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Species-conserved *SYNGAP1* phenotypes associated with neurodevelopmental disorders

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ABSTRACT

SYNGAP1 loss-of-function variants are causally associated with intellectual disability, severe epilepsy, autism spectrum disorder and schizophrenia. While there are hundreds of genetic risk factors for neurodevelopmental disorders (NDDs), this gene is somewhat unique because of the frequency and penetrance of loss-of-function variants found in patients combined with the range of brain disorders associated with *SYNGAP1* pathogenicity. These clinical findings indicate that *SYNGAP1* regulates fundamental neurodevelopmental processes that are necessary for brain development. Here, we describe four phenotypic domains that are controlled by *Syngap1* expression across vertebrate species. Two domains, the maturation of cognitive functions and maintenance of excitatory-inhibitory balance, are defined exclusively through a review of the current literature. Two additional domains are defined by integrating the current literature with new data indicating that *SYNGAP1/Syngap1* regulates innate survival behaviors and brain structure. These four phenotypic domains are commonly disrupted in NDDs, suggesting that a deeper understanding of developmental *Syngap1* functions will be generalizable to other NDDs of known or unknown etiology. Therefore, we discuss the known molecular and cellular functions of *Syngap1* and consider how these functions may contribute to the emergence of disease-relevant phenotypes. Finally, we identify major unexplored areas of *Syngap1* neurobiology and discuss how a deeper understanding of this gene may uncover general principles of NDD pathobiology.

1. *SYNGAP1* gene function is important in health and disease

The advent of genomic sequencing in previously undefined patients with NDDs has demonstrated that there is a subset of autosomal genes that confer near 100% risk for developing ID, ASD and/or epilepsy (Deciphering Developmental Disorders, 2015, 2017; Epi et al., 2013). There is considerable interest in understanding the molecular and cellular mechanisms regulated by this subset of high-impact genetic risk factors (Hoischen et al., 2014; Zhu et al., 2014). In-depth study of animal models harboring pathogenic variants common to patient populations is a powerful approach for determining linkages between molecular and cellular functions of distinct disease risk factors, and perhaps more importantly, how possible convergent molecular mechanisms contribute to disease-relevant phenotypes. Through in-depth biological investigations of highly-penetrant risk genes in model systems, it may be possible to identify common molecular mechanisms that converge to influence disease-relevant phenotypes. With such knowledge, therapeutic approaches developed through modeling of one gene may be successfully applied to other NDDs of known or unknown etiology.

The NDD risk-factor, *SYNGAP1*, is a major cause of genetically-defined childhood brain disorders and an attractive candidate for in-depth investigations that span multiple model systems. The *SYNGAP1* gene has emerged as a high-risk locus for neuropsychiatric disorders that cross diagnostic barriers (Hoischen et al., 2014; Zhu et al., 2014). Indeed, causal rare variants are found in enriched populations with ID (Deciphering Developmental Disorders, 2015, 2017; Hamdan et al., 2009; Rauch et al., 2012), ASD (Hamdan et al., 2011; O'Roak et al., 2014), severe epilepsy (Carvill et al., 2013; von Stulpnagel et al., 2015) and schizophrenia (Purcell et al., 2014). Severe de novo variants in *SYNGAP1* resulting in haploinsufficiency lead to a defined phenotype characterized by ID with epilepsy [termed Mental Retardation-Type 5(MRD5); OMIM#603384] that may explain up to 1% of ID cases (Berryer et al., 2013; Deciphering Developmental Disorders, 2015,

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2017). A recent study has found that there are no severe or obvious pathogenic *SYNGAP1* variants in > 60,000 subjects lacking any known neuropsychiatric conditions, solidifying the notion that pathogenic *SYNGAP1* loss-of-function variants are both highly penetrant and sufficient to cause NDDs (Kosmicki et al., 2017).

SynGAP proteins, the products encoded by the *SYNGAP1/Syngap1* gene (Chen et al., 1998; Kim et al., 1998), lie at a critical intersection of protein signaling networks strongly linked to a spectrum of NDDs. Mechanisms that drive brain dysfunction associated with neuropsychiatric disorders intersect at excitatory synapse regulation in glutamatergic neurons. For example, the NMDA receptor signaling complex within dendritic spine synapses is enriched with proteins encoded by a high-proportion of genes with pathogenic variants linked to a range of neuropsychiatric disorders marked by cognitive impairment (Bayes et al., 2014; Volk et al., 2015). SynGAP is both a core postsynaptic density (PSD) protein and a major constituent of the NMDA receptor signaling complex (Bayes et al., 2012; Chen et al., 1998; Kim et al., 1998). SynGAP protein-protein interactions are believed to promote organization of macromolecular complexes within dendritic spines (Walkup et al., 2016; Zeng et al., 2017). Moreover, SynGAP also regulates mRNA translation machinery (Barnes et al., 2015; Wang et al., 2013) that regulates excitatory synapse plasticity, which is a cellular process believed to contribute to ASD pathogenesis (Huber et al., 2015; Richter et al., 2015).

2. Species-aligned phenotypic domains regulated by *Syngap1* function

A relatively homogenous human phenotype emerges in patients harboring pathogenic variants causing *SYNGAP1* haploinsufficiency (Berryer et al., 2013; Mignot et al., 2016; Parker et al., 2015). Indeed, ~85% of known patients with pathogenic *SYNGAP1* variants have rare, loss-of-function variants predicted to cause reduced protein expression or function (Mignot et al., 2016). The high proportion of loss-of-function variants, combined with a relatively homogenous core phenotype in humans, indicates that *SYNGAP1* has essential natural functions during brain development.

