over time between memory and gross motor capabilities was made particularly obvious to us in a female patient with an odd mild variant of Rett syndrome and a mutation in MECP2. At age 8 years, she was able hyperactively and skillfully to ride on a tricycle, whereas at age 25 years, she was dysplastic, kyphotic, neuromuscularly prematurely aged, and disabled in motor terms. In contrast, she had retained an impressive memory, even for complicated details. These reported examples are only a few of many similar experiences, indicating limited yet nevertheless surprisingly well-preserved detailed long-term memory functions.

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References

Original Article

MECP2 Abnormality Phenotypes: Clinicopathologic Area With Broad Variability

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ABSTRACT

Rett syndrome is a neurodevelopmental disorder that occurs worldwide and predominantly affects girls. The MECP2 gene has been put forward as the underlying gene. Interestingly, other clinical presentations in addition to Rett syndrome have been reported to be the results of deviations in MECP2. This prompted us to outline a working hypothesis of how these diverse phenotypes are connected. Our aim was to summarize the clinical picture of deviations in MECP2 at this moment to obtain a comprehensive overview. Thus, we have attempted to create a gradient, starting at the left with the most severely affected MECP2-deviant subgroups, represented by boys who are diseased in the intrauterine phase or as neonates, and at the right, the most mildly affected subgroup, female asymptomatic carriers. In the center, with dominant numbers, we
have placed classic Rett syndrome presentations, together with the late-onset Rett syndrome variant and preserved speech variant. In conclusion, we feel that it is important to emphasize that Rett syndrome is a strictly clinical diagnosis that is not identical to the far broader concept of MECP2 deviations. (J Child Neurol 2005;20:727–732).

Rett syndrome is a condition with a strict clinical definition. In contrast, MECP2 mutations result in a broad spectrum of phenotypes, of which Rett syndrome is merely one example. In our experience, this distinction is important. Girls affected by Rett syndrome display a wide variety of somewhat peculiar and easily missed developmental deficiencies, early delineation of which is essential to ensure the correct understanding, genetic counseling, and adequate implementation of different habilitation efforts. Here we present a provisional working concept to illustrate the range of MECP2-deviant phenotypes (Figure 1) within this field, which is rapidly expanding as our understanding of the underlying biology of these conditions continues to grow and, in turn, influence patient care. This report contributes to the ongoing discussion regarding MECP2 phenotypes versus the clinical diagnosis of Rett syndrome. Our focus is not to include all known MECP2 mutations but to summarize the current clinical picture of deviations in MECP2 and offer a comprehensive overview. This information will be useful for clinicians and families who seek an explanation and a name for the complex disability affecting their child.

WORKING HYPOTHESIS

We present a clinical outline designed to illuminate the clinical deviations in MECP2 mutations from our point of view, in the hope of starting a discussion on this difficult subject and eventually extending awareness of the different clinical MECP2-deviant presentations. First, we hypothesize that there are clinically defined groups with a more or less Rett syndrome–like appearance, all related to each other. For the end points we have set, boys diseased at birth with Rett syndrome with XXY.6 The left side is thought to represent congenital phenotypes, whereas the center and right side represent the more typical (noncongenital) variants. The disposition of the following presentation matches that of the groups in the Figure; we start from the left end and work through our model, referring to the groups one by one.

LEFT SIDE: SUBGROUPS WITH CONGENITAL DISORDERS

Boys Diseased Neonatally With an Unexplained Etiology

In Figure 1, we have placed boys diseased neonatally and with an unexplained etiology at the far end on the left. We speculate that an as yet unknown number of boys, probably only a few, die during the intrauterine phase or at birth because of MECP2 mutations. In 1998, Schanen and coworkers reported on a boy who died after an apneic event at the age of 5 days.7 The boy’s mother was an obligate carrier, and, after MECP2 mutation analysis, he was found to have had the same mutation (806delG) as his mother, sister, and aunt.8 This is an example of a familial case, which is rare in itself, and we subsequently assume that male patients with MECP2 mutations in Rett families are very rare. In 2004, another boy was described by Leuzzi and coworkers.9 He had the same mutation as the boy described by Schanen and colleagues in 1998,7 but this time, it was sporadic, with a milder phenotype. Consequently, from these observations, we assume that boys with MECP2 mutations can present any of the phenotypes described above. If boys with sporadic MECP2 deviations are born into a family without any association with Rett syndrome, it is not unlikely that the MECP2 defect will remain undiscovered. It is still interesting to determine the frequency of these cases to learn whether there are enough to justify a future screening of MECP2 for this group. Once again, in the case of the affected family, a diagnosis would be valuable and would provide a way of dealing with sorrow and feelings of guilt.

