

Rett Syndrome: From the Gene to the Disease

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Key Words

MECP2 gene • Neurodevelopmental disorder • Rett syndrome

Abstract

Rett syndrome (RTT, MIM No. 312750) is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation. It is transmitted as an X-linked dominant trait, therefore almost exclusively affecting females. About 80% of RTT cases are sporadic caused by mutations in the *MECP2* gene located on Xq28. The gene codes for two isoforms of the methyl-CpG-binding protein (MeCP2, MeCP2B) which are involved in transcriptional silencing through DNA methylation. The gene has 4 exons. The fourth one is the largest. Almost all mutations in *MECP2* occur de novo. Although mutations are dispersed throughout the gene, about 67% of all *MECP2* mutations, caused by C>T transitions at 8 CpG dinucleotides, are located in the third and fourth exon. The most common mutation is R168X. So far, there is no clear evidence on genotype-phenotype correlations. There are also reports claiming that the same mutation can provoke different phenotypes. It was shown that MeCP2 can silence certain genes. One of them, brain-derived neurotrophic factor, is essential for neural plasticity, learning and memory. This discovery revealed the role of MeCP2 in the control of neuronal activity-dependent gene regulation and suggested that the pathology of RTT may result from deregulation of this process.

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Introduction

Rett syndrome (RTT, No. MIM 312750) is a progressive neurodevelopmental disorder and one of the most common causes of mental retardation in females. Its name comes from Andreas Rett who first described it in 1966 [1] but the syndrome was internationally recognized after Hagberg et al. [2] described 35 cases in 1983. RTT is characterized by apparently normal development for the first 6–18 months of life, followed by a period of regression in language and motor skills. The patients lose purposeful hand use and replace them with repetitive stereotyped hand movements. They usually have normal head circumference at birth followed by postnatal deceleration of head growth. Social withdrawal, communication dysfunction, loss of acquired speech and cognitive impairment are also characteristic of RTT patients. The impairment of locomotion is also very common. Additional characteristics include autistic features, panic-like attacks, respiratory dysfunctions (episodic apnea and/or hyperpnea), bruxism, impairment of sleeping patterns, progressive kyphosis or scoliosis, decreased somatic growth and hypotrophic small and cold feet and/or hands [3, 4]. After initial regression, the condition stabilizes. Patients may recover some skills and usually survive into adulthood, but the incidence of sudden, unexplained death is significantly higher than in controls of a similar age. It may be due to longer corrected QT intervals and T wave abnormalities [5]. Mutations in the *MECP2* gene which encodes the methyl-CpG-binding protein (MeCP2) cause approximately 80% of RTT cases [6]. MeCP2 is in-

volved in transcriptional silencing through DNA methylation. It is expressed in all tissues; therefore, this was an unexpected finding because RTT was thought to be primarily a neurodevelopmental disorder.

MECP2 maps to the X chromosome; so mutations in this gene are usually lethal in males and they can rarely survive. The occurrence of mutations in the *MECP2* gene in living males is usually connected with different types of mosaicism or mild forms of mutations.

Structure and Function of the *MECP2* Gene and Protein

MECP2 maps between *L1CAM* and the *RCP/GCP* loci in Xq28 and undergoes X chromosome inactivation (XCI) [7, 8]. The gene has 4 exons. MeCP2 is an abundant, ubiquitously expressed nuclear protein of 486 amino acids most frequently encoded by a second, third and fourth exon. However, in 2004, Mnatzakanian et al. [9] identified the previously unknown isoform of MeCP2 called MeCP2B; it employs exons 1, 3 and 4, skipping exon 2. They identified a deletion of 11 bp in exon 1 in 1 affected girl after screening 19 girls with typical RTT without mutations in exons 2, 3 or 4.

The fourth exon of the *MECP2* gene is the largest one; it contains a long (>8.5 kb) 3'-untranslated region, with several polyadenylation sites that enable the generation of multiple transcripts of different lengths. Alternative polyadenylation in the 3'-untranslated region results in a highly expressed 10.1-kb transcript in the fetal brain and a 5-kb transcript in the adult brain [10].

