Rett Syndrome

Exploring the Autism Link

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The presence of autism in individuals with neurodevelopmental disorders, whether transient as in Rett syndrome (RTT) or enduring as in fragile X syndrome or Down syndrome, suggests the possibility of common neurobiologic mechanisms whose elucidation could fundamentally advance our understanding. This review explores the commonalities and differences between autism and RTT at clinical and molecular levels with respect to current status and challenges for each, highlights recent findings from the Rare Disease Network Natural History study on RTT, and summarizes the broad range of phenotypes resulting from mutations in the methyl-CpG-binding protein 2 gene (*MECP2*), which is responsible for RTT in 95% of individuals with the disorder. For RTT, animal models have been critical resources for advancing pathobiologic discovery and promise to be important test beds for evaluating new therapies. Fundamental understanding of autism based on unique genetic mechanism(s) must await similar advances. *Arch Neurol. 2011;68(8):985-989*

Rett syndrome (RTT) is a neurodevelopmental disorder, predominantly affecting females, resulting from mutations in the methyl-CpG-binding protein 2 gene (*MECP2*) (OMIM 312750) located at Xq28 in at least 95% of individuals meeting clinical criteria for the disorder.[1-5] Rett syndrome is characterized by profound cognitive impairment, poor communication skills, stereotypic hand movements, and pervasive growth failure beginning between ages 6 and 18 months after a period of apparently normal development including acquisition of fine motor skills and language. During the regression period, fine motor skills, effective eye contact, and communication are lost. Features of autism, including limited eye contact and poor socialization or interaction, along with inconsolable crying or irritability, occur at this time. Typically, autistic features are transient, lasting from weeks to many months. By school age, intense eye gaze and interaction with others return, yet language does not. As such, individuals with RTT demonstrate a rather predictable temporal profile: apparently normal early development; arrest of developmental progress at 6 to 18 months followed by frank regression of social contact, language, and finger skills; and subsequent improvement in social contact and eye gaze by age 5. Effective interaction persists throughout life, yet motor functions gradually slow in adulthood.

Autism is a complex neurodevelopmental disorder involving poor communication and socialization and a limited repertoire of behaviors and interests.[6] Unlike RTT and a growing number of other gene-based neurodevelopmental disorders discussed here, mutations in a gene or specific set of genes have not been identified in nonsyndromic autism. The diagnosis of autism has been confirmed in several neurodevelopmental disorders including fragile X syndrome, Down syndrome, Angelman syndrome (AS), Prader-Willi syndrome, Smith-Magenis syndrome, Williams syndrome, neurofibromatosis type 1, tuberous sclerosis, Sanfilippo syndrome, phenylketonuria, adenylosuccinate lyase deficiency, and Smith-Lemli-Opitz syndrome. Specifically, up to 60% of individuals with fragile X syndrome and up to 7% of those with Down syndrome may have autism. The diagnosis of autism in some, but not all, individuals with these disorders, whether transient as in RTT or enduring as in fragile X syndrome or Down syndrome, suggests the possibility of common neurobiologic mechanisms whose elucidation could fundamentally advance our understanding. However, concern has been raised

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Table 1. Comparative Features of Autism and Rett Syndrome

Characteristic	Autism	Rett Syndrome
Regression	Some	Universal
Eye gaze	Poor	Good, except during regression period
Socialization	Poor	Good, except during regression period
Head circumference	Infants: large; adults: normal	Postnatal deceleration
Hand skills	Generally good	Poor to absent
Gait	Good	Dyspraxic/none
Periodic breathing	Uncommon	Common

as to whether individuals with neurodevelopmental disorders demonstrating severe or profound cognitive impairment can be assigned an autism diagnosis.[6]

In this review, the phenotypic variability and molecular complexities associated with *MECP2* gene abnormalities and the clinical and molecular convergence between RTT and autism are explored.

RTT AND AUTISM

Clinical Considerations

Rett syndrome and autism are regarded as neurodevelopmental disorders characterized clinically by apparently normal early development, failure of normal developmental progress, and absence of progressive deterioration; neurobiologic characteristics include fundamental failure of normal neuronal maturation and absence of progressive neuronal or glial pathologic changes. As such, these disorders are potentially reversible. Autism and RTT share many common features, but clear differences exist (**Table 1**). Autism occurs predominantly in males, is associated at least initially with accelerated rate of head growth, lacks a specific genetic basis, and has a greater incidence than RTT (1:100 births vs 1:10 000 female births), which occurs mainly in females and is associated with postnatal deceleration in the rate of head growth and *MECP2* mutations.

