



Linoleic acid improves PIEZO2 dysfunction in a mouse model of Angelman Syndrome

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Angelman syndrome (AS) is a neurogenetic disorder characterized by intellectual disability and atypical behaviors. AS results from loss of expression of the E3 ubiquitin-protein ligase UBE3A from the maternal allele in neurons. Individuals with AS display impaired coordination, poor balance, and gait ataxia. PIEZO2 is a mechanosensitive ion channel essential for coordination and balance. Here, we report that PIEZO2 activity is reduced in *Ube3a* deficient male and female mouse sensory neurons, a human Merkel cell carcinoma cell line and female human iPSC-derived sensory neurons with *UBE3A* knock-down, and de-identified stem cell-derived neurons from individuals with AS. We find that loss of UBE3A decreases actin filaments and reduces PIEZO2 expression and function. A linoleic acid (LA)-enriched diet increases PIEZO2 activity, mechano-excitability, and improves gait in male AS mice. Finally, LA supplementation increases PIEZO2 function in stem cell-derived neurons from individuals with AS. We propose a mechanism whereby loss of *UBE3A* expression reduces PIEZO2 function and identified a fatty acid that enhances channel activity and ameliorates AS-associated mechano-sensory deficits.

Angelman syndrome (AS) is a neurogenetic disorder characterized by cognitive, motor, and behavioral abnormalities¹. Individuals with AS display impaired motor coordination (e.g., unable to reach objects), abnormal gait (i.e., instability while walking), sensory ataxia, scoliosis, seizures, an abnormally happy disposition, and intellectual disability^{2–4}. Likewise, AS mouse models have clearly defined phenotypes resembling behavioral dysfunction, such as ataxic gait, seizures, and learning deficits, making this model useful for testing therapeutics^{5–8}. The AS phenotype results from the loss of expression of an E3 ubiquitin-protein ligase (*UBE3A*) from the maternal allele^{9–11}.

UBE3A is regulated by genomic imprinting, a process that causes genes to be monoallelically expressed in neurons¹². The cellular role of the UBE3A ubiquitin ligase is to transfer a single ubiquitin moiety from the E2 protein to a substrate protein¹³. UBE3A targets proteins for degradation, regulates their trafficking, and/or modulates their function^{14–16}.

The abnormal gait associated with this disorder is debilitating, as most children have difficulty walking². Importantly, gait (ataxic or broad-based) is among the most common behaviors (88%) observed in children with AS⁴. *UBE3A* is imprinted in most brain neurons¹. For

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instance, *UBE3A* exhibits maternal allele-specific expression in the Purkinje cell layer of the cerebellum and cell bodies of hippocampal CA1 – CA2 neurons in mice and humans^{17–19}. Bruinsma et al. found that cerebellar function is only partly responsible for the behavioral deficits (e.g., ataxic-like gait, problems with balance) displayed by a mouse model of AS²⁰. On the other hand, in the peripheral nervous system, it has been shown that proprioceptive and mechanosensitive dorsal root ganglia (DRG) neurons express the maternal *UBE3A* allele, while the paternal allele is silenced by an antisense transcript²¹. Hence, the loss of function of the maternal allele, in sensory neurons, could contribute to the phenotypes observed in AS.

Proprioception confers the ability to sense movement, tension, balance, and limb position²². The mechanosensitive ion channel PIEZO2 is expressed in sensory neurons innervating muscle spindles and Golgi tendon organs, where it mediates proprioception and balance^{23–26}. A previous study demonstrated that *PIEZO2* deficiency in humans profoundly decreases proprioception, leading to sensory ataxia²³. For example, a premature stop codon in *PIEZO2* causes unsteady gait and increased stride-to-stride variability in step length and force, among other deficits^{23,27}. Likewise, mice lacking *Piezo2* display abnormal limb position and coordination, unstable gait, and balance deficits^{24,26,28}. Moreover, *PIEZO2* is highly expressed in human Merkel cells and their afferent sensory neurons, where it mediates gentle touch and vibration^{29–32}. Individuals carrying loss-of-function variants in *PIEZO2* have a selective loss of discriminative touch perception²³. Similarly, mice deficient for *Piezo2* in the skin display reduced behavioral responses to gentle touch^{29,30,32}.