The MeCP2 protein contains four functional domains: (1) a methyl-CpG-binding domain (MBD, 85 amino acids) which binds to 5-methyl cytosine with a high affinity; (2) a transcriptional repression domain (TRD, 104 amino acids) which interacts with histone deacetylase and transcriptional corepressor SIN 3A; (3) the nuclear localization signal which may be responsible for the transport of MeCP2 into the nucleus, and (4) the C-terminal segment which facilitates its binding to the nucleosome core. Interactions between this transcription repressor complex and chromatin-bound MeCP2 lead to deacetylation of core histones H3 and H4 by histone deacetylases resulting in compaction of the chromatin, making it inaccessible to components of the transcriptional machinery. In addition, MeCP2 can perform histone deacetylase-independent transcriptional repression [11]. DNA-methylation-dependent repression is important for XCI and genomic imprinting.

MeCP2 is expressed in all tissues and it was first hypothesized to act as a global transcriptional repressor [7, 12]. Interestingly, recent studies show that MeCP2 does not act as a general repressor. Colantuoni et al. [13] found that in postmortem brain tissues of RTT patients the expression of multiple neuron-specific mRNA was decreased. Tudor et al. [14] investigated the difference in global gene expression between mutant (MeCP2-null) and wild-type mice. They hypothesized that mutant mice should have transcriptional deregulation throughout the genome. However, the results showed only subtle gene expression changes in the brains of mutant mice. In addition, it was thought that in the brain *MECP2* is expressed only in neurons and not in nonneuronal cells. However, *MECP2* is also expressed in nonneuronal cells, presumably astrocytes, and not only in the nucleus but also in the cytosol [15]. These findings are important because nonfunctional *MECP2* may cause a decrease in the neuronal body size and dendritic trees; this may be connected with microcephaly and specific behavior in RTT. Furthermore, it has been suggested that MeCP2 plays a central role in neuronal maturation. The study of postnatal brain development in an *Mecp2*-deficient mouse model showed that *Mecp2* was not critical for the production of immature neurons but for neuronal maturation. In addition, analysis of gene expression profiles showed abnormal expression levels of a number of genes important for synaptogenesis [16].

Interestingly, Nikitina et al. [17] reported that hMecp2 binds to the linker DNA region of nucleosomes and protects it from micrococcal nuclease action. Thus, *Mecp2* mutants show a different level of protection depending on the type of the mutation.

Nan et al. [18] have recently shown that *Mecp2* interacts with the ATRX protein which is a SWI2/SNF2 DNA helicase/ATPase mutated in the ATRX syndrome (alpha-thalassemia/mental retardation, X-linked). In addition, ATRX localization is disrupted in neurons of *Mecp2*-null mice. They also showed that point mutations within the DNA binding domain of *MECP2* that would normally cause RTT inhibit interactions with ATRX in vitro and its localization in vivo.

RTT-Causing Mutations

Almost all mutations in *MECP2* occur de novo. The exceptions are familial cases where the mutation has been inherited from a healthy or mildly affected mother; they either have a gonadal mosaicism or favorable XCI. About

67% of all *MECP2* mutations are caused by C>T transitions at 8 CpG dinucleotides (R106, R133, T158, R168, R255, R270, R294 and R306) which are located in the third and fourth exon. The most common mutation is R168X. Although mutations are dispersed throughout the gene, a clustering of missense mutations occurs 5' of the TRD, mostly in the MBD; they all involve evolutionarily conserved amino acids in functional domains of the protein. Nonsense, frameshift and splicing mutations appear distal to the MBD and result in premature termination of the protein. Larger multinucleotide deletions occur in the C-terminal domain. Although deletions tend to affect the same region, entirely identical deletions are rare. It is hypothesized that truncated proteins still bind methylated DNA but cannot interact with the corepressor SIN 3A [19].

Three studies on RTT patients of Slavic origin, including ours [20–22], have been published so far. In all studies, one of the most common mutations, T158M, R168X, R270X and R133C, has been found as well as rather common C-terminal deletions. These data do not differ to a great extent from the data collected in other studies conducted on patients of different origins [23–28].

Since in some RTT patients no *MECP2* mutations could be found, it was proposed that there is at least one other locus responsible for RTT. Indeed, two groups of authors described cases of RTT-like phenotypes caused by truncating frameshift and missense mutations in the gene for cyclin-dependent kinase-like 5 (*CDKL5*; OMIM No. 300203), which is also known as serine/threonine protein kinase 9 (*STK9*) and is mapped to the Xp22 region [29, 30]. It was suggested that *CDKL5* and *MECP2* play a role in a common pathogenic process [31]. Mutations that cause the disease can be found in the catalytic domain and in the COOH region. Bertani et al. [32] showed that the wild-type *CDKL5* autophosphorylates and is responsible for *MECP2* phosphorylation. On the other hand, the mutated protein shows impaired and increased catalytic activity.