A clear understanding of the relationship between autism and RTT is lacking. Does brain function differ in autism and RTT? Are similar brain regions affected, and do these change over time? Innovative technologies such as functional magnetic resonance imaging should be able to provide important insights into such questions.

Molecular Convergence

A potential molecular convergence exists for autism and RTT involving *MECP2* and early growth response gene-2 (*EGR2*) (OMIM *129010), an activity-dependent immediate-early gene.[7] *EGR2*, the only member of the EGR family restricted to central nervous system neurons, encodes a DNA-binding zinc finger protein important for cerebral development and synaptic plasticity. Expression of EGR2 and MECP2 increase coordinately in mouse and human cortex; regulation of *EGR2* and *MECP2* is disrupted in autism and RTT; MECP2 expression is decreased in the cortex of people with autism; and EGR2 expression is decreased in the cortex of individuals with autism or RTT, as well as in the cortex of an *Mecp2* knockout mouse. EGR2 has a predicted binding site in the *MECP2* promoter region, and the MECP2 family of methyl-binding proteins binds the *EGR2* enhancer region.

Angelman syndrome, due principally to 15q11-q13 deletions, represents another potentially important link with autism and RTT. Mutations in ubiquitin protein ligase E3A (*UBE3A/E6AP*) (OMIM *601623), a gene contained within this deletion, result in AS. Expression of *UBE3A/E6AP* and the β3 subunit of γ-aminobutyric $acid_A$ receptors located within the deleted region (*GABRB3*) (OMIM +137192) reduced in *Mecp2*-deficient mice and in the brains of humans with RTT, AS, or autism.[8]

FUNCTIONAL ROLE OF *MECP2*

Methyl-CpG-binding protein 2, a member of the methyl-binding protein family, is capable of transcriptional regulation. However, specific gene targets are not defined fully.[9] Methyl-CpG-binding protein 2 is ubiquitous in mammalian tissues and is highly expressed in the brain, functioning in the development and maintenance of neurons. During brain development, MECP2 appears in a caudal-rostral gradient, with cortical neurons being the last to express. In the early prenatal period, forebrain expression is limited to Cajal-Retzius neurons. Recent evidence[9] suggests that MECP2, initially proposed as a transcriptional repressor, participates in upregulation or downregulation of many genes.

BRAIN MORPHOLOGIC CHARACTERISTICS IN RTT

In the human brain, morphologic characteristics of RTT include reduced volume, particularly of the frontotemporal lobes and caudate, reduced brain weight, and reduced or absent melanin pigmentation, notably in substantia nigra.[10] At the microscopic level, cortical neurons are small and demonstrate simplified dendrites with reduced and immature dendritic spines.[10] No evidence of recognizable disease progression, especially neuronal loss, is evident in RTT. This pattern appears to be a common theme among other neurodevelopmental disorders. Autism is associated with increased packing density, decreased cell size, and increased spine density; Down syndrome with reduced dendritic branches and spines after early infancy; AS with reduced dendritic branches and immature spines; and fragile X syndrome with immature spines. These commonalities suggest a convergence of molecular mechanisms underlying their pathobiologic characteristics.

