTT and its variants are correctly diagnosed as PDD: among these, the Preserved Speech Variant (PSV) is characterized by a relatively benign course [Zappella, 1992]. It shows the same stage system and a number of symptoms (initial absence of speech, hand-washing stereotypic activities, etc.) common to classic RTT. However, during stage 3, these girls slowly improve the use of their hands and start to talk in short or longer phrases. Their mental abilities usually increase to levels between 2 and 4 years of mental age but their behavior and spoken language is autistic according to DSM IV criteria. Moreover, they differ from RTT in having normal head circumference, normal or even exceeding weight, slight kyphoscoliosis, and hardly ever epilepsy [Zappella et al., 2001]. The diagnosis of PSV, which has been considered as part of a ‘complex’ of disorders including classic RTT and other RTT variants [Zappella et al., 1992], rests on the inclusion criteria for RTT variants [Hagberg and Skjeldal, 1994] in addition to the course and clinical features described above. In summary, both RTT and its variants are correctly diagnosed as PDD: most PSV show an autistic-like behavior often associated with a peculiar ‘musical’ aptitude.

RTT is usually due to de novo mutations in MECP2 gene [Amir et al., 1999]. MeCP2 is a nuclear protein that binds to methylated DNA and may act as a silencer of gene expression interacting with other proteins such as Sin3A and histone deacetylase complex. MECP2 mutations account for about 80% of RTT cases [Vacca et al., 2001]. We and others have shown that MECP2 mutations are found in PSV [Amir et al., 2000; De Bona et al., 2000; Hupprke et al., 2000; Zappella et al., 2001].

The opportunity to extend the search for MECP2 mutations in girls with Autism was a consequence of the above quoted studies: it is possible that girls with other forms of Autistic Disorders have MECP2 mutations or, alternatively, only girls with a clinical course and symptoms compatible with RTT have this genetic alteration. Until now, only two studies have attempted to address this question. In the first, analysis of 21 autistic females led to the identification of a putative splice site mutation in one case [Lam et al., 2000]. In the second, analysis of 58 autistic patients, 42 males and 17 females, failed to detect any mutation in MECP2 [Vourc’h et al., 2001]. In the second, analysis of 21 autistic females led to the identification of a putative splice site mutation in one case [Lam et al., 2000]. In the second, analysis of 58 autistic patients, 42 males and 17 females, failed to detect any mutation in MECP2 [Vourc’h et al., 2001]. Here we report a search for MECP2 mutations in 19 girls with Autism. Two of them had clinical features of PSV but reached over time an IQ close to 45.

MATERIALS AND METHODS

Patients

Nineteen girls ranging from 5 to 17 years with a diagnosis of Autism were studied. Autism was assessed by us using DSM IV criteria [American Psychiatric Association, 1994], Autistic Behavior Checklist (ABC) [Krug et al., 1979], and Childhood Autism Rating Scale (CARS) evaluation [Schopler et al., 1980]. ABC score was over 57 in all except three cases in which a rating of 40–47 was found. CARS score ranged from 30 to 43 with a mean score of 37. These criteria for Autism were independently assessed by two child neuropsychiatrists and were concordant. IQ was obtained either by Wechsler scale (Wechsler Pre-school Performance Scale for Intelligence, WPPSI, for children below 6, and Wechsler Intelligence Scale for Children-Revised, WISC-R, for children aged 6–18), or by the Leiter test, or the Stanford Binet L-M scale. Different tests were administered because the evaluation was realized independently in distinct centers. All patients were mentally retarded. Most had an IQ below 50 but four had values above 50, with the maximum IQ being 70. In 16 girls no metabolic or known genetic disorders were detected. Among the remaining cases, one had features of Sotos syndrome. In the other two patients, an accurate clinical follow-up suggested the possibility of a diagnosis of PSV. In these two patients, autistic behavior was present in infancy and early childhood but progressively remitted at the end of the first decade of life.