Female Intrauterine Microcephaly

Related to severely compromised boys with MECP2 mutations (neonatally and unexplained diseased), there is a group of very rare girls who are also affected in utero by microcephaly but display no other prenatal signs. One of our female patients falls into this category. She has microcephaly and a severe learning disability, although her socialization and learning capacity have increased slowly with age. This girl is now 14 years old but still lacks the expected motor skills, although she has successfully started to use her hands (unlike girls with Rett syndrome). She has, for example, learned to paint and loves to do so, vigorously and with bright colors. After several years, she slowly learned to walk, and she can now both walk and run. We have detected an MECP2 mutation (E397K) in this girl and her unaffected father. This mutation has been regarded as a normal variant for the past 5 years.8

We considered the possibility that it is a coincidence that a microcephalic girl with the unusual presentation described above also has E397K. To date, there have been 16 entries of E397K in RettBASE (<http://meCP2.chw.edu.au>), and of these, 3 are related to unaffected individuals. Furthermore, we have also observed E397K in another
**Males XY–Severe Mental Retardation**

The next group on the left side comprises boys with a normal karyotype (46,XY) and severe mental retardation. For example, a 21-year-old man was presented by Moog and coworkers. He had a severe learning disability and neurologic disorder and a de novo mutation in MECP2 (P225L). Three males from another family, all with mild X-linked mental retardation and an in-frame deletion of MECP2, were reported on in 2002. Furthermore, in a French study, four patients were described with nonspecific mental retardation and sporadic MECP2 mutations. In this study, 185 patients were examined, and the authors concluded that the frequency of sporadic mutations in MECP2 in patients with nonspecific mental retardation (∼2%) was comparable to the frequency of CGG expansions in FMR1 causing fragile X syndrome (3–4%). In a letter, Laccone and coworkers analyzed the pathogenicity of the MECP2 mutations that have been found in male patients with mental retardation. The authors felt uncertain about the pathogenicity of three of the four mutations found by Couvert and coworkers and concluded that additional studies had to proceed with caution in the evaluation of MECP2 mutations.

Recent publications put forward the hypothesis that MECP2 mutations in male patients with mental retardation are far more rare than was previously thought, and are now calculated at approximately 0.1% to 0.4%. These studies have been conducted with varying study designs and selection criteria, which might account for the discordant conclusions. We emphasize the importance of further studies aiming to determine the true frequency of male MECP2 mutations.

**Female Congenital Rett Syndrome Variants**

Female congenital Rett syndrome variants are rare. One example is a girl without recorded initial normal development who was found to have an MECP2 mutation (R306C). She had hypotension at 6 months of age, and at 3.5 years, she lost all of the words she had previously acquired. Some of her hand movements were still purposeful. The mutation in MECP2 was a hot-spot mutation, and it was not found in the girl’s mother. Another girl with congenital onset and MECP2 deletion (1163delG) has been reported. Patients with congenital Rett syndrome are interesting when it comes to addressing the question of whether patients with Rett syndrome are born healthy, and if they are, whether there is a window through which it might be possible in the future to give some kind of therapy to prevent or limit the effects of defective MECP2 protein.

**Angelman Syndrome–Related Mixed Group**

Angelman syndrome, which we have incorporated as a branch of congenital Rett syndrome variants in Figure 1, does have some clinical overlaps with Rett syndrome. There are several reports of patients with Angelman syndrome, both female and male, who present with MECP2 mutations. Four girls and one boy with a clinical diagnosis of Angelman syndrome have been found with mutations in MECP2. The boy from this series was a somatic mosaic, which means that he had a normal MECP2 in some of his leukocytes. Kleefstra and coworkers found another male patient mosaic with an MECP2 hot-spot mutation (T158M); he was also diagnosed as having Angelman syndrome. In the same report, the authors presented one girl with Angelman syndrome and R133C, as well as a fragile X–negative female patient with Q406X in MECP2. Hitchins and coworkers set out to search for an independent molecular mechanism for sporadic Angelman syndrome. They analyzed MECP2 in 24 patients (without detected mutations in UBE3A or abnormalities in chromosome 15q11-13 or 22q13.3-qter) and found only one male patient with a mutation; he was reported by Watson and colleagues in 2001.