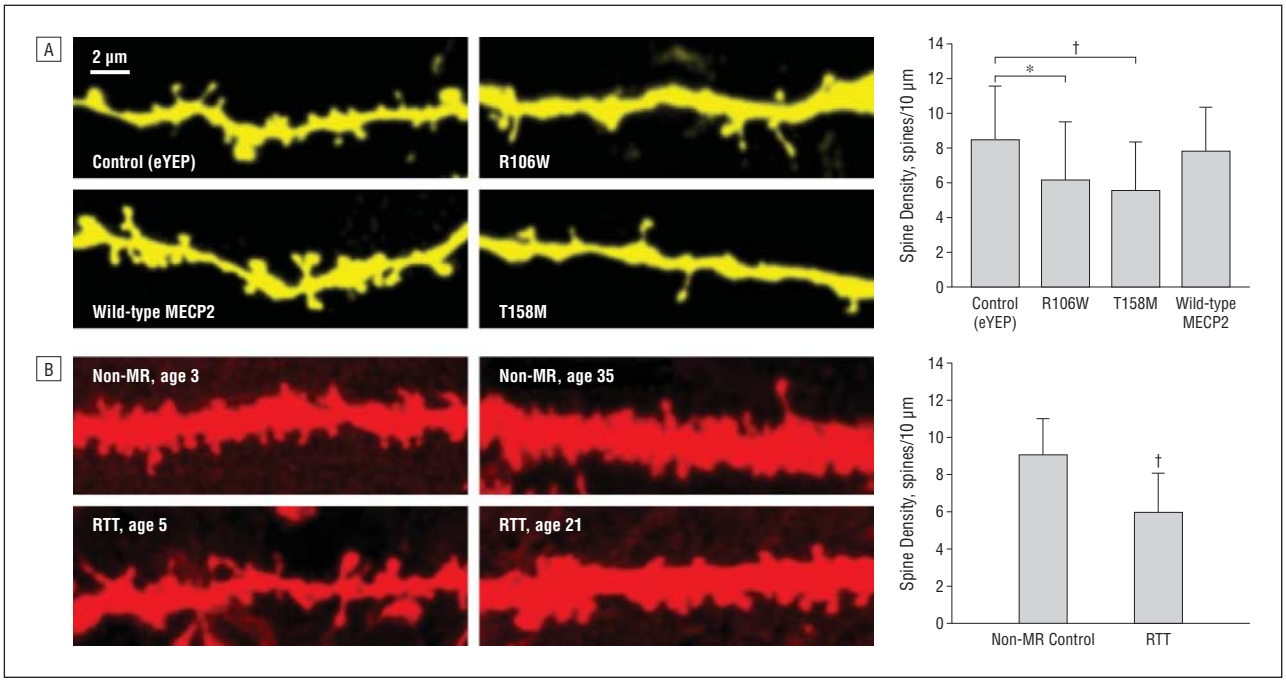

Figure. Pyramidal neuron dendritic spines from rat hippocampal slice cultures and human hippocampus. A, Rat CA1 neurons (96 hours posttransfection with enhanced yellow fluorescent protein, eYFP) from slice cultures showing spine density similar to that of controls with wild-type MECP2 overexpression (approximately 2-fold) and decrease in spine density with mutant R106W ($P<.05$ [asterisk]) and T158M ($P<.01$ [dagger]) MECP2 expression when compared with control slice cultures. The number of observations represented are control, 30 cells from 14 slices; T158M, 31 cells from 23 slices; R106W, 16 cells from 11 slices; and wild-type *MECP2*, 18 cells from 16 slices. B, CA1 pyramidal neurons from human hippocampus stained with DiI showing marked reduction and aberrant morphologic characteristics of dendritic spines ($P<.01$) from a child (age 5) and an adult (age 21) with Rett syndrome (RTT), compared with controls without mental retardation (non-MR; a child [age 3] and an adult [age 35]). Data in each bar graph represent the mean (SD) of 10 RTT and 9 control individuals. Unpublished data graciously provided by Christopher Chapleau, PhD, and Lucas Pozzo-Miller, PhD.

CELLULAR ROLE OF MECP2

Tightly regulated MECP2 dosing is critical to neuronal morphologic characteristics and dendritic spine density in RTT and animal models. In the human brain, dendritic spine abnormalities occur in CA1 hippocampal neurons. In cultured rat embryonic hippocampal neurons, knockdown of normal MECP2 produces shorter dendrites with normal axon length, whereas mutant MECP2 results in shorter axons and dendrites.[11] Conversely, overexpression (approximately 2-fold) of MECP2 yields longer axons and dendrites. Both knockdown and overexpression (approximately 2-fold) of MECP2 produce higher brain-derived neurotrophic factor levels; overexpression of *Bdnf* partially corrects the adverse effect of mutant MECP2. In postnatal rat hippocampal slice cultures, knockdown and mutant MECP2 reduce spine density, whereas spine density in wild-type MECP2 (approximately 2-fold) overexpression is similar to that of controls (**Figure**).[12]

MECP2 ROLE IN RTT

Before 1999, diagnosis of RTT was strictly clinical. In 1999, *MECP2* mutation testing became available, and identification of an *MECP2* mutation implied that the diagnosis was RTT: maybe yes . . . maybe no.

