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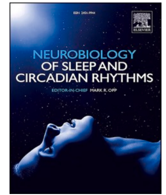

Pleiotropic effects of a high confidence Autism Spectrum Disorder gene, *arid1b*, on zebrafish sleep

Fusun Doldur-Balli [a,*], Amber J. Zimmerman [a], Brendan T. Keenan [a], Zoe Y. Shetty [a], Struan F.A. Grant [b,c,d,e], Christoph Seiler [f], Olivia J. Veatch [g,1], Allan I. Pack [a,1]

[a] Division of Sleep Medicine, Department of Medicine, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
[b] Center for Spatial and Functional Genomics, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA
[c] Department of Pediatrics, The University of Pennsylvania Perelman School of Medicine, Philadelphia, PA, 19104, USA
[d] Divisions of Human Genetics and Endocrinology & Diabetes, Children's Hospital of Philadelphia, Philadelphia, PA, 19104, USA
[e] Department of Genetics, University of Pennsylvania, Philadelphia, PA, 19104, USA
[f] Aquatics Core Facility, Children's Hospital of Philadelphia, Philadelphia, PA, USA
[g] Department of Psychiatry and Behavioral Sciences, University of Kansas Medical Center, Kansas City, KS, USA

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ABSTRACT

Sleep fulfills critical functions in neurodevelopment, such as promoting synaptic plasticity, neuronal wiring, and brain connectivity which are critical phenomena in Autism Spectrum Disorder (ASD) pathophysiology. Sleep disturbance, specifically insomnia, accompanies ASD and is associated with more severe core symptoms (e.g., social impairment). It is possible that focusing on identifying effective ways to treat sleep problems can help alleviate other ASD-related symptoms. A body of evidence indicates shared mechanisms and neurobiological substrates between sleep and ASD and investigation of these may inform therapeutic effects of improving sleep at both behavioral and molecular levels. In this study, we tested if sleep and social behavior were different in a zebrafish model with the *arid1b* gene mutated compared to controls. This gene was selected for study as expert curations conducted for the Simons Foundation for Autism Research Institute (SFARI) Gene database define it is as a 'high confidence' ASD gene (i.e., clearly implicated) encoding a chromatin remodeling protein. Homozygous *arid1b* mutants displayed increased arousability and light sleep compared to their heterozygous and wild type counterparts, based on testing a mechano-acoustic stimulus presenting different vibration frequencies of increasing intensity to detect sleep depth. In addition, decreased social preference was observed in *arid1b* heterozygous and homozygous mutant zebrafish. The behavioral phenotypes reported in our study are in line with findings from mouse models and human studies and demonstrate the utility of zebrafish as a vertebrate model system with high throughput phenotyping in the investigation of changes in sleep in models relevant to ASD. Furthermore, we demonstrate the importance of including assessments of arousal threshold when studying sleep using *in vivo* models.

1. Introduction

The role of sleep in brain development is evident (Wintler et al., 2020). Sleep promotes synaptic plasticity (Abel et al., 2013; Tononi and Cirelli 2014; Seibt and Frank 2019), neuronal wiring (Azeez et al., 2018), and brain connectivity (Chee and Zhou 2019). Rapid development of the human brain occurs during infancy and early childhood and is coincident with heightened sleep need (Wintler et al., 2020). Notably, sleep disturbances are highly prevalent in individuals with neurodevelopmental disorders (Shelton and Malow 2021). Given the evidence that sleep plays a critical role in brain development, the need to inform treatment of sleep disturbances via understanding risk factors underlying the relationship between expression of sleep problems and neurodevelopmental conditions is crucial. Insomnia is the most common sleep disturbance reported in children with Autism Spectrum Disorder (ASD), with prevalence rates ranging between 40% and 86% (Wintler et al., 2020). Insomnia is characterized by difficulty in falling and staying asleep, which often results in short sleep duration, decreased

* Corresponding author.
E-mail address: fusun.balli@pennmedicine.upenn.edu (F. Doldur-Balli).
[1] Denotes equal contributions for dual-senior authorship.

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sleep efficiency and long sleep onset latency (Wintler et al., 2020). Notably, sleep disordered breathing is also commonly reported in individuals with ASD (~28%) (Tomkies et al., 2019). Studies report that improving sleep in children with ASD also improves daytime symptoms (Malow et al., 2014; Cuomo et al., 2017). The interplay between sleep and ASD pathophysiology indicates shared mechanisms and neurobiological substrates (Harvey et al., 2011). Despite heterogeneity in ASD, the high prevalence of sleep problems offers a potential avenue for therapies addressing sleep behaviors.