The notable similarities between proprioception phenotypes in individuals with AS or *PIEZO2* loss of function (LOF) mutations, as well as their associated mouse models, raise the intriguing hypothesis that mechanotransduction could be impaired in AS. However, it is unclear if the loss of maternal *Ube3a* expression in DRG neurons affects *PIEZO2* activity. Here, we show that *PIEZO2* function is reduced in AS and that a safflower oil diet, enriched in linoleic acid (LA), increases *PIEZO2* activity, mechano-excitability, and ameliorates gait ataxia in a mouse model of AS.

Results

Ube3a^{m-/p+} DRG neurons have decreased mechano-currents and -excitability

We used mice carrying a LOF *Ube3a* mutation on the C57BL/6 genetic background⁵. Heterozygous mice with a maternal deficiency (*Ube3a^{m-/p+}*) display AS-associated phenotypes, including lack of balance and gait ataxia. Conversely, mice with a paternal deficiency (*Ube3a^{m+/p-}*) do not show imbalance, unsteady gait, or sensory ataxia phenotypes^{5,6,33}. Since mice lacking *Piezo2* experience severe mechanosensory and proprioceptive deficits²⁴, we hypothesized that DRG neurons from the *Ube3a^{m-/p+}* mice would display impaired mechanical responses. Parenthetically, ~ 80% of cultured mouse DRG neurons display mechanically activated currents³⁴. These mechanocurrents display various inactivation kinetics (i.e., rapidly, intermediate, and slowly inactivating currents). The rapidly inactivating currents ($\tau < 10$ ms) have been previously assigned to mouse *PIEZO2*³⁵, whereas the intermediate ($10 < \tau < 30$ ms) and slowly inactivating currents ($\tau > 30$ ms) have not yet been identified.

We measured mechanocurrents in the whole-cell patch-clamp configuration from wild-type (WT), *Ube3a^{m-/p+}*, and *Ube3a^{m+/p-}* mouse DRG neurons. All experiments in this work were performed with male mice unless noted. All the mechanocurrents (including the rapidly inactivating currents assigned to *PIEZO2*) from the *Ube3a^{m-/p+}* neurons were significantly reduced compared to WT or *Ube3a^{m+/p-}* DRG neurons (Fig. 1a-b and Supplementary Fig. 1a-b). The displacement threshold required to elicit mechanocurrents in the *Ube3a^{m-/p+}* neurons was slightly higher than WT or *Ube3a^{m+/p-}* (Fig. 1c). The percentage of DRG neurons featuring *PIEZO2* mechanocurrents (i.e., rapidly inactivating

currents) was similar between WT, *Ube3a^{m-/p+}*, and *Ube3a^{m+/p-}* cultures (Supplementary Fig. 1c). Moreover, the capacitance distribution of recorded neurons was skewed towards medium to large diameter cells (i.e., mechanoreceptors³⁶) and, importantly, was similar for WT, *Ube3a^{m-/p+}*, and *Ube3a^{m+/p-}* neurons (Supplementary Fig. 1d-e). Parenthetically, DRG neurons from female *Ube3a^{m-/p+}* mice also displayed reduced mechanocurrents compared to WT or *Ube3a^{m+/p-}* (Supplementary Fig. 1f-g). *PIEZO2* mediates most of the mechano-activated excitatory currents in mouse DRG neurons^{24,31}. As neurons from *Ube3a^{m-/p+}* mice display decreased mechanocurrents and an increase in displacement threshold, we sought to determine the ability of these neurons to elicit mechanically-activated action potentials. *Ube3a^{m-/p+}* neurons required larger indentation steps to elicit action potentials than WT or *Ube3a^{m+/p-}* (≥ 12 μ m; Fig. 1d-e). Importantly, *Ube3a^{m-/p+}* neurons have similar neuronal electrical excitability compared to WT neurons (membrane capacitance, action potential amplitudes, input resistances, and minimal current threshold required to elicit an action potential; Supplementary Fig. 1d, h-k). These results demonstrate that mechanocurrents (including those from *PIEZO2*) and mechano-excitability are reduced in *Ube3a^{m-/p+}* DRG neurons.