A recent publication presents a new mechanism for gene regulation by MECP2, linking MECP2 to Angelman’s syndrome. The results suggest that MECP2 protein regulates imprinting by the formation of a silent chromatin loop. Defective MECP2 protein (by mutations) caused the loss of imprinting of the target gene Dlk5. MECP2 might therefore be epigenetically linked to Angelman and Prader-Willi syndromes because they are imprinting disorders. It...
is interesting to note that the \textit{MECP2} target gene \textit{Dlk5} is involved in the production of \gamma-aminobutyric acid (GABA)ergic neurons.\textsuperscript{21} Numerous genes are involved in the GABAergic systems. Besides \textit{Dlk5}, some of them have been associated with autism, Angelman syndrome, or Rett syndrome. \textit{GABRB3}, a GABA-A receptor subunit, has been associated with autism, and the gene is located in chromosome region 15q11-13, which is also involved in Angelman syndrome.\textsuperscript{22} Meguro and colleagues demonstrated that three human GABA-A receptor subunit genes are imprinted and located in chromosome 15q11-13 and are hence possible candidate genes for Prader-Willi syndrome.\textsuperscript{23} Interestingly, the human \textit{GABRA3} gene is located on chromosome Xq28.\textsuperscript{24} The chromosomal location and status as a neurotransmitter made it a candidate gene for Rett syndrome (this was before \textit{MECP2} was put forward as the Rett syndrome gene). A study of expression levels in postmortem brain tissue from patients with Rett syndrome showed no reduction in patients versus controls.\textsuperscript{25} In spite of this, it is interesting that GABA-related genes are commonly found in the chromosomal regions associated with the clinically related disorders Angelman syndrome, Rett syndrome, and autism.\textsuperscript{22}

\textbf{Complex Severe Mental Retardation Mixed Group}

The complex severe mental retardation mixed group comprises both male and female individuals with the features of Rett syndrome. This group might be interesting for future studies, but we have not dealt with it here.

\textbf{CENTER AND RIGHT SIDE: SUBGROUPS WITHOUT CONGENITAL ONSET}

\textbf{Clinically Defined Classic Rett Syndrome}

Most \textit{MECP2}-defective patients are assembled in the group of clinically defined classic Rett syndrome and Rett syndrome variants. This is by far the largest group of patients with \textit{MECP2} defects, comprising classic Rett syndrome and also including the late-onset and preserved speech variants. The period of initially normal development distinguishes the two directions in our model, with this large section of clinically defined Rett syndrome or Rett syndrome variants in the center. The preserved speech variant has been shown to be an allelic variant of classic Rett syndrome.\textsuperscript{10,26}

After improved mutation detection, 85% to 100% of patients with classic Rett syndrome have been shown to have mutations in \textit{MECP2}.\textsuperscript{27,28} When analyzing two of the earliest recognized women with Rett syndrome in Sweden, with phenotypes representing typical cases of classic Rett syndrome, we found \textit{MECP2} mutations in both women.\textsuperscript{24} Case S-1 had a complex rearrangement, with a deletion covering exon 3 (detected by multiplex ligation-dependent probe amplification, P015C kit) and a second deletion in exon 4 (1005del188nt; NM_004592; detected by DNA sequencing and confirmed by multiplex ligation-dependent probe amplification). Because of the clinical presentation of case S-1 (classic Rett syndrome), her two deletions might be located on the same chromosome, hence leaving the other \textit{MECP2} gene copy intact, an occurrence that is in consensus with our thoughts on the phenotype presented by male individuals with the 47,XXX karyotype or somatic mosaicism. Case S-2 had a hot-spot mutation (R270X) commonly found in the Rett syndrome population, both for classic and variant cases according to RettBASE.

\textbf{Male Rett Syndrome}

Male individuals with Rett syndrome with the 47,XXX karyotype or somatic mosaicism have a phenotype similar to that of classic Rett syndrome.\textsuperscript{19,20,30} The phenotype of classic Rett syndrome probably requires both a functional \textit{MECP2} gene in at least some of the cells and a mutated \textit{MECP2} gene on the other allele. A mutation in one \textit{MECP2} copy, without the combination of a normal gene, results in diverse phenotypes ranging from severe mental retardation to mild, nonspecific mental retardation and appears to be distinctly different in presentation compared with patients who also carry a complementary \textit{MECP2} gene copy.\textsuperscript{3}

\textbf{Female Forme Fruste Rett Syndrome Variants}

Females with the forme fruste Rett syndrome variant have been noted with \textit{MECP2} mutations.\textsuperscript{16} For example, we refer to a female patient previously described by us in a long-term follow-up study.\textsuperscript{32} She is 25 years old, with a 1154del44 \textit{MECP2} mutation. Her development was initially normal, but when she was examined in her fourth year, she was considered to be motor hyperactive. Her right hand had stereotypic movements, and her hand functions subsequently became dyspraxic. She was very capable with conversation and managed well in a class for developmentally subnormal children. In her twenties, she lost some of her former skills: she was no longer capable of participating in dressing herself or writing her name.