Borg et al. [33] reported a girl with characteristics of the RTT bearing translocation-involved chromosomes 1 and 7. On chromosome 1, the *Netrin G1* gene was disrupted while on chromosome 7 all genes were intact. Since *Netrin G1* is mostly expressed in the brain and is important in the central nervous system development it was suggested that *Netrin G1* is a potential novel gene candidate for the RTT. On the other hand, Archer et al. [34] tested *Netrin G1* for mutations on 115 patients with the RTT but found only few sequence variants and none of them appeared to be pathogenic. Therefore, they concluded that mutations in *Netrin G1* very rarely cause RTT.

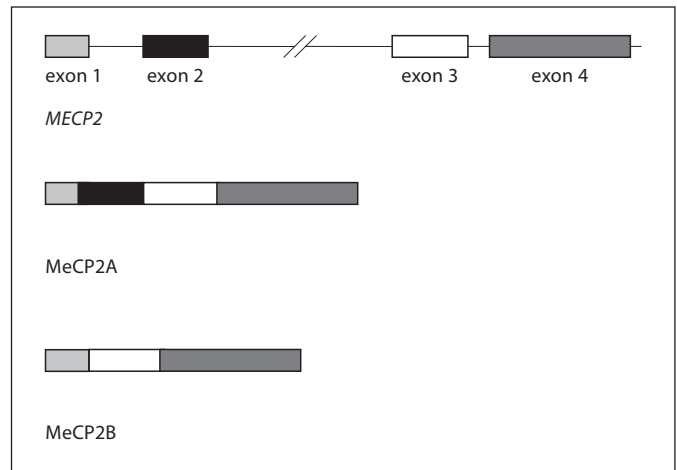


Fig. 1. The structure of *MECP2*. The *MECP2* gene consists of 4 exons and there are 2 splice variants: MeCP2A which includes all 4 exons and MeCP2B which skips exon 2 and includes only exon 1, exon 3 and exon 4.

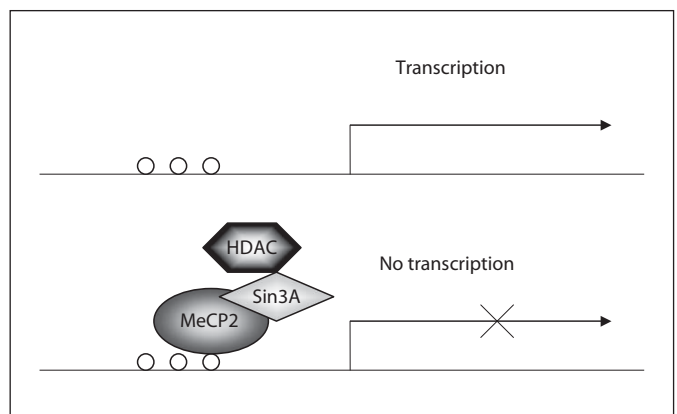


Fig. 2. MeCP2 function. MeCP2 acts as a transcription repressor. It binds to methylated CpG and through the Sin3A corepressor recruits histone deacetylases (HDAC), which leads to deacetylation of the core histones H3 and H4. The result is compacted chromatin which the transcription machinery cannot access.

Genotype-Phenotype Correlations

Genotype-phenotype correlation studies have so far yielded conflicting results. Some researchers have not found a genotype-phenotype correlation between the type of mutation and clinical features but some of them have found such a correlation [34–37]. Later on, a milder form of the disease was found in patients carrying missense mutations as compared to those with truncating mutations; milder disease was also associated with late

but not with early truncating mutations [38, 39]. However, large deletions can also be found in very mildly and in severely affected patients [39]. Huppke et al. [40] showed that patients who carry either missense mutation or deletion located within the hotspot region for deletions, an area between 1,030 and 1,207 bp of the *MECP2* gene, have a milder phenotype than other patients. They also found that all mutations that lead to either a complete or partial truncation of the region coding for the nuclear localization signal are associated with a severer phenotype than other truncating mutations. They did not find a significant difference between the patients with mutations in the MBD and those with mutations in the TRD but concluded that the mutation type and location are connected with the phenotype in the RTT.

There are also reports claiming that the same mutation can provoke different phenotypes [32, 41]. This suggests that not only the type of mutation influences phenotype severity; XCI might be an additional factor.

