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The Role of CNTNAP2 in Itch Sensation

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To the Editor

Itch is a hallmark symptom associated with atopic dermatitis (AD), which is an allergic disorder accentuated by both immunological dysregulation and epidermal barrier defect (Wahlgren, 1991). Autism spectrum disorders (ASD) are complex neurobehavioral and neurodevelopmental conditions which cause a variety of phenotypes including impaired social communication, stereotyped behaviors, and altered sensory processing (Dawes et al., 2018, Vahia, 2013). A longitudinal study and a systemic review reveal an association between early AD that subsequently leads to ASD (Lee et al., 2016, Tongo et al., 2015), but how itch is regulated in ASD individuals remains unknown. In addition, excessive itching leads to sleep disorders, which accentuate neuropsychiatric illness (Mazzone et al., 2018).

ASD is a group of diseases caused by many environmental as well as germline and somatic mutation. Contactin associated protein 2 (CNTNAP2 or CASPR2) is a neural adhesion molecule of the neurexin superfamily and the *CNTNAP2* gene may be linked to one of several mutations associated with some types of autism spectrum disorder (ASD) (Zweier, 2012). However, the functional role of CNTNAP2 in the neural transmission of itch is unknown. Itch is a noxious sensation detected by the nociceptors expressed on peripheral afferents, which on activation release neurotransmitter/neuropeptide into the spinal cord, resulting on conveying itch signals to the brain (Mishra and Hoon, 2013). Since CNTNAP2 is linked to pain hypersensitivity in ASD and expressed in almost all dorsal root ganglia (DRG) sensory neurons (Dawes et al., 2018), we wondered whether CNTNAP2 might contribute to itch behavior. Experiments using mice followed the North Carolina State University laboratory animal care protocols approved by an Institutional Animal Care and Use Committee (IACUC) as per NIH guidelines. Mice lacking the *CNTNAP2* gene demonstrated enhanced pain-related hypersensitivity to noxious mechanical stimuli, heat, and algogens (Dawes et al., 2018). Further, this study showed that both primary afferent excitability and subsequent nociceptive transmission within the dorsal horn were increased in *Cntnap2 knockout (KO)* mice. In a separate report, the dorsal root ganglion (DRG) from *Cntnap2* KO mice showed hyperactive Akt-mTOR signaling that leads to

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enhanced pain. By blocking this signaling using Akt inhibitor LY94002 or the mTOR inhibitor rapamycin, pain-related hypersensitivity to noxious mechanical stimuli, heat, and inflammatory substances were attenuated (Xing et al., 2020). Here, we sought out to address whether *CNTNAP2* plays a role in itch sensation. Itch is broadly classified into two types- histaminergic and non- histaminergic- based on distinct neural pathways (Dong and Dong, 2018). After intradermal injection of histamine (100μg/10 μl) in the dorsal nape of the neck (DNN), a robust scratching behavior in control mice was observed, but interestingly *Cntnap2 KO* mice exhibited a significant decrease in scratching (Figure 1A). Similarly, we evaluated the non-histaminergic response to the compound chloroquine (CQ; 100μg/10 μl). Again, we observed a significant decrease in CQ-induced itch behavior in *Cntnap2* KO mice compared to their control littermates (Figure 1B) but was not completely abolished. This could be due to some off-target effect of CQ. Surprisingly, CQ (50μg/10 μl) scratching response was comparable to CQ (100μg/10 μl) in control mice (Figure 2B), but in *Cntnap2* KO mice, we found a lower dose (50μg/10 μl) does not induce itch response and is comparable to the vehicle (1XPBS) injected mice (Figure 2C). Thus, these results suggest that both histamine and CQ-induced itch responses are dependent upon *CNTNAP2* gene; however, it is still unclear what role, if any, CNTNAP2 plays in the attenuation of itch behavior.