Genetic Analysis

Mutation screening of MECP2 gene was performed by SSCP as reported elsewhere [De Bona et al., 2000]. DNA sequencing was performed with Big dye terminator cycle sequencing kit (Applied Biosystems) on an ABI 310 Automated Sequencer. X-chromosome inactivation analysis was performed as described elsewhere [Meloni et al., 2000].

RESULTS

Nineteen girls with a clinical diagnosis of Autism were screened for mutations in MECP2 gene by SSCP analysis. A pathogenic mutation was found only in the two cases that fulfilled the criteria for PSV. Case one had a c.1357C > T transition, which caused the replacement of arginine 453 with a stop codon in the C terminal portion of MECP2 (p.R453X), skipping the last 34 amino acids. This mutation has never been reported before. Case two had the common missense mutation p.R133C in the methyl-binding domain of the protein, due to a c.397C > T transition. Both mutations were absent in the parents. X-chromosome inactivation studies revealed a borderline pattern in case one (70:30) and a balanced pattern in case two (55:45). Due to the unique clinical course, phenotypic description of these two cases will be given in more detail and is summarized in Table I.

Case one (#386): she is an only child, presently 12 years old. Pregnancy and delivery were normal. She said her first words at 18 months and walked alone at 21 months with a wide basis. At 18 months she appeared less responsive than before: she showed eye avoidance and would not turn if called by name. In subsequent months she became unable to hold objects in her hands and let them fall easily. At 20 months she lost her words and the use of her hands. At 2 years of age, she was severely mentally retarded, and hand-washing stereotypic activities began and remained active for most of the day in the following years. At 5 years of age, it was noticed that the use of her hands was slowly improving with an initial, awkward use of spoon and
fork and she started again to say words. At 6 years she had been tested with WPPSI with an IQ of 45 (VIQ 60; PIQ 48), while on the Leiter test she had an IQ of 38. On the Peabody Picture Vocabulary test her abilities corresponded to 3 years 8 months: she was frequently echolalic and had a tendency to repeat questions over and over again. Her ability to interact with her peers was very poor. She was seen by one of us (M.Z.) at 8 years, when hand-washing stereotypic activities were still frequently present. She was able to draw a sun and a face and she was playing with a doll like a normal 2 year-old girl. She fulfilled the criteria for Autism at the DSM IV (in group A she scored positive for (1) b, c; (2) b, c, d; (3) a, b, c) and her ABC score was 58. Her head circumference (53 cm), weight (23 kg), and height (126 cm) were all in the normal range. She had flat feet: otherwise, her vertebral column was straight with no signs of kyphosis or scoliosis, and no genu valgum. She fulfilled the main criteria of a variant of RTT [Hagberg and Skjeldal, 1994] and was diagnosed as having a PSV. In the following year, at age 9, she was tested again with the Stanford Binet L-M scale and showed an IQ of 49. She was seen again at 12 years. She had no more signs of ataxia and moved around quite well. Her abilities had increased further: she was able to draw simple pictures (Fig. 1A) and spoke more correctly in long sentences. Her face had become more expressive and she was interacting appropriately with children in the ward. DSM IV for Autism was negative except items (2) b and (3) b, ABC was 34 and CARS 24.5 [Schopler et al., 1980]. Hand-washing stereotypic activities had disappeared at 9 years and were now absent. Head circumference (55 cm), weight (39 kg), and height (149 cm) remained within the norm. An EEG showed the presence of sharp waves in the right parieto-temporal region, more intense during sleep. A MNR showed slightly asymmetric ventricles.

