Neuron

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Previews

Unraveling the mysteries of MYT1L: From reprogramming factor to multifaceted regulator of neuronal differentiation

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https://doi.org/10.1016/j.neuron.2021.11.006

In this issue of *Neuron*, Chen et al. (2021) generated a mouse model for haploinsufficiency of *MYT1L*. MYT1L is widely used in neuronal reprogramming, and *de novo* mutations have been linked to a neurodevelopmental syndrome. Extensive characterization in this study better delineates MYT1L's role in transcriptional regulation and neuronal differentiation.

Brain development involves an intricate pattern of gene expression that ebbs and tides through tightly regulated critical windows. Transcription factors (TFs) represent pivotal nodes in this cascade as changes in their levels can affect many downstream molecular targets and alter or delay cell differentiation. Haploinsufficiency of transcriptional regulators, TFs, or chromatin remodeling factors has often been involved in the genetic etiology of neurodevelopmental disorders, including intellectual disability (ID) and autism spectrum disorder (ASD) (De Rubeis et al., 2014; Satterstrom et al., 2020; Scandaglia and Barco, 2019).

Recently, *de novo* mutations in *myelin transcription factor 1-like* (*MYT1L*), a CCHC zinc finger TF highly expressed during brain development, have been described in patients affected by a syndrome characterized by ID and endocrine disruptions leading to obesity/hyperphagia, as well as other partially penetrant traits, including ASD, attention-deficit/hyperactivity disorder (ADHD), epilepsy, brain malformations, and dysmorphic features (Blanchet et al., 2017; Loid et al., 2018; Windheuser et al., 2020). MYT1L is best known in stem cell biology for its instructive role in reprogramming fibroblasts into neurons (Pang et al., 2011), largely attributed to widespread repression of non-neuronal genes. However, conflicting data were found on its *in vivo* functions as a transcriptional repressor or activator, and a loss-of-function model in the mouse had not been developed.

In their study in this issue of *Neuron*, Chen et al. (2021) generated and characterized a CRISPR-edited mouse strain designed to match a stop-gain patient mutation and closely compare the mouse phenotypes at both molecular and behavioral levels to the clinical hallmarks found in individuals with *MYT1L* syndrome. While homozygous mutants (−/−) die at birth, *Myt1l* heterozygotes (+/−) displayed haploinsufficiency, mirroring the heterozygous loss of function in humans. This genetic loss closely recapitulates several features of *MYT1L* syndrome, including obesity, hypotonia, and clinodactyly. Brain weight, cortical volume, and corpus callosum volume are also reduced, reminiscent of microcephaly and white matter thinning described in a subset of affected individuals. Magnetic resonance imaging and diffusion tensor imaging analyses indicate that white matter tracts are properly organized and myelinated, suggesting a phenotype akin to microcephaly vera, where the total number of cells is reduced, leading to a smaller brain. Chen et al. (2021) found that the spatiotemporal expression pattern of *Myt1l* increases during neurogenesis peaking at postnatal day 1 (P1), with the highest protein expression in differentiating neurons. They showed that proliferation rates are reduced in the embryonic day 14 (E14) cortex in both *Myt1l* +/− and −/− embryos, though only *Myt1l* −/− cortices show marked reduction in cell number and in the number of Sox2-positive apical progenitors.

Profiling chromatin state and expression changes using parallel ATAC sequencing and RNA sequencing at E14 indicates that the loss of MYT1L impacts neurogenesis, leading to early differentiation. Chen et al. (2021) found widespread changes in chromatin accessibility showing both increased and decreased accessible regions. Corresponding changes in gene expression led to downregulation of cell cycle genes along with the concomitant upregulation of cell differentiation genes. By leveraging existing chromatin immunoprecipitation sequencing (ChIP-seq) data, they also showed that there was limited overlap with ChIP-seq peaks, suggesting that MYT1L can also indirectly affect chromatin at sites where it is not bound to DNA. In addition, because many ChIP target regions were closed in the *Myt1l* +/− and −/− brains, MYT1L appeared to act as an activator as opposed to functioning as a repressor, as predominantly shown in the overexpression. The comparison between homozygous and heterozygous loss also showed that *Myt1l* +/− mice had a more substantial decrease in chromatin accessibility, raising the possibility that haploinsufficiency may not just have a less severe effect than loss of function but lead to a different pattern of transcription (Figure 1).