CNTNAP2 is required for synaptic function and ASD-related behavior in mice (Sacai et al., 2020). Knocking down the CNTNAP2 in the developing brain shows reduced excitatory synaptic transmission, leading to impaired social interaction and induced mild vocalization abnormality. Pharmacological enhancement of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor effectively restored impaired social behavior. We wondered how CNTNAP2 might operate in itch signal transduction. One of the hypotheses is that the loss of function of the CNTNAP2 gene in the DRG diminished glutamate release in the spinal cord produces a consequent reduction in itch responses. Both AMPA and glutamate binds to the AMPA receptor, an ionotropic transmembrane receptor that mediates fast synaptic transmission in the central nervous system (Kaczor and Matosiuk, 2010). To test this postulate, AMPA (to mimic glutamate) was delivered exogenously in the spinal cord in the intrathecal space between the lumbar 4 and 5 (L4–L5) and, 10-minutes later, histamine was injected at the DNN, with scratching behavior quantified for every 30 minutes. At lower exogenous dose, AMPA (0.001mM/20 μl) had no effect on histamine-induced itch, but AMPA (0.1mM/ 20 μl) application partially restored the histamine (100μg/10 μl)-induced itch behavior in *Cntnap2* KO mice (Figure 2). Importantly, injection of AMPA (0.1mM/ 20 μl) alone evoked no itch response. In addition, spinal cord delivery of AMPA had no effect on histamine-induced itch behavior in naïve mice (Figure 2B).

CNTNAP2 plays many different roles in the nervous system. Loss of CNTNAP2 in mice reduced the number of inhibitory neurons, abnormal positioning and migration of excitatory neurons, and neural network dysfunction (Penagarikano et al., 2011). CNTNAP2 also plays a role in neurotransmitter release (Zou et al., 2017) and receptor trafficking. Silencing CNTNAP2 expression *in vitro* or *in vivo* decreases the synaptic expression of AMPA receptors (AMPAR) and the amplitude of AMPA receptor-mediated currents (Fernandes et al., 2019). These data suggest that CNTNAP2 in the DRG plays an important role in the excitatory synaptic function. The loss of CNTNAP2 inhibits the pre-synaptic release of glutamate, an excitatory neurotransmitter resulting in loss of itch behavior. Our data is

consistent with a recent study by Kiguchi et al. They showed that peripheral induction of histaminergic and non-histaminergic itch was prevented by intrathecal (i.t.) administration of the AMPA antagonist NBQX (Kiguchi et al., 2020). Interestingly, the data presented here discusses the role of primary afferent neurons; however, we do not exclude the possibility that CNTNAP2 might be involved in synaptic release of neurotransmitters from spinal interneurons and could be responsible for the effect that we see in our experiments. Overall, our data suggest a previously unknown role for CNTNAP2 in the neurotransmission of itch, mediated through an excitatory glutamatergic pathway.

Taken together, the CNTNAP2 gene is linked to itch sensation and may provide strategies for the treatment of itch. To the best of my knowledge, there is no single report that suggests a link between ASD and itch behavior. Given that ASD patients often experience AD, we would expect to see an increase in itch behavior in *Cntnap2*-deficent mice, but our results did not demonstrate that expectation. The reasons could be: first, due to species difference between mice and humans; and second, CNTNAP2 might contribute directly to AD in ASD by affecting neuropeptides release; therefore, a lack of CNTNAP2 might impact development of AD in ASD. Interestingly, this study sheds light on the possible linkage between ASD and itch. Our study's limitation is that the result is primarily based on behavioral experiments, and cellular and molecular aspects of these behavioral deficits are not explained. Further studies may be required to dissect the molecular underpinnings of CNTNAP2 and itch sensation.

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CONFLICT OF INTEREST:

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DATA AVAILABILITY STATEMENT:

No datasets were generated or analyzed during the current study.