UBE3A knockdown alters the cytoskeleton and decreases *PIEZO2* function

We asked whether knocking down the expression of *UBE3A* in a human cell line by silencing RNA (siRNA) could recapitulate our findings in DRG neurons. *PIEZO2* is expressed in Merkel cells (tactile epithelial cells) and their innervating afferents, where it transduces skin indentation^{29,30,32}. To support functional and biochemical experiments, we utilized the human Merkel cell carcinoma cell line (MCC13), in which *PIEZO2* mediates all endogenous mechanosensitive currents³⁷. MCC13 cells displayed a decrease in *PIEZO2* currents after knocking down the expression of *UBE3A* or *PIEZO2*, when compared with mechanocurrents from the scrambled siRNA treatment (47% and 74%, respectively; Fig. 2a). We validated the siRNA treatment by performing RT-qPCR (Supplementary Fig. 2a-b). Quantification of mRNA levels demonstrates that knocking down *UBE3A* does not affect *PIEZO2* transcripts (Supplementary Fig. 2b). As expected, decreasing the mRNA levels of *UBE3A* reduced *UBE3A* protein expression (Supplementary Fig. 2c-d). Notably, knocking down the expression of *UBE3A* decreased 23% of *PIEZO2* membrane levels in MCC13 cells (Fig. 2b and Supplementary Fig. 2e). For electrophysiology experiments, we patched cells expressing the transfection marker siGLO green, whereas for western blots, we extracted membrane protein from a mixed culture of transfected and untransfected cells. This could explain the difference in effect size between currents and membrane protein expression reduction (47% vs. 23%). Conversely, *UBE3A* over-expression by transient transfection of *UBE3A* in MCC13 cells showed increased *PIEZO2* currents and membrane expression (Fig. 2c-d and Supplementary Fig. 2f). Taken together, in both mouse DRG neurons and human cell lines, downregulation of *UBE3A* expression decreases *PIEZO2* currents.

Ube3a deficient mice display reduced filamentous actin (F-actin) in cultured hippocampal neurons³⁸. Moreover, using proteomic profiling, we have previously shown in *D. melanogaster* that *Ube3a* homozygous mutants have less F-actin, consistent with the identification of actin targets regulated by *Ube3a*³⁹. *PIEZO1* and *PIEZO2* channel function is modulated by cytoskeletal elements^{40–43}. We previously demonstrated that disrupting actin filaments with latrunculin A decreased *PIEZO2* currents in N2A cells⁴². Therefore, we tested the hypothesis that loss of *UBE3A* expression could decrease *PIEZO2* currents by impairing F-actin. MCC13 cells treated with latrunculin A display reduced *PIEZO2* currents compared to untreated cells (Fig. 2e). Of note, we measured a decrease in *PIEZO2* membrane expression levels in MCC13 cells after latrunculin A treatment, as well as a reduction in F-actin content (Fig. 2f and Supplementary Fig. 2g-i). Importantly,