Based on these clinical observations, we hypothesize that variants in high confidence risk genes for ASD have pleiotropic effects on sleep (Veatch et al., 2017; Doldur-Balli et al., 2022). We selected the *ARID1B* high confidence ASD gene from a broad class of genes that are enriched for associations with ASD, i.e., the chromatin remodeling complexes. *ARID1B* encodes the AT-rich interactive domain containing protein 1B (Moffat et al., 2019). It is a subunit of SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeling complex (Pagliaroli and Trizzino 2021), important for regulating access of transcriptional machinery to DNA by modifying DNA-histone interactions within nucleosomes in an ATP-dependent manner (Pagliaroli and Trizzino 2021). *ARID1B* belongs to npBAF and nBAF complexes which are specific to neural progenitors and neuron-specific chromatin remodeling complexes, respectively (Staahl et al., 2013). Previous work has demonstrated human *ARID1B* is involved in neurite outgrowth and maintenance (Moffat et al., 2019) indicating it may be important during early developmental patterning of behavioral circuitry.

As a high confidence ASD gene, *ARID1B* has been associated with multiple neurodevelopmental and behavioral deficits. In mice, *Arid1b* haploinsufficiency is associated with ASD-relevant phenotypes such as elevated anxiety-like behaviors and behavioral inflexibility, together with impaired motor coordination and impaired social behavior (Shibutani et al., 2017). Other studies of *Arid1b* haploinsufficient mice reported decreased social behavior, increased repetitive movements and anxiety like behaviors (Celen et al., 2017; Jung et al., 2017; Moffat et al., 2022). In humans, *ARID1B* haploinsufficiency is associated with Coffin-Siris syndrome (Santen et al., 2012; Tsurusaki et al., 2012) and ASD (Nord et al., 2011). Moreover, individuals carrying *ARID1B* mutations have symptoms of sleep apnea (van der Sluijs et al., 2019) and increased number of awakenings at night (Kruizinga et al., 2020). Sleep and social behavior have not been described in *arid1b* mutant zebrafish, and fish provide a rapid tool for examining these conserved behaviors.

To investigate whether a loss of function of *ARID1B* alters both sleep and social interaction, we studied a zebrafish mutant that has a deletion in this gene. We assess sleep phenotype, arousal threshold and social preference in these mutant fish and compared results to wild-type controls. Our results show that this mutation does result in differences in both sleep depth and social behavior.

2. Materials and methods

2.1. *arid1b* mutant zebrafish line and animal use

The zebrafish *arid1b* mutant line was purchased from Zebrafish International Resource Center (https://zebrafish.org/) (ZFIN 2006). The allele designation of the *arid1b* mutant line is y607. The line was generated in Harold Burgess lab. This information can be found on the following ZFIN page (https://zfin.org/ZDB-ALT-190403-3) (ZFIN 2006). This mutant line was generated using CRISPR/Cas9 and contains a 13 base-pair (bp) deletion (open reading frame nucleotides 1865–1877, 5′-TTCCTCCGATGCC/-3′) which shifts the translational reading frame after amino acid 391 and results in a premature stop codon. To confirm zygosity, mutant animals were genotyped using the primers 5′-GAGCTTTGTTTTCTGAGTAATCTTTAG-3′ and 5′-CACTGCTCACCGATATCCAA-3′, which produce a 125 bp or 112 bp band for the wild type and mutant allele, respectively (Supplementary Fig. S1). Embryos of F2 generation were used in the experiments. Animals heterozygous for *arid1b* mutations were crossed yielding wildtype (*arid1b* +/+), heterozygous mutant (*arid1b* +/−) and homozygous mutant (*arid1b* −/−) sibling progeny. Sex is not yet determined at larval stages of zebrafish. Behavioral experiments were carried out blinded to genotype. Animals were then genotyped using PCR and agarose gel electrophoresis after each experiment.

All experiments were performed in accordance with the University of Pennsylvania Institutional Animal Care and Use Committee guidelines. Fish were raised on a 14-h:10-h light:dark cycle with lights on at 9 a.m. (ZT0) and lights off at 11 p.m. (ZT14) at optimum growth temperature of 28.5 °C.