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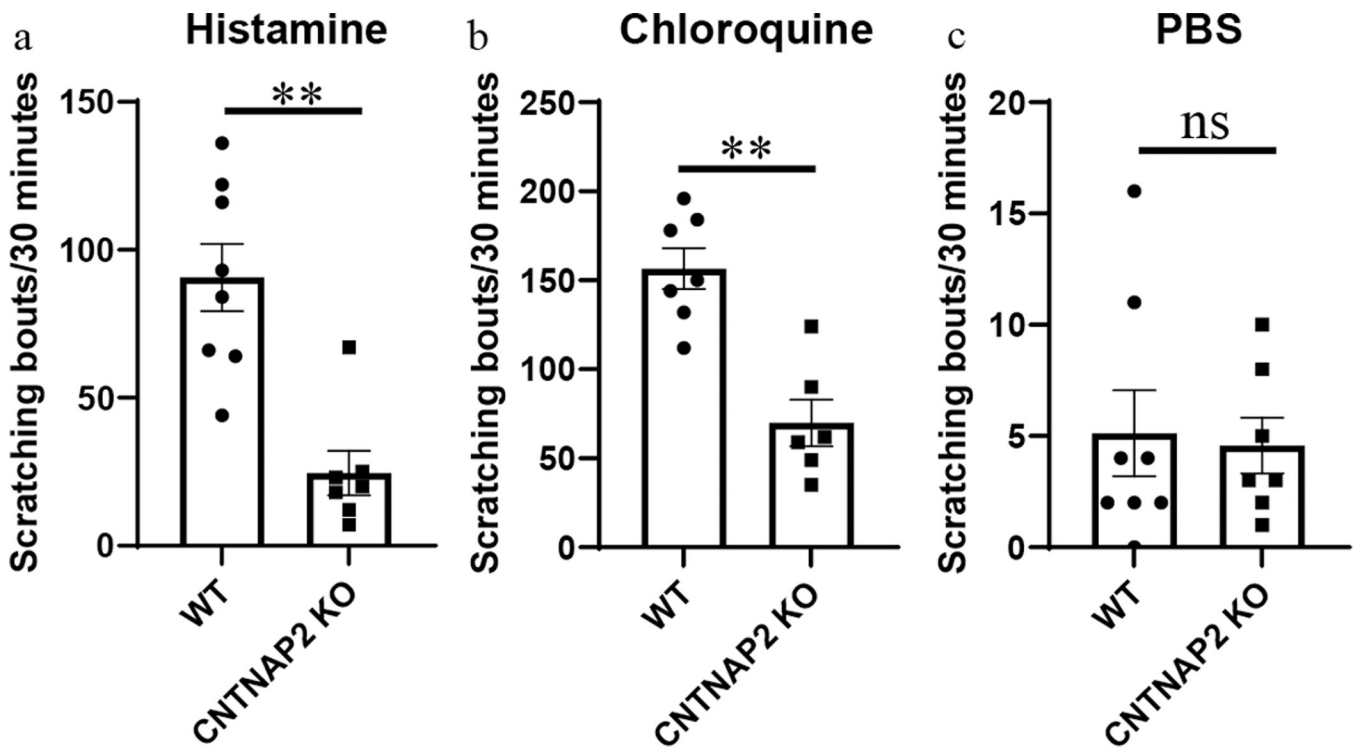

Figure 1. Scratching after intradermal injection of itch mediators in mice.

(*a*) Histamine injection (100ug/10 ul) (*b*) chloroquine injection (100ug/10 ul). (c) vehicle (1xPBS in 10 ul volume). Values represent the means ± SEM, n ≥ 6 mice. **p<0.01, student t-test, nonparametric comparison, n= 3–4 male and female mice were used.