Recently, Scala et al. [42] have reported a case of one identical intragenic *MECP2* deletion, probably due to gonadal mosaicism, found in two sisters with a contradictory phenotype: one classic and one 'highly functioning' preserved speech variant. In a later case, epigenetic or genetic causes other than *MECP2* were most certainly modulators of phenotype severity.

Effect of XCI

The inactivation of one of the X chromosomes occurs randomly in differentiating embryonal cells in females, resulting in cells that are mosaic with respect to which chromosome is active. The purpose is to equalize X-linked gene products between XX females and XY males.

The *MECP2* gene undergoes X inactivation. In one study, it was shown that of 34 patients with classic RTT 91% had random XCI [36]. Nonrandom XCI was associated with milder phenotypes; preferential inactivation of the X chromosome with mutated *MECP2* gene protects against the deleterious effects of mutations. Females, carriers of RTT-causing mutations but asymptomatic or who suffer from only mild learning disability, had a nonrandom XCI [43].

Most studies of XCI done so far involved determination of the XCI pattern using lymphocytes of peripheral blood or established lymphoblastoid cell lines. However, it turned out that blood cells are not the right model for researching epigenetic and genetic mechanisms of RTT pathogenesis; there is no evidence that the exact XCI pat-

tern is found in the brain tissues. On the contrary, there is at least one case that demonstrates that there is no connection between XCI of leukocytes and XCI of the brain. It was observed that a girl with the classic RTT, where the mutation was on the paternal X chromosome, had an inactive paternal X chromosome in peripheral leukocytes. It is known that in highly differentiated brain cells, especially neurons, different genes are expressed than in peripheral blood lymphocytes. Archer et al. [44] have recently reported that there is a correlation between clinical severity in patients with the RTT (p.R168X or p.T158M *MECP2* mutation) and a proportion of the active mutated allele. They pointed out that XCI may vary in neurological and hematological tissues, and showed a relationship between the degree and direction of XCI in leukocytes and clinical severity in the RTT.

RTT Mutations in Males

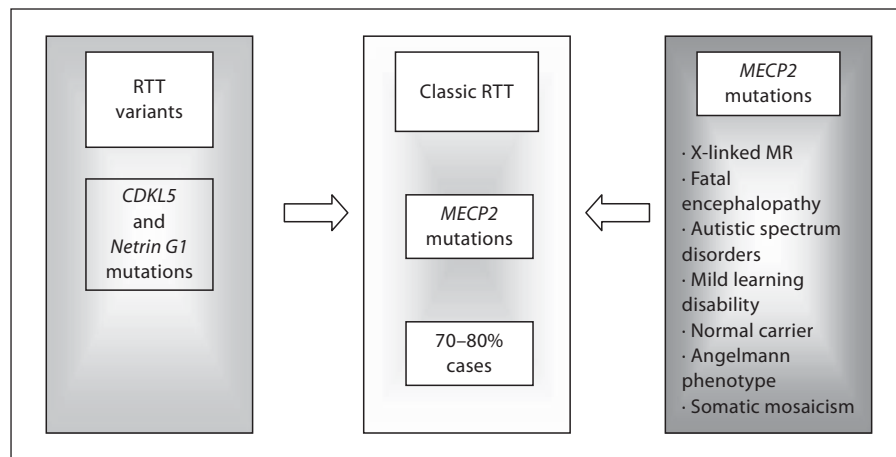
The frequency of potentially disease-causing *MECP2* mutations in the population of mentally retarded male patients is between 1.3 and 1.7% [45]. Males with mutations in the *MECP2* gene usually suffer from severe neonatal encephalopathy and die within 1–2 years of birth. However, males with a mild *MECP2* mutation that would most likely produce a very mild phenotype in girls (late truncating mutations and some missense mutations) can survive but they develop severe mental retardation associated with motor abnormalities. There are also few examples of males who developed an RTT phenotype; they either have somatic mosaicism for the mutation [46] or a partial or complete Klinefelter (47,XXY) karyotype [47]. Maiwald et al. [48] reported a 46,XX male with RTT caused by a mutation in the *MECP2* gene; the clinical manifestation of the boy resembled female RTT cases, which was explained by the karyotype. Also, the rather mild phenotype was due to the preferential expression of the normal allele.