Diagnosis of RTT is based on specific clinical criteria (**Table 2**). Approximately 95% of girls with classic RTT have an *MECP2* mutation.[4] More than 250 specific mutations are associated with RTT: 4 missense mutations, 4 nonsense mutations, several 3′ deletions, and entire exon deletions taken together account for 70% to 80%. Mutations of *MECP2* are generally sporadic (de novo), with the majority being of paternal origin. Familial RTT represents much less than 1%. Rett syndrome is not synonymous with *MECP2* mutations, and *MECP2* mutations occur without RTT. Thus, *MECP2*-related phenotypes involve both males and females and extend well beyond classic RTT (**Table 3**).

CONTINUUM OF *MECP2*-ASSOCIATED PHENOTYPES

In females, mutation-related phenotypes range from normal to learning disabilities to RTT to congenital variants (Table 3). Rett syndrome composes the largest group; the other presentations are greatly underrepresented because of their phenotypic differences from RTT. Individuals with normal function, learning disabilities, or mild cognitive impairments are transmitting females, with both female and male offspring affected adversely by the *MECP2* mutation. The favorable status of transmitting females generally results from skewed or unbalanced X chromosome inactivation. Other non-RTT presentations represent a combination of factors: milder or more severe mutations, skewed X chromosome inactivation, or perhaps variable distribution of normal MECP2 within critical brain regions. In males, the picture is quite different (Table 3). Severe encephalopathy with markedly shortened survival was first noted[13] in a boy from a multiplex family including 2 females with RTT and 1 with mild learning

Table 2. RTT Clinical Criteria[5]

Characteristic	Criteria
Classic or typical RTT	Regression followed by recovery or stabilization
	Main and exclusion criteria required[a]
	Main criteria
	Partial or complete loss of acquired purposeful hand skills
	Partial or complete loss of acquired spoken language
	Gait abnormalities: impaired or absent
	Stereotypic hand movements
	Exclusion criteria
	Brain injury: trauma, metabolic disease, or infection
	Abnormal psychomotor development in first 6 mo
Variant or atypical RTT	Regression followed by recovery or stabilization
	2 of the 4 main criteria
	5 of 11 supportive criteria
	Supportive criteria
	Breathing disturbances when awake
	Bruxism when awake
	Impaired sleep pattern
	Abnormal muscle tone
	Peripheral vasomotor disturbances
	Scoliosis/kyphosis
	Growth retardation
	Small cold hands and feet
	Inappropriate laughing/screaming spells
	Diminished response to pain
	Intense eye communication: "eye pointing"

Abbreviation: RTT, Rett syndrome.
[a]Supportive criteria are not uniformly present and are not required for diagnosis.

Table 3. *MECP2*-Related Phenotypic Variants

Sex	Phenotype
Female	RTT
	Preserved speech variant
	Delayed-onset variant
	Congenital or early-onset seizure variant
	Autistic-like variant
	Angelman syndrome
	Mild learning disability
	Normal carriers
Male	Severe encephalopathy
	Classic RTT: Klinefelter syndrome or somatic mosaicism
	X-Linked MR with or without progressive spasticity
	MECP2 duplications with or without frequent respiratory infections

Abbreviations: MR, mental retardation; RTT, Rett syndrome.

disability. Subsequently, more than 15 males with severe encephalopathy have been described.[14] Typical RTT has been diagnosed in males with Klinefelter syndrome (47XXY) and somatic mosaicism, both yielding populations of cells with distinct X chromosomes. More recently, the much more common (>100 reported) *MECP2* duplication disorder in males was described,[15] involving duplication of variable portions of the X chromosome, including Xq28. This disorder presents a phenotype distinct from RTT, including recurrent respiratory infections in the majority of individuals.