To explore the long-term effects of *Myt1l* haploinsufficiency, Chen et al. (2021) included an additional chromatin and expression profiling study in the adult

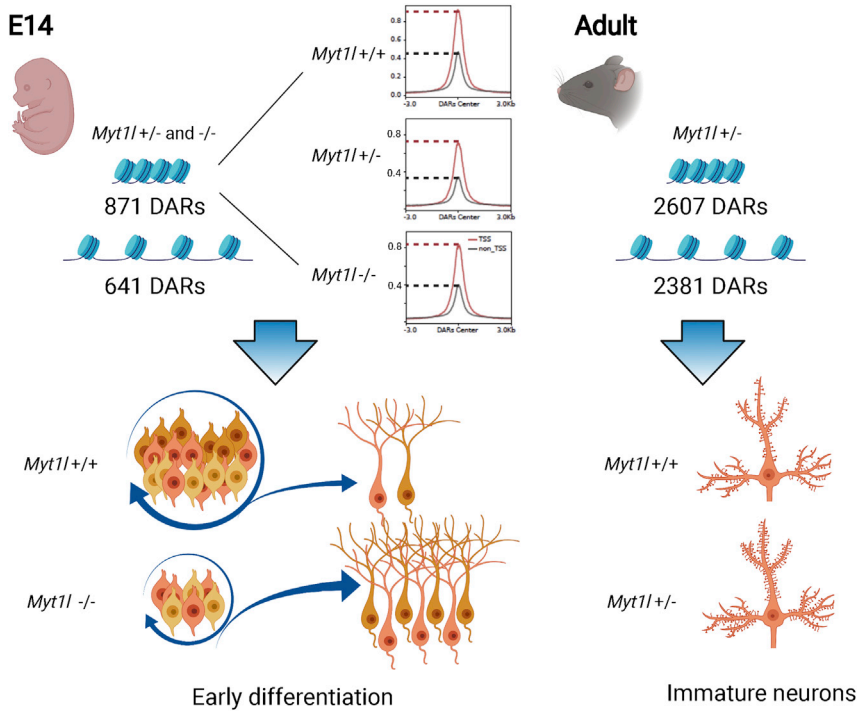

Figure 1. MYT1L differentially affects transcriptional regulation and neuronal maturation during development
DAR, differentially accessible regions. Adapted from Chen et al. (2021). Created with BioRender.

cortex, revealing distinct targets at different developmental stages. Despite their precocious differentiation, adult neurons appeared to be stuck at a more immature stage (Figure 1). Indeed, in *Myt1l* +/− mice, layer 2/3 pyramidal neurons exhibited both structural and electrophysiological deficits with increased density of thin immature spines, more depolarized membrane properties, and a shift in excitatory-inhibitory balance.

Finally, Chen et al. (2021) conducted a comprehensive behavioral characterization of male and female *Myt1l* +/− mice both before weaning and in adults exploring motor function, multiple cognitive domains, and sociability. Mice of both sexes showed an array of behavioral phenotypes primarily related to motor strength, hyperactivity, and arousal. While there were no gross delays in attaining developmental milestones, *Myt1l* +/− pups had reduced strength and hypotonia and produced increased ultrasonic vocalization in response to maternal separation that were attributed to hyperarousal. Adult *Myt1l* +/− mice showed signs of hyperactivity but only limited cognitive deficits in multiple tests. Of note was the extensive analysis of social deficits. While memory of a previously encountered mouse was preserved in the three-chamber test, the initial approach to a conspecific was reduced. Chen et al. (2021) asked whether this deficit could be due to a reduction in social motivation, applying a modified operant paradigm using social interaction as a reward and showing that male *Myt1l* +/− mice sought fewer interactions.

Overall, *Myt1l* +/− mice recapitulate many aspects of the human presentation including obesity, microcephaly, hypotonia, and variable clinodactyly. This mouse mutant strain will be a valuable tool to help answer open questions on the molecular determinants of pathogenesis, in particular as it pertains to the neuronal or muscular origin of the hypotonia or to the endocrine dysfunction leading to obesity. As it is common in animal models of ID and ASD, the interpretation of neurobehavioral phenotypes remains more nuanced. While cognitive deficits are fully penetrant and often severe in individuals with MYT1L syndrome, *Myt1l* +/− mice only show deficits in cued and contextual fear conditioning and mostly present with hyperactivity and variable social deficits. Yet, behavioral changes in rodents still represent a strong clue that there is an underlying disruption in neuronal function, as was shown in the molecular and cellular analyses.