Case two (#362): she is a 13 year-old girl with a healthy older sister. Pregnancy and delivery were normal. She walked alone and said her first words at 12 months. In the second year of life her development is described as slow. At 2 years there was a regression: she lost the use of her hands concomitantly with the appearance of hand-clapping stereotypic activities, became avoidant and isolated, and lost the few words she had been able to say, in a context of severe mental retardation. Her parents report that at 4 years contin-

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**TABLE I. Clinical Features of the Two Cases**

<table>
<thead>
<tr>
<th>Case 1</th>
<th>Case 2</th>
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<tbody>
<tr>
<td>Normal pre-perinatal period</td>
<td>+</td>
</tr>
<tr>
<td>Normal development in first year</td>
<td>+</td>
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<tr>
<td>First words at</td>
<td>18 m</td>
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<tr>
<td>Walked alone at</td>
<td>21 m</td>
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<tr>
<td>Loss of hand use at</td>
<td>20 m</td>
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<td>Loss of words at</td>
<td>20 m</td>
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<tr>
<td>Stereotypic hand washing at</td>
<td>24 m</td>
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<tr>
<td>Stereotypic hand washing fades at</td>
<td>9 y</td>
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<tr>
<td>Stereotypic hand clapping at</td>
<td>–</td>
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<tr>
<td>Improvement in hand abilities at</td>
<td>5 y</td>
</tr>
<tr>
<td>Starts again to say words at</td>
<td>5 y</td>
</tr>
<tr>
<td>Autism starts at</td>
<td>18 m</td>
</tr>
<tr>
<td>Autism fades at</td>
<td>12 y</td>
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<tr>
<td>Severe mental retardation at 2–4 y</td>
<td>+</td>
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<tr>
<td>Final IQ</td>
<td>49</td>
</tr>
<tr>
<td>Normal head circumference (constant)</td>
<td>+</td>
</tr>
<tr>
<td>Kyphosis (slight)</td>
<td>–</td>
</tr>
<tr>
<td>Scoliosis (slight)</td>
<td>–</td>
</tr>
<tr>
<td>Flat feet</td>
<td>+</td>
</tr>
<tr>
<td>Genu valgum</td>
<td>–</td>
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<tr>
<td>Sleep EEG as in RTT</td>
<td>+</td>
</tr>
<tr>
<td>Convulsions</td>
<td>+</td>
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m, months; y, years.

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**Fig. 1.** Pictures of the two cases with MECP2 mutation. Case one at the age of 12 years while she is drawing a house (panel A); case two at the age of 13 years while she is writing single letters of her name (panel B).
uous hand-washing stereotypic activities became evident together with hand-clapping and persisted in subsequent years. At 5 years she started again to say some words and her vocabulary continued to slowly increase in the following years; similar progress was reported in her manual abilities and at seven she was able to say some short sentences and to feed herself with a spoon and fork. At 9 years she had two generalized convulsions and was put on a regular valproate treatment. An EEG showed sharp waves in the left hemisphere and in the right parieto-frontal regions. A CT of the brain was normal. At this age (nine), she was seen in our department. She showed a persistent hand-washing activity and spoke in short sentences, frequently echolalic, with an altered tone of speech mainly out of context. She did not play with dolls but was able to draw triangles and squares. A difficulty in opposition movements of thumb and index finger was noticed bilaterally: nerve conduction appeared to be slower, both in the sensory and motor component, in the abduces and opponent nerves of both thumbs. Evaluated with the DSM IV for Autism, she had all items positive in group A, except (3) d, and with CARS (35) she scored in the moderate range of an autistic disorder. She had flat feet, genu valgum, and a slight kyphoscoliosis. She fulfilled the main criteria for a variant of RTT [Hagberg and Skjeldal, 1994] and a diagnosis of PSV was made. She was seen again at 11 years 7 months when her progress had advanced considerably: she was speaking in long correct sentences, although with some out of context expressions, had achieved the capacity of symbolic play and interacted better with her peers. An EEG showed a basic rhythm of seven–eight c/s with frequent slow bursts of five c/s and occasional sharp waves in the right centro-temporal regions in sleep. Her head circumference (52.6 cm), weight (51.5 kg), and height (142 cm) were within normal values. At present, at 13 years, she has further improved her mental abilities. A WISC-R test was administered scoring below 40, with a VIQ below 45 and a PIQ below 45. Her ABC is 17, at CARS she scores 23 and at DSM IV for Autism in group A she has only (3) c as a positive item. She is a lively girl, speaks a lot, has a sense of humor, likes to sing, and is able to engage in a fully reciprocal conversation using long phrases. She knows some of the common metaphors of a part of a continuum in the improvement of their abilities.