MEDICAL ISSUES IN RTT

Despite emphasis on neurodevelopmental aspects of RTT, attention should also be given to associated medical issues. These are multisystemic, but particularly involve gastrointestinal functions including ineffective chewing and swallowing, gastroesophageal reflux, delayed gastric emptying, constipation, and an unusually high frequency of gallbladder disease. Growth and nutrition are problematic. In addition to abnormal deceleration in the rate of head growth in infancy, weight and height also demonstrate a fall-off, typically by the second year of life. Longevity is less than normal, but average survival is longer than 50 years.[16] Other issues requiring attention are epilepsy,[17] scoliosis,[18] sleep, and anxiety. Differentiation of seizures from anxiety or other behaviors is often difficult, requiring video electroencephalographic assessment. Results of electroencephalograms are uniformly abnormal by age 3 years, with background slowing and multifocal epileptiform patterns, especially during sleep. Cardiac conduction may be abnormal, with prolonged corrected QT interval and nonspecific ST-segment changes that should be evaluated annually. Onset of menstruation mirrors general female patterns. As such, appropriate measures are required to protect this vulnerable group.

ANIMAL MODELS

Several knockout and knockin mouse models for RTT are available for study. Knockin models representing 2 common human mutations, R168X and R255X, are now emerging, as well as the less common A140V mutation associated with cognitive impairment in males, but not RTT in females.

Notable findings have emerged from these models. One study[19] addressed the question of whether *Mecp2* mutation is reversible. Using an estrogen receptor–controlled *Mecp2* promoter, the *Mecp2* knockout phenotype could be reversed in both male and female mice, indicating that identification of effective therapies could be beneficial in reversing some, if not all, of the clinical abnormalities. This study, although not applicable to humans mechanistically, provided proof of principle that RTT, and perhaps other neurodevelopmental disorders, could be reversible, regardless of stage of involvement.

Two studies in a knockin model demonstrated impaired hippocampus-dependent social, spatial, and contextual fear memory; impaired long-term potentiation and depression; and reduced postsynaptic densities.[20] Extending these studies to the hypothalamus, enhanced anxiety and fear were related to elevated blood concentrations of corticosterone and of corticotropin-releasing hormone (Crh) in the hypothalamus, central nucleus of amygdala, and bed nucleus of stria terminalis. Mecp2 binds to the *Crh* promoter methylated region, producing enhanced Crh expression and underscoring the central role of anxiety in RTT.[21] Furthermore, amygdala have direct input into hypothalamus and brainstem auto-

nomic nuclei, correlating with clinical problems of respiratory and gastrointestinal function. Trials directed specifically at Crh expression or at downstream effects associated with anxiety treated with Food and Drug Administration–approved selective serotonin reuptake inhibitors are ongoing.

Three available read-through compounds show promise in providing a full-length protein in individuals with nonsense or stop mutations. These agents are being evaluated in the knockin models R168X and R255X.

COMMENT

At present, our understanding of clinical aspects of RTT has been accelerated by a National Institutes of Health–funded RTT Natural History Study (NCT00299312) and an international investigator consortium. Medical and behavioral management have improved, and advances in molecular genetics have refined diagnostic methodologies and broadened recognition of clinical and molecular heterogeneity. Development of animal models has expanded neurobiologic understanding and provided test beds for evaluating promising treatment strategies. Current challenges include adequate information dissemination to broader medical communities, implementation of consensus criteria, refinement of medical management, and development of fundamental therapies, both targeted and gene-based. Clinical trials of new and repurposed agents are hampered by a lack of reliable behavioral, cognitive, and neurophysiologic outcome measures.

Recognition of autism has penetrated the public domain widely and differs dramatically from that of RTT with respect to national focus and commitment, strong collaborative networks and interdisciplinary approaches, and standardized, broad-based evaluations. Identifying specific molecular markers is a national priority with autism. Innovative in vivo investigations including functional magnetic resonance imaging and interactive computer techniques are increasingly being conducted, and strong emphasis has been directed toward behavioral management. Nonetheless, significant challenges exist, including lack of unifying genetic defect(s), paucity of human neuroanatomic and functional data, lack of a gene-based animal model as in RTT, and clinical trial challenges similar to those of RTT. Although an inbred animal model with autistic features exists, and recent pharmacologic interventions demonstrated reduced repetitive behaviors but not improved socialization in this model, a specific gene defect has not been elucidated in this model.[22] Before clinical trials can be conducted, strategic targets, how they should be stratified, and appropriate outcome measures must be identified.

Autism and RTT may share common neurobiologic mechanisms; however, fundamental understanding of both remains a critical topic for study.

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