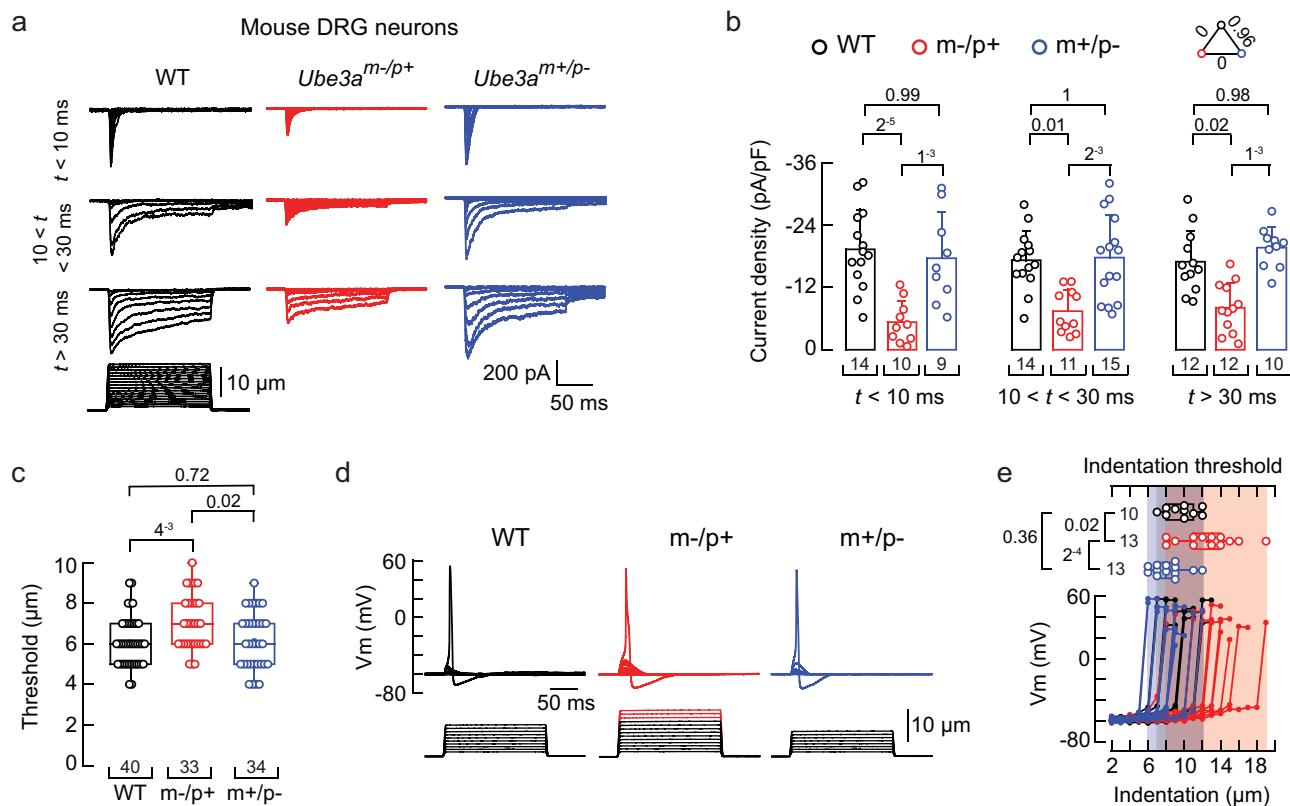


Fig. 1 | *Ube3a^{m-/p+}* DRG neurons display reduced mechano-currents and -excitability. **a** Representative whole-cell patch-clamp recording elicited by mechanical stimulation (-60 mV) of rapidly, intermediate, and slowly inactivating currents of WT, *Ube3a^{m-/p+}*, and *Ube3a^{m+/p-}* DRG neurons. **b** Current densities elicited by maximum displacement of DRG neurons classified by their time constant of inactivation. Bars are mean \pm SD. Two-way ANOVA ($F = 35.44$, $p = 2.63^{-12}$) and Tukey multiple-comparisons test. **c** Boxplots show the displacement thresholds required to elicit mechanocurrents of DRG neurons. Boxplots show mean (square), median (bisecting line), bounds of box (75th to 25th percentiles), outlier range with 1.5 coefficient (whiskers), and minimum and maximum data points. Kruskal-Wallis