2.2. Sleep/wake assay

Zebrafish larvae collected from in-cross of *arid1b* ± animals were used in sleep/wake assays. Sleep/wake assays began on day 4 into larval development (i.e., 4 days post fertilization [dpf]) and stopped when larvae were 7 dpf. Analyzed data corresponded to locomotor activity measured over two days and nights from zebrafish larvae that were 5 dpf and 6 dpf, respectively. Larval zebrafish were individually pipetted into each well of a 96 well plate (7701-1651, Whatman) containing 650 μl of standard embryo medium (E3 embryo medium, 5 mM NaCl, 0.17 mM KCl, 0.33 mM $CaCl_2$, 0.33 mM $MgSO_4$, pH 7.4) at 4 days post fertilization according to previously established protocols (Rihel et al., 2010). E3 medium levels were topped off each morning. An automated video tracking system (Zebrabox, ViewPoint Life Sciences Inc.) in which 96 well plates are housed was continuously illuminated with infrared lights for data acquisition and illuminated with white light only under the 14 h light followed by 10 h dark, thereby providing day and night cycles. Constant temperature of 28.5 °C was ensured by recirculating water in the chamber of the equipment. Zebrafish larvae were allowed to acclimate in the equipment for approximately 24 h before data acquisition. DNA was extracted following manufacturer's instructions (Quanta bio, Beverly, MA) immediately after completion of sleep/wake assays. Sleep/wake assays were repeated 5 times using a different group of embryos, providing 5 biological replicates.

ZebraLab software version 5.8.0.0 (Viewpoint Life Sciences Inc.) was used to analyze video tracking and capture locomotor activity of zebrafish larvae (Rihel et al., 2010; Palermo et al., 2023). The following parameters were used to detect activity: detection threshold: 20; burst: 29; freeze: 3; bin size: 60 s (Lee et al., 2019). Data were analyzed using custom MATLAB scripts (Lee et al., 2022) to compare sleep and activity parameters across the 3 different genotypes. Sleep was defined as any 1-min period of inactivity with less than 0.1 s of total movement (Rihel et al., 2010). A sleep bout was defined as a continuous sequence of sleep minutes (Rihel et al., 2010). Latency to sleep was measured by detecting the length of time starting from lights off until the start of the first sleep bout (Rihel et al., 2010). Multiple insomnia-related sleep traits were analyzed. These included sleep duration (total minutes in dark cycle or light cycle), activity duration (seconds/min, due to activity being more dynamic than sleep), waking activity (seconds of activity/awake minute), sleep bout number (number in dark cycle or light cycle), sleep bout length (average duration in minutes, min/bout) and sleep latency (minutes) (Rihel et al., 2010). These parameters were calculated for each day (14 h of light cycle between 9 a.m. and 11 p.m.) and night (10 h of dark cycle between 11 p.m. and 9 a.m.) of the experiments.

2.3. Arousal threshold assay

Arousal threshold was tested using a mechano-acoustic stimulus presenting ten different vibration frequencies of increasing intensity, as previously described (Singh et al., 2015, Zimmerman et al., 2022). Briefly, individual larvae were pipetted into wells of a 96-well plate. Ten distinct frequencies were applied beginning at 1 a.m. (during dark cycle) (ZT16) and ending at 6 a.m. (ZT 21). Each stimulus was presented for 5 s every 3 min (5 s on, 2 min 55 s off). Each stimulus frequency was

presented 10 times across the 5 h window for a total of 100 trials (10 frequencies x 10 repetitions). The response to stimuli was measured by automated video tracking (ViewPoint Life Sciences) to assess if arousal threshold was elevated in all genotypes during sleep and data were analyzed using MATLAB (Mathworks). A 5-s pre-stimulus baseline was used to calculate the proportion of fish considered to be asleep (activity <0.01s/5s), and the response fraction was calculated as the proportion of larvae whose activity increased over baseline during the stimulus presentation. Thus, only fish which were asleep immediately prior to stimulus onset were included in the analysis. Data were averaged across all fish in each genotype to give an average response at each stimulus intensity across the 10 trials. Experimenters were blinded to the genotype; genotypes were assessed by PCR following assays.

