Rett Syndrome: From the Gene to the Disease

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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-
olved 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 LICAM 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 deacyethylase 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 deacyetylases resulting in compaction of the chromatin, making it inaccessible to components of the transcriptional machinery. In addition, MeCP2 can perform histone deacyetylase-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.
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.

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 pattern 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
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 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 Schule 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 Gli2/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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