The methyl binding domain containing protein MBD5 is a transcriptional regulator responsible for 2q23.1 deletion syndrome

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2q23.1 microdeletion syndrome is a recently described rare disease that includes intellectual disability, motor delay, autistic-like behaviors, and craniofacial abnormalities. Dosage insufficiency of the methyl-CpG-binding domain protein 5 (*MBD5*) gene was suggested as the genetic cause, since all the described patients carry a partial or total heterozygous deletion of *MBD5*. We reported the generation and characterization of a mouse model with haploinsufficiency for Mbd5 that confirmed this hypothesis. As in human 2q23.1 microdeletion syndrome, the $MBD5^{+/GT}$ mouse model exhibited abnormal social behavior, cognitive impairment, and motor and craniofacial abnormalities, supporting a causal role for MBD5 in 2q23.1 microdeletion syndrome. The use of mouse neuronal cultures uncovered a deficiency in neurite outgrowth, suggesting the participation of MBD5 in neuronal processes. The study of the $MBD5^{+/GT}$ mouse advanced our understanding of the abnormal brain development associated with behavioral and cognitive symptoms.

2q23.1 Microdeletion Syndrome

Copy number variations have been associated to many neurodevelopmental disorders, such as the recently described 2q23.1 microdeletion syndrome. First identified in 2009,[1] the syndrome was further defined in 2010.[2] The typical presentation of patients with 2q23.1 microdeletion includes intellectual disability, behavioral difficulties such as autistic spectrum disorder or attention-deficit/hyperactivity disorder, seizures, sleep disturbances, ataxia/unusual gait, failure to thrive, and specific craniofacial abnormalities.

The 2q23.1 locus contains several genes associated with genetic disorders. Analysis of the minimal region of deletion overlap in patients with 2q23.1 microdeletion syndrome identified *MBD5* as a candidate causal gene.[3]

Interestingly, microduplications in the same 2q23.1 region have been observed in cases with autistic or Angelman-like features and developmental delay, indicating that either decreases or increases in expression of wild-type *MBD5* can result in clinical manifestations.[4-6]

MBD5

Methylation of DNA is an epigenetic mark essential for mammalian development, with functional impact on tissue-specific gene expression, X chromosome inactivation, genomic imprinting, and suppression of transposable elements.[7,8]

MBD5 is a newly recognized member of a family of proteins carrying a defining domain, methyl-CpG-binding domain (MBD), which binds preferentially to methylated DNA. Other members of this group of methyl-CpG binding proteins include MeCP2, MBD1, MBD2, MBD3, MBD4, MBD6, setdb1 and setdb2, and BAZ2A, and BAZ2B.[9]

In spite of having a recognizable MBD, the preferential binding of MBD5 to methylated DNA has yet to be proved. The MBD domain of MBD5 was not able to bind to methylated DNA in vitro.[10] Thus, although the presence of MBD in MBD5 suggests it might play a role as

mediator of DNA methylation outcomes,[11] its functions need to be experimentally tested.

Our data suggest that the main isoforms of MBD5 in the mouse brain contain a proline and tryptophan-rich domain called PWWP domain, in addition to the MBD domain. The PWWP domain was initially described as a DNA binding domain[12] and more recently identified as a recognition domain for histone marks predominantly associated with actively transcribed chromatin.[13] Mammalian proteins that contain a PWWP domain are mostly chromatin binding proteins, including DNMT3A, DNMT3B, bromodomain-containing protein 1 (BRD1), bromodomain and PHD finger-containing protein 1 (BRPF1), 2 (BRPF2) and 3 (BRPF3), and DNA mismatch repair protein MSH6.