It is important to note the converging development of the mildly affected forme fruste girls into women with adult Rett syndrome; forme fruste women display considerable similarities to women with the adult classic Rett syndrome according to Professor Hagberg (personal communication).

\textbf{Female Autism Variants}

Female autism variants have also been found with \textit{MECP2} mutations. In a study conducted by Lam and colleagues, 1 of 21 examined female patients with infantile autism was confirmed as having a splice-site mutation in \textit{MECP2}.\textsuperscript{29} A second study included 69 female patients with autistic disorder and identified \textit{MECP2} mutations in 2 patients.\textsuperscript{34} However, Beyer and colleagues analyzed 202 autistic patients (154 male and 48 female) and found 14 mutations leading to amino acid substitutions. As the authors felt that the mutations resulted in polymorphic variants, not underlying the disease, they concluded that infantile autism and Rett syndrome probably represent two distinct entities at the molecular level.\textsuperscript{35}

\textbf{Female Normal Carriers}

Female normal carriers are sometimes observed. Examples include mothers in families with Rett syndrome or with mild nonspecific mental retardation who have been shown to be obligate carriers.\textsuperscript{37} The normal phenotypes of the carrier women are probably due to skewed X-chromosome inactivation.\textsuperscript{11,36} The hypothesis that skewed X-chromosome inactivation could mask normal carriers of \textit{MECP2} mutations has been difficult to evaluate because most studies have been performed with peripheral blood leukocytes and not neuronal tissue.\textsuperscript{35} However, the results of a mouse study suggest the possibility of normal female carriers spread within the population, whom we are not able to identify at this stage.\textsuperscript{37}
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DISCUSSION

In conclusion, we emphasize the difficulties involved in evaluating and recognizing the phenotypic effects of MECP2 mutations. Considering the broad spectrum of MECP2 phenotypes we have described, it is likely that modifiers (i.e., X-chromosome inactivation, the number of X chromosomes in patients or the patients’ genetic makeup) in different forms are involved.11,38 Furthermore, it would be intriguing to see whether an MECP2 mutation is associated with disease or whether it represents a polymorphism normal variant.12 Recently, CDKL5 has been put forward as a gene underlying severe neurodevelopmental disorders with infantile spasms and mental retardation; thus, the phenotype of MECP2 mutations overlap.37,40

We have created a working concept for the MECP2-deviant phenotypes, which we hope can be useful as an overview of the various clinical manifestations that we have seen so far. We are aware that there are other ways of combining the groups of clinical manifestations originating from MECP2 mutations. However, it is important in the discussion to emphasize the fact that Rett syndrome is a strictly clinical diagnosis that is not identical to the far broader concept of MECP2 deviations.

FINAL CONSIDERATIONS

In the Swedish series of patients with Rett syndrome, we currently have more than 260 registered female patients, including those with forme fruste Rett syndrome and other well-defined Rett syndrome variants, in addition to approximately 100 other female cases who are clinically Rett syndrome related but were not investigated in detail in relation to MECP2 or adequately followed up in clinical terms. Half a dozen of them represent congenital-onset phenotypes in clinical terms, and they are currently the subject of extensive clinical genetic exploration and neurologic follow-up.

References

The possibility of detecting mutations in MECP2 in patients with Rett syndrome has changed the face of this unique disorder and has accelerated research in this field. Several articles have already been written about the genetics of Rett syndrome. In this article, we do not attempt to discuss the genetics of Rett syndrome in great detail but instead summarize knowledge that is important for clinicians caring for these patients and their parents.

DESCRIPTION OF THE FIRST MUTATIONS IN MECP2

It had been speculated for many years that genetic defects in the X chromosome are involved in the pathogenesis of Rett syndrome because almost only female individuals are affected. It was assumed that there is male lethality in this condition. However, because greater than 99% of Rett syndrome cases are sporadic, linkage studies were not possible until 1998, when a family identified with a maternal inheritance pattern of Rett syndrome permitted exclusion mapping studies to be performed that then defined chromosome Xq28 as a candidate region for the Rett syndrome gene. In 1999, Amir and colleagues identified the first mutations in MECP2.