2.4. Social preference assay

Social preference of 3–4 weeks post fertilization (wpf) zebrafish spawned from in-crossing *arid1b* ± animals was assessed. It was shown that social preference toward other zebrafish emerges at this developmental stage (Dreosti et al., 2015). Methodology reported by Ruzzo et al. (2019) was adopted to perform experiments and calculate phenotype (Ruzzo et al., 2019). The experimental animals were individually placed into the middle wells of columns of a 12 well plate (CC7672-7512, CytoOne). One of the two neighboring wells include a wild type zebrafish which were at the same age and size with experimental animals as a social cue and the other well is left empty. Animals were allowed to acclimate to the environment for 5 min (habituation period I) and then their swimming behavior was recorded by automated video tracking for 10 min (baseline period). Dividers between wells were in place during first habituation and baseline periods. After baseline recording, dividers were removed and fish were allowed to swim freely for 5 min (habituation period II). Swimming behavior of larvae was recorded by automated video tracking for another 10 min (post-baseline period). Constant temperature of 28.5 °C in the water was ensured by recirculating water provided by a temperature control unit (Corio CP BC4, Julabo GmbH). Tracking mode of the (Viewpoint Life Sciences Inc) software was utilized to capture swimming behavior of zebrafish (Ruzzo et al., 2019). All experiments were performed between 2 and 4 pm (ZT5-7). Location of social cue and empty well were alternated in each assay. DNA was extracted following manufacturer's instructions (Quanta bio, Beverly, MA) immediately after completion of social preference assays.

Two rectangular zones on the middle well which face to the neighboring wells were virtually defined on the software prior to each assay. Both areas were of equal size. Time spent by the experimental animals in virtual areas were recorded by the software and served as the data to calculate their social preference. Using these data, social preference index (SPI) of each experimental zebrafish was calculated by the following formula (Ruzzo et al., 2019):

$$\frac{\text{Time spent in zone near the wild type larva (social zone)} - \text{Time spent in zone near the empty well (nonsocial zone)}}{\text{Time spent in both zones}}$$

SPI ranges between +1 and −1. A positive SPI means the experimental animal preferred to continue interaction with its conspecific (another animal from the same species) and a negative SPI value means experimental animal preferred to stay far from this social cue. Notably, the social cue is a wild type zebrafish of the same age and size.

To evaluate whether any differences in social preference were due to developmental delay, we measured and compared zebrafish body length on animals, euthanized by rapid cooling, from the tip of the mouth to the end of the tail fin manually after completion of the assay (Ruzzo et al., 2019).

2.5. Statistical analysis

Statistical analyses were performed using Stata/SE 14.2 (StataCorp LLC, College Station, TX). Comparisons of sleep and activity traits among *arid1b* genotypes were performed utilizing a repeated measures analysis of variance (RM-ANOVA), followed by between genotype comparisons if the RM-ANOVA found significant differences among genotypes (unadjusted $p < 0.05$). The mixed model included two measurements per animal (corresponding to the two data collection timepoints when summarized [i.e., night 1 and 2 or day 1 and 2]), a random animal effect, and a categorical covariate for experimental batch (1, 2, 3, 4 or 5). Results were summarized using the model estimated genotype-specific mean and 95% confidence interval (CI) and between genotype differences and 95% CIs, when applicable. Standardized mean differences (SMD) estimated as the between group differences in standardized (Z-score) outcomes are also reported to understand the relative magnitude of effect, with values of 0.2, 0.5 and 0.8 representing small, medium and large effect sizes based on definitions provided by Cohen (1988).

To control type I error across our different hypotheses of interest, a Hochberg step-up procedure was utilized to control for multiple comparisons when performing among genotype (ANOVA) and between genotype (pairwise) comparisons (Hochberg 1988; Huang and Hsu 2007). To implement the Hochberg method, the p-values for the set of multiple null hypotheses are ordered from largest to smallest, and each p-value is compared to a sequentially decreasing alpha-level to determine whether the null hypothesis (and, potentially, subsequent hypotheses) should be rejected. Symbolically, for the set of p-values (p_1, …p_k) ordered from largest to smallest and testing the corresponding set of null hypotheses (H_{o1}, …H_{ok}), the Hochberg procedure is implemented as.

1. Evaluate whether $p_1 < \alpha$. If yes, reject H_{o1} and all subsequent null hypotheses (H_{o2}, …H_{ok}). Else, do not reject H_{o1} and go to Step 2.
2. Evaluate whether $p_2 < \alpha/2$. If yes, reject H_{o2} and all subsequent null hypotheses (H_{o3}, …H_{ok}). Else, do not reject H_{o2} and go to Step 3.
   […]

K. Evaluate whether $p_k < \alpha/k$. If yes, reject H_{ok}. Else, none of the null hypotheses (H_{o1}, …H_{ok}) are rejected and stop.