Two main features are noteworthy in these two girls: (1) The progressive disappearance of autistic features. (2) The improvement of cognitive level after the regression/deterioration period with a final IQ close to 45. These data confirm, in part, previous observations, conducted on clinical grounds only, which already showed some PSV girls with moderate mental retardation but with persistent autistic behavior [Zappella et al., 1998]. The two girls described here in their younger years displayed a moderate but clear autistic picture with marked reduction of previous autistic traits. However, RTT, PSV and the two cases described here all appear part of a continuum in the improvement of their abilities.

A clinical review of the remaining 17 patients revealed that Autism and mental retardation had remained stable over the years in all. None showed the criteria suggested by Hagberg and Skjeldal [1994] for RTT variants. One of them had Sotos syndrome, a co-morbidity which had already been reported [Zappella, 1990]. MECP2 analysis revealed a likely non-pathogenic variant in two cases. One girl (#196) had a homozygous c.1202G > A transition causing the replacement of Serine 401 with Asparagine in the C terminal portion of the molecule. The same change was found in the hemizygous father and in the heterozygous state in the paternal grand-

mother, in a paternal aunt and in the mother. Another girl (#184) had a heterozygous transversion, c.720C > G, leaving Threonine 240 invariant. The change was inherited from the unaffected mother, who did not show a skewed X-inactivation (not shown). Neither of the two changes was found in 50 healthy controls, suggesting that they are not common polymorphisms but instead private variants.

**DISCUSSION**

In the present study a MECP2 mutation was not found in 16 girls with Autism and in one girl with Sotos syndrome and Autism. A mutation was found only in two girls which fulfilled the main criteria for RTT variants [Hagberg and Skjeldal, 1994], had a diagnosis of PSV, and lost autistic behavior by early adolescence increasing cognitive and social skills. These two girls shared with other PSV a typical course structured in stages as in RTT, accompanied by hand-apraxia in stage 2 and by a parallel development of hand-washing stereotypic activities that continued along stage 3. Five of the 6 main criteria for RTT variants [Hagberg and Skjeldal, 1994] were present in both, and sleep EEG abnormalities were also compatible with this definition. Other clinical features were relatively milder than in most PSV, an improved use of their body and of their hands along the years was observed in both and no evidence of scoliosis was found (only a slight kyphosis in case two). Head circumference was normal as in the majority of the other recently reported cases of PSV with MECP2 mutation [De Bona et al., 2000; Zappella et al., 2001]. From a neurological point of view, both girls had convulsions and one showed signs of peripheral neuropathy. The main difference between these two cases and those reported previously lies in the higher intelligence level reached at the end of the first decade of life, and in a parallel improvement in interpersonal behavior with marked reduction of previous autistic traits. However, RTT, PSV and the two cases described here all appear part of a continuum in the improvement of their abilities.
difference of these girls from most cases of Autism where abnormal behavior remains constant over time.

The other girls with Autism described in this study, who did not have MECP2 mutations, maintained stable autistic behavior and mental retardation over time. In a study of 21 autistic females, a MECP2 mutation was found only in one girl, aged 4 years [Lam et al., 2000]. It was a putative 5’ splice site mutation in intron 2 but it has not been proven to be pathogenic. Unfortunately, authors gave a very limited clinical description of this case and did not have the opportunity to re-evaluate her later. In another recent study, neither mutations nor polymorphisms were identified in 59 autistic patients (42 males and 17 females) [Vourc’h et al., 2001]. In our 17 patients with stable autistic behavior a clear pathogenic MECP2 mutation was not found. It is more difficult to understand the role of the inherited MECP2 rare variants found in two cases. The change c.720C > G (patient 184) does not cause an amino acid change and it is very unlikely that it exerts an even mild variation of MeCP2 function. However, a splice effect and/or influence on mRNA stability cannot be excluded without mRNA analysis. Likewise, a slight variation of MeCP2 function can not be excluded for the p.S401N change (patient 196) in that Serine 401 is conserved in mouse, and is substituted by a neutral amino acid (Glycine) in Xenopus (while in chicken the entire C terminal domain is missed). At present a role of MECP2 as modifier gene in the context of a potential polygenic and multifactorial disorder like Autism cannot be ruled out.