Interestingly, there are mutations that are only found in males with the RTT phenotype. For example, the A140V mutation has never been reported in a girl with a classical RTT phenotype but it was found in 4 severely retarded males from the same family [49].

MeCP2 Target Genes

It was shown that MeCP2 can silence certain genes (table 1). LINE1 but not Alu retrotransposons are repressed by MeCP2 in transfection assays [50]. The leukosialin gene

Fig. 3. Overlapping and nonoverlapping between RTT and *MECP2*. 70–80% of classic RTT patients have mutations in the *MECP2* gene but RTT can also be caused by mutations in the *CDKL5* and *Netrin G1* gene. On the other hand, mutations in *MECP2* have been connected with several other diseases (e.g. X-linked MR, autistic spectrum disorders) as well as with carriers who have no apparent phenotype.



is also repressed by MeCP2 when the promoter is methylated but it was not confirmed that MeCP2 binds to this promoter in vivo [51]. However, the MeCP2 target genes associated with RTT pathogenesis could not be found.

Two groups of authors have discovered that MeCP2 regulates the expression of the gene encoding brain-derived neurotrophic factor (BDNF) [52, 53]. BDNF is essential for neural plasticity, learning and memory. MeCP2 binds selectively to BDNF promoter III and represses the expression of the BDNF gene. Membrane depolarization causes the influx of calcium ions and consequent phosphorylation of MeCP2, which is released from BDNF promoter III; this facilitates activation of BDNF transcription. This discovery revealed the role of MeCP2 in the control of neuronal activity-dependent gene regulation and suggested that the pathology of RTT may result from deregulation of this process. In 2006, Chang et al. [54] discovered that the overexpression of *Bdnf* with a conditional *Bdnf* transgene in mice extends the life span, rescues a locomotor defect, and reverses an electrophysiological deficit observed in *Mecp2* mutants. Their results showed that a functional interaction between *Mecp2* and *Bdnf* exists in vivo.

Another possible MeCP2 target gene is the so-called distal-less homeobox 5 (*DLX5*; OMIM No. 600028) [55]. This gene was thought to be an imprinted gene and to regulate the production of enzymes that synthesize γ -aminobutyric acid (GABA). Namely, the transcription of *DLX5* was roughly two times greater in brains of *Mecp2*-null mice than in wild-type mice. An imprinted gene is the one with a methylated promoter and is usually silent. If the MeCP2 protein is defective or missing it fails to silence an imprinted allele so the expression of the gene would be two times greater. Failure to repress imprinted

Table 1. Potential MeCP2 target genes and their function

Potential MeCP2 target genes	Function	Ref.
<i>LINE-1</i> (Long interspersed nuclear elements)	retrotransposons	46
<i>Leukosialin</i>	major sialoglycoprotein on the surfaces of hematopoietic cells; role in signal transduction and cell adhesion	47
<i>BDNF</i> (brain-derived neurotrophic factor)	neural plasticity, learning and memory	48, 49
<i>DLX5</i> (distal-less homeobox 5)	production of enzymes that synthesize γ -aminobutyric acid (GABA)	51
<i>Crh</i> (corticotropin-releasing hormone)	corticotropin-releasing hormone production	53
<i>FXYD1</i> (FXD domain-containing transport regulator 1)	Phospholemman production (single spanning membrane protein that controls cell excitability by modulating Na^+/K^+ -ATPase activity)	54

alleles has been implicated in several neurological disorders. Horike et al. [55] reported that the maternally expressed *DLX5* showed a loss of imprinting in lymphoblastoid cells from RTT patients, possibly resulting in changed GABAergic neuron activity in individuals with the RTT. They also found a completely new mechanism of gene regulation mediated by MeCP2 through the formation of a

silent chromatin loop. In wild-type mice, *Mecp2* forms a loop of silent, methylated chromatin near the location of the *DLX5* and *DLX6* genes; this loop regulates the expression of the *DLX5* gene. However, in knockout mice that cannot produce *Mecp2*, this region of the chromatin is acetylated and active so different looping brings together widely separated chromatin segments. Consequently, the *DLX5* gene expression is highly increased. However, recently Schüle et al. [56] reported that *DLX5* and *DLX6* are not imprinted and are not influenced by MeCP2 deficiency. Their results showed that the expression of *Dlx5* and *Dlx6* and the imprinted gene *Peg3* in the mouse forebrain was highly variable and *Mecp2*-null mutant or control status had no actual influence on their expression. They concluded that *DLX5* and *DLX6* are not imprinted in humans and probably are not MeCP2 target genes.