The comparison between *Myt1l* −/− and *Myt1l* +/− animals has already revealed interesting differences in chromatin accessibility that are dynamic throughout development and may be specifically affected by haploinsufficiency. Future studies should examine the mechanisms that lead to these distinct deficits in knockout compared to heterozygous mice and the limited overlap with the downstream molecular targets of MYT1L overexpression used for reprogramming. Sex biases in how deficits presented were also observed in *Myt1l* +/− mice with increased obesity and hyperactive behaviors in females and social deficits in males. These discrete biases likely point toward possible overlap between the MYT1L gene regulatory network and sex-specific gene networks. There is a prominent male bias in the diagnosis of ASD/ID, and mouse models recapitulating other ASD/ID mutations have shown occasional sex-specific deficits in different brain circuits, but it remains unknown how this sex specificity emerges (Mossa and Manzini, 2021).

In closing, this expansive study provides multiple insights into the complexities of MYT1L function that can be further followed up to better define its role in neuronal differentiation, maintenance, and reprogramming.

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Excessive mitophagy for anxiety

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https://doi.org/10.1016/j.neuron.2021.11.007

In this issue of *Neuron*, Duan et al. (2021) identified excessive mitophagy in BLA neurons synapsing onto adBNST neurons as a mechanism for reduced neurotransmission and social defeat-induced anxiety-like behaviors.

Each of us experiences anxiety and stress at some point in our lives. They share many emotional and physical symptoms. Yet, anxiety is different from stress. Picture yourself taking your dog out on a walk: one would experience anxiety at the idea of losing our dog because of a problem with the leash, but one would experience stress if we actually lost our dog. Anxiety is a feeling of dread or unease in response to a stressful situation and may present an evolutionary advantage by allowing an organism to anticipate and avoid danger. While beneficial in the acute setting, anxiety, when it becomes constant, can also be maladaptive, interfering with our lives and ability to function. Human functional imaging and rodent behavioral studies have implicated different brain regions, including the basolateral amygdala (BLA), bed nucleus of stria terminalis (BNST), hippocampus, nucleus accumbens (NAc), prefrontal cortex (PFC), and locus coeruleus (LC), as neuroanatomic substrates of anxiety and stress (Tovote et al., 2015). In particular, BLA emerges as a key structure encoding emotional valence and is critical to responses to stress and anticipated threats. It receives sensory inputs from the thalamus and sensory cortex and forms reciprocal connections with other stress/anxiety-related brain regions to control behavioral responses (Tovote et al., 2015). Overall activation of BLA projection neurons enhances anxiety-like behavior, although different populations of BLA neurons may vary (Felix-Ortiz et al., 2013). However, the intracellular pathological mechanisms of how BLA neurons respond to stress are not well understood.

In a recent study in *Neuron*, Duan et al. (2021) demonstrated that stress in mice causes mitochondria impairment in BLA projection neurons, revealing a novel subcellular substrate in anxiogenesis. Mitochondria not only are the powerhouse of the cell, producing ATP, but also control redox homeostasis, cell apoptosis, and Ca^{2+} buffering. Particularly, mitochondria in axons and dendrites regulate synapse development and synaptic plasticity (Zhao et al., 2021). The function of mitochondria relies on the dynamic balance between fusion and fission (Zhao et al., 2021) and between biogenesis and mitophagy, a selective form of autophagy, to degrade damaged mitochondria (Onishi et al., 2021). In the brain, mitophagy helps to maintain the quality of mitochondria. When it is impaired, damaged mitochondria accumulate, altering normal cell function and causing cell death. Mitophagy can also be induced by stress stimuli such as oxidative changes, starvation, or hypoxia.

To investigate potential pathological mechanisms of anxiety disorders, Duan et al. (2021) subjected mice to chronic social defeats (CSDs), a paradigm believed to mimic psychosocial stress suffered by human beings (Krishnan et al., 2007). In a series of elegantly designed experiments, they found that mitochondria in BLA neurons of mice subjected to CSDs are damaged, showing decreased uptake of TMRE (tetramethylrhodamine ethyl ester), a chemical that accumulates in mitochondria proportionally to mitochondrial membrane potential (MMP), decreased size and mass as observed