($H = 9.51$; $p = 0.0086$) and Dunn's multiple comparisons test. **d** Representative current-clamp recordings of membrane potential changes elicited by mechanical stimulation in DRG neurons. **e** Membrane potential peak vs. mechanical indentation of independent mouse DRG neurons. At the top, boxplots show the displacement threshold required to elicit an action potential in these neurons. Boxplots show mean (square), median (bisecting line), bounds of box (75th to 25th percentiles), outlier range with 1.5 coefficient (whiskers), and minimum and maximum data points. One-way ANOVA ($F = 10.54$; $p = 2.89^{-4}$) and Tukey multiple-comparisons test. n is denoted in each panel. Post hoc p values are denoted in the corresponding panels. Source data are provided as a Source Data file.

we also observed a reduction in actin content after knocking down the expression of *UBE3A* in MCC13 cells (Fig. 2g and Supplementary Fig. 2j). Our data support that knocking down *UBE3A* expression reduces actin filaments, leading to a decrease in PIEZO2 membrane expression and currents.

UBE3A could alter actin dynamics by regulating the content of actin-binding protein(s). Cofilin is an actin-binding protein that promotes rapid actin filament disassembly⁴⁴. We determined an increase in cofilin content after knocking down the expression of *UBE3A* in MCC13 cells (Fig. 2h and Supplementary Fig. 2k). Moreover, over-expression of cofilin in MCC13 cells, by transient transfection, decreased PIEZO2 currents compared to control cells (Fig. 2l). Previous works have shown that cofilin can be ubiquitinated by the E3 ubiquitin ligases Cbl and AIP4⁴⁵; however, whether *UBE3A* ubiquitinates cofilin is unknown. Using a stringent cell-culture based ubiquitination assay^{46,47}, we demonstrated that cofilin ubiquitination is increased by WT *UBE3A* but not by a ligase dead (LOF) version of this E3 enzyme (Fig. 2j and Supplementary Fig. 2l). Our findings support a model whereby loss of *UBE3A* expression increases cofilin, which severs actin filaments and decreases PIEZO2 membrane expression and currents.

Ube3a^{m-/p+} DRG neurons display reduced F-actin, and promoting actin polymerization increases PIEZO2 currents

Based on our findings in MCC13 cells, we reasoned that *Ube3a^{m-/p+}* mouse DRG neurons could have a reduced F-actin content. Indeed,

cultured *Ube3a^{m-/p+}* neurons fixed and stained with Alexa Fluor 488 phalloidin (selective actin filament stain) displayed a reduced mean fluorescence intensity compared to WT (Fig. 3a). This result is further supported by the increase in the G/F actin ratio in *Ube3a^{m-/p+}* DRG neurons, as determined by western blots (Fig. 3b and Supplementary Fig. 3a). Jasplakinolide is a peptide that promotes actin polymerization and stabilizes actin filaments⁴⁸. Notably, jasplakinolide treatment significantly increased PIEZO2 currents in *Ube3a^{m-/p+}* mouse DRG neurons (Fig. 3c). An increase in cofilin could account for the decrease in actin in the *Ube3a^{m-/p+}* mouse DRG neurons. We found elevated levels of cofilin (a target of *UBE3A*) in *Ube3a^{m-/p+}* mouse DRG neurons when compared to neurons from WT mice (Fig. 3d and Supplementary Fig. 3b). Importantly, knocking down the expression of *cofilin* in *Ube3a^{m-/p+}* DRG neurons increases PIEZO2 currents similar to WT levels (Fig. 3e). Taken together, our results demonstrate that *Ube3a^{m-/p+}* DRG neurons have an impaired actin cytoskeleton and treatments that stabilize actin filaments or reduce cofilin expression increase PIEZO2 function.

Linoleic acid increases PIEZO2 currents

There are no agonists available for PIEZO2⁴⁹. We have previously shown that PIEZO1 (a close homolog of PIEZO2) displayed slower inactivation and more mechanocurrents in plasma membranes containing high levels of linoleic acid (LA, ω -6 C18:2)⁵⁰ and Supplementary Fig. 4a. Considering the similarities between the PIEZO channels,