Thus, the presence of MBD and PWWP domains in MBD5 suggests a possible role of this protein in transcriptional regulation. We found that, when fused to the DNA-binding domain of GAL4, MBD5 activates transcription of a reporter gene containing a GAL4 binding sequence. Notably, a truncated version lacking the PWWP domain showed higher transcriptional activation capabilities. Thus, in these in vitro assays, the presence of a PWWP domain usually associated with active transcription does not seem to contribute to the activator function of MBD5. In agreement with our data, Tao et al reported that the PWWP domain was unessential for MBD5's activation of the Fth1 promoter, a purported endogenous target of MBD5.[14] Interestingly, MBD5 was shown to interact with the human Polycomb group protein complex PR-DUB through their MBD in a manner that is independent of the PWWP domain. Thus, the functionality of the PWWP domain in MBD5 remains to be identified.

Mbd5-haploinsufficient Mice

We have recently showed that mice carrying an insertional mutation in the Mbd5 gene generated through gene-trap mutagenesis ($MBD5^{+/GT}$) constitute a model for Mbd5 haploinsufficiency.[15] These mice express a mutant Mbd5 transcript composed of the first 2 exons of Mbd5 fused to a lacZ reporter gene. We showed that the $MBD5^{+/GT}$ mice express around 60% of endogenous MBD5. In concordance with a recent report of Mbd5-null mice,[16] the homozygous $MBD5^{GT/GT}$ mutant animals die soon after birth, suggesting and essential role for MBD5 in postnatal survival. Whether deficiency of MBD5 in humans is also inconsistent with survival is not clear yet, but, to our knowledge, no homozygous inactivating mutations in MBD5 have been reported.

Mbd5- haploinsufficient Mice Recapitulates Critical Aspects of The 2q23.1 Deletion Syndrome

Neurobehavioral characterization of the $MBD5^{+/GT}$ mice revealed impaired learning and memory, and altered social behavior, reminiscent of 2 of the most characteristic clinical features of the 2q23.1 microdeletion patients: developmental delay/intellectual disability, and autistic-like manifestations. The $MBD5^{+/GT}$ mice exhibited deficiencies in both hippocampus and amygdala-dependent fear conditioning, suggesting that the general ability to learn basic associations is impaired. These mice also showed increased self-grooming, a stereotyped behavior in mice that is often compared to the restrictive repetitive and stereotyped patterns of behavior seen in autism.[17] Of interest is the observation that, upon introduction of an object into their home cage, the excessive self-grooming of the $MBD5^{+/GT}$ mice was replaced by a compulsive interaction with the novel object. We also observed that $MBD5^{+/GT}$ mice spent more time interacting with stranger mice than with their wild-type littermates. Thus, these data suggested to us that the $MBD5^{+/GT}$ mice demonstrated an abnormal insistence on sameness evocative of human autistic features.

Strength, balance, and movement coordination were affected in $MBD5^{+/GT}$ mice, in line with the diminished motor skills observed in the majority of the 2q23.1 microdeletion syndrome patients.[1-4,18]

2q23.1 microdeletion syndrome patients are often described as having craniofacial anomalies,[3,6,19] however, these are not completely consistent. This was generally supposed to be the result of different deletion size and location and the possible presence of polymorphic cis- and trans-acting modifiers. $MBD5^{+/GT}$ mice present craniofacial abnormalities mimicking the alterations found in 2q23.1 microdeletion syndrome patients. Notably, in spite of these mice having a common mutation and genetic background, we observed deviation of the snout toward the left or the right side in the same proportion in the $MBD5^{+/GT}$ mice. We therefore speculated that the craniofacial phenotype might result from delayed ossification combined with an environmental effect. This may explain the inconsistency of the craniofacial anomalies of the 2q23.1 microdeletion syndrome patients.[3,6,19] The mutant mice have reduced abdominal fat, consistent with the failure to thrive/growth retardation observed in 2q23.1 microdeletion syndrome.