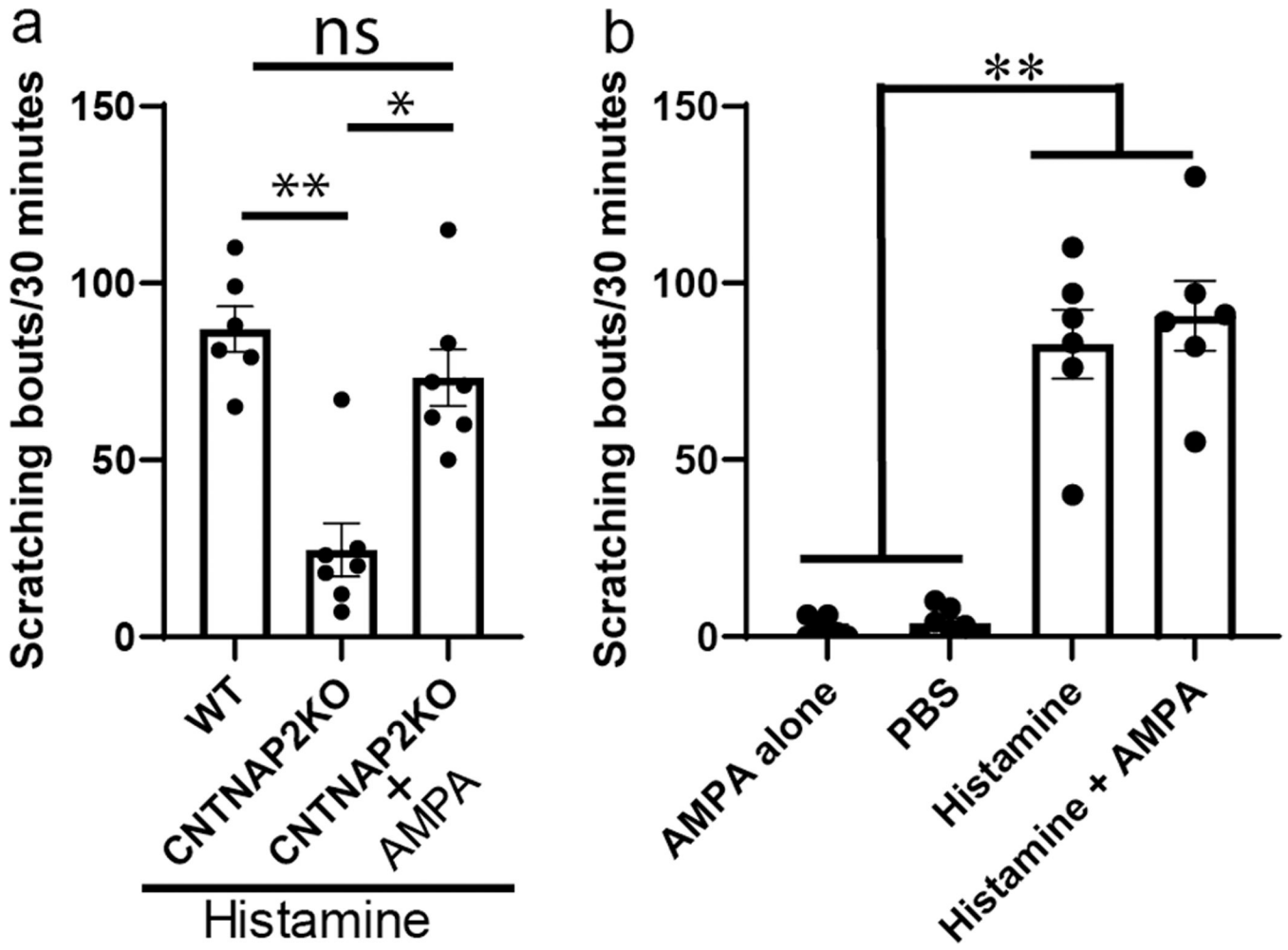

Figure 2. Exogenous AMPA restore histamine-induced itch response.

(*a*) Vehicle injected in the intrathecal space between lumbar (L) L4–L5 and histamine injection in the DNN (100ug/10 ul) in control and CNTNAP2 KO mice. Another set of CNTNAP2 KO mice AMPA was injected in the intrathecal space followed by histamine in the DNN. (b) Control littermates were injected with AMPA (i.t., 0.1 mM/ 20μl), vehicle (i.t.), vehicle (i.t.) + histamine in DNN, and AMPA (i.t., 0.1 mM/ 20μl) + histamine DNN. Values represent the means ± SEM, n ≥ 6 mice. **p<0.01, Kruskal-Wallis multiple comparison test, n= 3–4 male and female mice were used.