Three types of mutations are reported in MECP2 gene: missense mutations determining an amino acid change, early truncating mutations leading to a very short protein, and late truncating mutations leading to a protein with some preserved domains. It is important to note that the mutations reported here are one missense (p.R133C) and one late truncating mutation (p.R453X).

The p.R133C is common to both RTT and PSV while p.R453X was never reported before, even if it was predicted to be the fifth Arginine which can change to a stop codon by C to T transition in CpG dinucleotide [Wan et al., 1999]. Both missense and late truncating mutations lead to a protein able to translocate to the nucleus where it may exert some residual function. On the contrary, early truncating mutations lead to a protein lacking the nuclear localization signal and part of the transcription repression domain; consequently, this protein is predicted to be inactive and this might determine a more severe prognosis. We and others reported that missense and late truncating mutations lead to both classic RTT and PSV, while early truncating mutations only to classic RTT [Obata et al., 2000; Auranen et al., 2001; Umansky et al., 2001; Zappella et al., 2001]. The two cases described here, one with a missense and one with a late truncating mutation, are in line with this rule and strengthen the hypothesis that a missense or late truncating mutation is necessary to improve the behavioral phenotype and reach a diagnosis of PSV, while a skewed X inactivation in blood cells is not necessary [Auranen et al., 2001; Nielsen et al., 2001]. A partially preserved MeCP2 function may, however, not be sufficient and the improvement may be favored by social facilitation and educational support, and/or by the presence of one (or more) modifier genes.

**Limitations**

One limitation of this study is the reduced size of the sample analyzed (19 patients). A larger sample size would have strengthened the conclusions. A second limitation concerns the short time span during which the two PSV girls have been observed. In fact, in other reported cases a final mental and motor deterioration occurred, even in the second decade [Zappella et al., 2001]. Consequently, we cannot exclude that one or both girls may undergo a progressive deterioration with loss of the present improvements in the future years. Another limitation of this study is represented by the sensitivity of the mutation screening technique. In fact SSCP has a sensitivity of about 80% and consequently some mutations could have been missed. We can not exclude that the employment of more sensitive techniques, like DGGE and DHPLC, would lead to the identification of additional mutations [Buyse et al., 2000]. However, a recent DGGE analysis on 59 autistic patients failed to detect MECP2 mutations [Vourc’h et al., 2001].

**Clinical Implications**

Both the present and previous studies suggest that MECP2 mutations are not likely to cause Autism in girls. This is likely to be a different complex of genetic disorders in which more than one susceptibility gene is involved [Persico et al., 2001]. Furthermore, our data suggest that MECP2 mutations can be found in PSV girls reaching, in time, a moderate mental retardation and losing previous autistic features.

From a clinical point of view, signs such as mild stereotypic hand activities and moderate dispraxia (possibly following a developmental regression occurred at early age) deserve attention and careful assessment for underlying causes. In these cases molecular investigation (i.e., search for MECP2 mutations) may lead to the correct diagnosis and appropriate educational activities.

In conclusion, while MECP2 mutations are not seen in Autism, they could have relevance to understand the genetic basis of autistic behavior, albeit transient.

**NOTE ADDED IN PROOF**

Case one: Following the last visit, this girl developed a severe epileptic syndrome, difficult to treat and accompanied by profound mental deterioration with an extensive loss of language abilities which, one year later, were reduced to short phrases and single words.

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