In 2006, McGill et al. [57] identified another potential MeCP2 target gene. They studied mice bearing a truncation mutation (*Mecp2*^{308/y}) and found that the expression of the gene for the corticotropin-releasing hormone (*Crh*) is increased in the paraventricular nucleus of the hypothalamus, the central amygdala, and the bed nucleus of the stria terminalis. In addition, they found that while wild-type *Mecp2* binds to the methyl CpG-rich *Crh* promoter, truncated *Mecp2*³⁰⁸ does not.

In 2007, Deng et al. [58] identified another potential MeCP2 target gene, FXYD domain-containing transport regulator 1 (*FXYD1*). This gene codes for phospholemman (PLM, *FXYD1*), a small, single-spanning membrane protein that controls cell excitability by modulating Na⁺/K⁺-ATPase activity. They showed that *FXYD1* expression is increased in the frontal cortex of RTT patients and *Mecp2*-null mice. In addition, they showed that MeCP2 binds to sequences in the *FXYD1* promoter and represses its transcription [58].

Mouse Models for the RTT

To clarify the mechanisms of this disease, several animal models have been generated. *Mecp2*-null mice and conditional mouse mutants with selective loss of *Mecp2* in the brain develop clinical features that resemble some of the symptoms of RTT patients. *Mecp2*-null mice were developing normally until 5 weeks of age, when they started developing the disease which led to death between 6 and 12 weeks. In addition, the brain weight and neuronal cell size were reduced with no structural defects or signs of neurodegeneration. In one conditional mutant the *Mecp2* gene was deleted early in neurons while in the

other it was deleted only in postmitotic, differentiated neurons. The phenotype of the first mutant was identical to the null mutation phenotype; this shows that the phenotype is caused by *Mecp2* deficiency in the central nervous system rather than in peripheral tissues. The deletion in the second mutant resulted in a similar phenotype but at a later age. These results indicate that the role of *Mecp2* is critical in mature neurons. It was suggested that an appropriate genetic mouse model for the RTT may be a female mouse heterozygous for the *Mecp2*-null allele [59]. Shahbazian et al. [60] developed a model of the RTT in male mice by replacing the wild-type allele with one encoding a truncated protein; the mice showed many features similar to those found in RTT patients.

Collins et al. [61] developed a model that transgenically expresses MeCP2 under the endogenous human promoter; this showed that even a mild overexpression of MeCP2 causes a progressive neurological disorder in mice and premature death. Additionally, they crossed the *Mecp2*-null mice with the overexpressing transgenic mice and demonstrated that expression of a human *MECP2* gene rescues the loss of the mouse *Mecp2*. They also showed that by removing the endogenous allele and therefore reducing the *Mecp2* levels, the overexpression phenotype is rescued; this revealed that the effects in transgenic mice were a result of the overexpression of *Mecp2*.

Mouse models have become a very useful tool for finding new *Mecp2* target genes as well as for new genes responsible for the RTT. Jordan et al. [62] performed a microarray-based global gene expression study in the cerebellum of *Mecp2*-mutant mice by comparing transcript levels in mutant/wild-type male sibs of two different *Mecp2*-deficient mouse models at 2, 4 and 8 weeks of age. They found 4 genes with increased expression in *Mecp2*-mutant mice that could be possible new targets for *Mecp2* research: *Irak1* (interleukin-1 receptor-associated kinase 1), *Fxyd1* (phospholemman, associated with Na⁺/K⁺-ATPase), *Reln* (encoding an extracellular signaling molecule essential for neuronal lamination and synaptic plasticity), and *Gtl2/Meg3* (an imprinted maternally expressed nontranslated RNA that serves as a host gene for C/D box snoRNAs and microRNAs).

Conclusion

It is now well known that most of the cases with RTT are caused by mutations in the *MECP2* gene but recent findings reported that two additional genes (*CDKL5* and *Netrin G1*) can also be involved in the pathogenesis of this

syndrome. There is also evidence for the hypothesis that *CDKL5* and *MECP2* together play an important role in the RTT since it was shown that *CDKL5* phosphorylates MeCP2. Additional research is required for these findings to be fully confirmed and further exploration should be directed towards the discovery of other possible MeCP2 target genes. Few of the potential genes have been discovered but we will certainly know more about this

disorder and about the MeCP2 protein itself following additional findings of target genes.

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