Here, we discus known human phenotypes (Berryer et al., 2013; Mignot et al., 2016; Parker et al., 2015) in the context of conservation across several vertebrate species to highlight the fundamental importance of *SYNGAP1/Syngap1* in sculpting brain function. Studies performed across species demonstrate that this gene has retained functions throughout vertebrate evolution to promote cognitive functions, excitatory-inhibitory (E-I) balance, brain structure, and innate behavioral adaptations (*see following sub-sections*). The alignment of *SYNGAP1/Syngap1* phenotypes across vertebrate species suggests that it controls fundamental cellular processes that promote the assembly and function of neural circuits that underlie behavior and cognition. Thus, in-depth study of *Syngap1* phenotypes in animal models may provide molecular insight into the shared pathobiology of NDDs.

2.1. Phenotype 1: cognitive function

Reduced cognitive function is a common feature of many NDDs, including intellectual disability, ASD, epilepsy and schizophrenia. In humans, proper *SYNGAP1* expression is essential for the development of cognitive abilities. *SYNGAP1* haploinsufficiency leads to an intellectual disability disorder characterized by severe cognitive impairment (Berryer et al., 2013; Mignot et al., 2016; Parker et al., 2015). Most patients have reduced capacity for language and are non-verbal. IQ is usually < 50 and patients have impaired executive functions. Animal models with disrupted *Syngap1* expression also display behaviors and neurophysiological abnormalities consistent with cognitive impairment (Guo et al., 2009; Komiyama et al., 2002; Muhia et al., 2010; Ozkan et al., 2014). For instance, *Syngap1* heterozygous KO mice (Hets) express disruptions in various forms of learning and memory, including impaired spatial learning, altered spatial working memory, weakened social memory, and deficits in remote contextual memory consolidation. These learning and memory phenotypes are highly reproducible in mice with *Syngap1* haploinsufficiency, having been observed across several laboratories using independently generated *Syngap1* knockout lines (Guo et al., 2009; Komiyama et al., 2002; Muhia et al., 2010; Ozkan et al., 2014).

Syngap1 also regulates synaptic plasticity in the same brain regions that support memory and cognition. Disruptions to this cellular process may contribute to the cognitive impairments displayed by *Syngap1* haploinsufficient animals. Indeed, reduced germline *Syngap1* expression causes deficits in various forms of hippocampal synaptic plasticity. Long-term potentiation (LTP) is a cellular correlate of learning and memory and the synaptic strengthening that accompanies LTP is believed to be a mechanism for storing newly acquired information within neural circuits (Lynch et al., 2007; Nicoll, 2017). Alterations in this cellular process is, therefore, an attractive substrate for cognitive impairments commonly observed in NDDs. Consistent with this, many animal models of NDD risk gene pathogenicity display impaired synaptic plasticity (Araujo et al., 2017; Lauterborn et al., 2007; Lee et al., 2014; Li et al., 2016), including LTP impairments in *Syngap1* Het mice (Clement et al., 2013; Kim et al., 2003; Komiyama et al., 2002; Ozkan et al., 2014). Similar to learning and memory impairments, several laboratories using independently generated *Syngap1* Het mouse lines and distinct induction protocols have routinely observed severely impaired LTP.

LTP deficits in *Syngap1* mice are associated with alterations in NMDAR-activated Ras signaling dynamics in dendritic spines (Ozkan et al., 2014). SynGAP is a GTPase activating protein (GAP) (Chen et al., 1998; Kim et al., 1998), which inactivates several small GTPases from the Ras superfamily, including Ras, Rap1/2 and Rab5 (Krapivinsky et al., 2004; Pena et al., 2008; Tomoda et al., 2004). SynGAP content within spines is dynamically reduced in response to neuronal activation (Araki et al., 2015). This process promotes a transient elevation in Ras activation, which is known to drive AMPA receptor membrane insertion required for LTP expression (Zhu et al., 2002). Whole brain extracts (Clement et al., 2013; Kim et al., 2003; Komiyama et al., 2002) and even hippocampal dendritic spines (Ozkan et al., 2014) within *Syngap1* Het mice display basally elevated Ras signaling, which occludes further Ras activation in response to synaptic stimulation. Restoring normal SynGAP protein levels in adult *Syngap1* Het mice rescues both LTP expression and Ras-related signaling impairments (Ozkan et al., 2014). Together, these data support the view that a function of SynGAP protein within dendritic spines is to maintain low basal level Ras-ERK signaling in an unstimulated state, which may be a mechanism to maximize the signal/noise ratio of this pathway upon synaptic stimulation to promote LTP during learning.

Studies in long-term depression (LTD) are consistent with the role of SynGAP to balance Ras-ERK signaling at synapses. LTD is a unique form of synaptic plasticity that weakens neural connections in response to activity and may act to enhance computational flexibility within neural networks (Pinar et al., 2017). In contrast to the clear impact of *Syngap1* on LTP, this gene has a more complex role in LTD. For example, LTD in response to a standard input-specific and synaptically-driven induction paradigm in CA1 is normal in *Syngap1* Hets (Kim et al., 2003). Additionally, no change was observed in CA1 tissue slices from *Syngap1* Hets after LTD induced by bath application of NMDA (Carlisle et al., 2008). In contrast, mGlur5-dependent CA1 LTD was enhanced and resistant to protein synthesis inhibitors (Barnes et al., 2015). This mGlur5-dependent LTD phenotype is similar to what was reported in *Fmr1* KO mice (Osterweil et al., 2010), which is an animal model of Fragile X syndrome. In both *Syngap1* Het and *Fmr1* KO mice, pharmacological targeting of elevated Ras signaling rescued LTD phenotypes (Barnes et al., 2015). This finding demonstrates a form of molecular convergence at the synapse caused by two distinct NDD risk factors and suggests that targeting aberrant Ras signaling may improve behaviors