Studies of Mbd5-haploinsufficient Mice Suggest Mechanisms of Pathogenesis for 2q23.1 Deletion Syndrome

Mbd5 is expressed throughout the brain, as revealed by positive β-gal activity in $MBD5^{+/GT}$ mice, a proxy for Mbd5 expression. Highest expression was observed in neurons of the cortex, olfactory bulb, striatum, hippocampus, and cerebellum. Thus, with the goal of shedding light into the pathogenesis of 2q23.1 deletion syndrome, we searched for neuronal structural deficits by analyzing neurite extension in the early phases of differentiation of cortical neurons in vitro. $MBD5^{+/GT}$ neurons showed decreased neurite length and less branching points compared to wild-type neurons, suggesting an important role for MBD5 in neuronal differentiation. The identification of this cellular phenotype points toward early processes of neuronal differentiation as instrumental in MBD5 brain pathology. Furthermore, the availability of a robust, quantifiable cellular phenotype constitutes a potential platform for future screening of compounds capable of phenotypic modulation.

The mechanisms by which MBD5 participates in the regulation of neurite
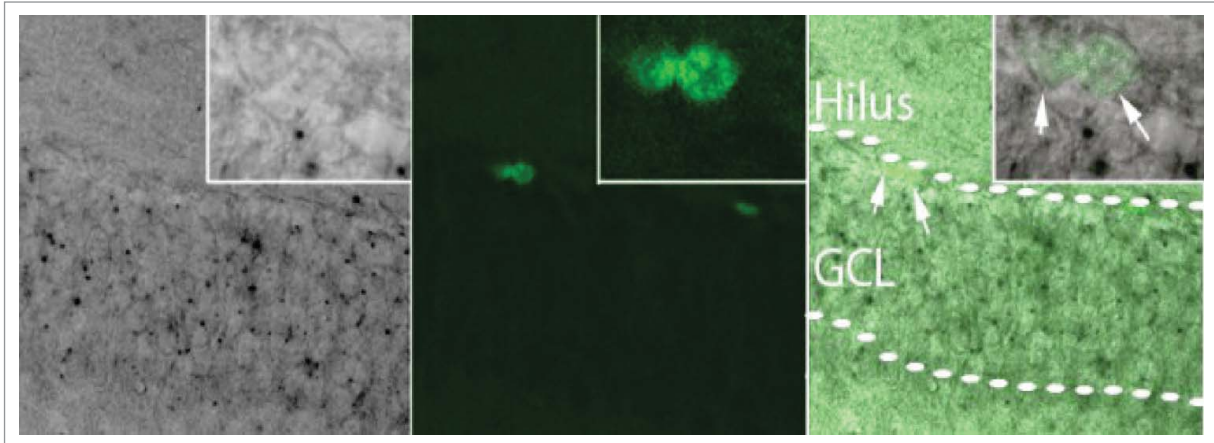
Figure 1. Proliferating neural stem cells do not express MBD5. 2 month old *Mbd5*$^{+/GT}$ mice were treated with BrdU 24 h before brain collection and processed for X-gal (left panel) and BrdU immunolabeling (center panel). BrdU positive cells (arrows, merged right panel and magnified inset) in the subgranular zone do not show X gal staining, suggesting that newly generated cells in the brain do not express MBD5. GCL: granule cell layer.

formation, progression, and maintenance remain to be addressed. Expression of MBD5 is not evident in proliferating BrdU-positive cells in the subgranular zone of the dentate gyrus (**Fig. 1**), suggesting that the onset of expression of MBD5 in neurons might correlate with neuronal differentiation processes.

Concluding Remarks

The study of the consequences of Mbd5 haploinsufficiency in mice confirmed a causal role for this gene in most of the clinical manifestations presented by 2q21.3 microdeletion syndrome. Our report established the *MBD5*$^{+/GT}$ mouse model as a valuable model for social disturbances and intellectual disabilities in patients with 2q21.3 deletions and will be useful for delineation of mechanisms of pathogenesis. The extent of involvement of transcriptional functions of MBD5 in pathogenic mechanisms is unknown and will be subject to future investigations.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest have been disclosed.

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