As only the smallest p-value is compared to the traditional Bonferroni-corrected α-level, the Hochberg method is more statistically powerful for controlling type I error in the context of testing multiple null hypotheses and better accounts for possible correlation among variables.

Curve fit and statistical analyses of arousal threshold assay were carried out using non-linear regression [Agonist vs Response—Variable slope (four parameters)]. EC50 (half-maximal response) was compared between genotypes using extra sum-of-squares F-test as described previously (Singh et al., 2015). In addition, the proportion of animals responding to low (≤5 a.u.) and high (>5 a.u.) intensity stimuli were averaged and compared among genotypes using an analysis of variance (ANOVA), followed by between genotype comparisons if the overall ANOVA $p < 0.05$.

In social preference studies, comparisons of the difference in SPI from baseline to post measurement and post value adjusted for baseline among genotypes were performed via an analysis of variance (ANOVA) followed by pairwise comparisons, consistent with other analyses. As all models were controlled for baseline SPI, these two analyses provide entirely consistent results when comparing values between groups; thus, the conclusions of the two analyses are identical and are used simply to provide complementary data presentations.

3. Results

3.1. Sleep-related traits in arid1b zebrafish line

We studied sleep/wake behaviors in 5- to 7-day old larvae (total n = 432, *arid1b* +/+ n = 118, *arid1b* ± n = 219, *arid1b* −/− n = 95) (Fig. 1A). Prior to adjusting for multiple comparisons, we observed differences in sleep bout length among genotypes (ANOVA p = 0.048), with reduced nighttime sleep bout length in *arid1b* −/− mutant zebrafish larvae compared to their *arid1b* +/+ siblings (mean [95% CI] = −1.58 [−2.91, −0.26] minutes; unadjusted p = 0.019), consistent with difficulties maintaining sleep bouts (Fig. 1B, Supplementary Table 1). Differences in other nighttime or daytime sleep characteristics including sleep duration (Supplementary Fig. S2A) were not significant based on an unadjusted $p < 0.05$ (Supplementary Table 1). In addition, daytime activity (ANOVA p = 0.048) was increased in *arid1b* −/− mutant zebrafish larvae compared to their *arid1b* +/+ siblings (0.26 [0.05, 0.47] seconds/min; p = 0.018) (Fig. 1C, Supplementary Fig. S2B, Supplementary Table 1). While these results were nominally significant ($p < 0.05$), they did not maintain statistical significance after Hochberg correction.

We observed no significant difference between genotypes (total n = 183, *arid1b* +/+ n = 44, *arid1b* ± n = 94, *arid1b* −/− n = 45) for the EC50 (half-maximal response) stimulus threshold which elicits a response half-way between the minimum and maximum response ($F_{(2,288)} = 0.58$, p = 0.56) (Fig. 2A). By segregating stimuli intensity into low (≤5 a.u.) and high (>5 a.u.) bins, we observed a significant difference in the proportion of larvae responding to low intensity stimuli ($F_{(2,27)} = 10.10$, p = 0.0005), with a significantly greater proportion of *arid1b* −/− larvae responding to low intensity stimuli compared to *arid1b* +/+ and *arid1b* +/− (Fig. 2B, Supplementary Table 2). This result is consistent with a decreased arousal threshold in homozygous *arid1b* mutants. There was no significant difference between *arid1b* +/+ and +/−, indicating that depth of sleep in zebrafish is only altered by complete loss of *arid1b*. Additionally, no differences were found between genotypes for high intensity stimuli (Fig. 2C, Supplementary Table 2) where most larvae were responsive to the stimuli regardless of genotype.

3.2. Social preference of arid1b mutants

Social behavior was assessed in 3- to 4- weeks old animals (total n = 85, *arid1b* +/+ n = 20, *arid1b* ± n = 47, *arid1b* −/− n = 18) (Fig. 3A). We observed a significant difference in social preference ($F_{(2, 81)} = 5.40$; p = 0.0063) among *arid1b* genotypes (Fig. 3B, Supplementary Table 3). Compared to wildtype (*arid1b* +/+), social preference was decreased in both the *arid1b* +/− (SMD = −0.66; p = 0.003) and *arid1b* −/− (SMD = −0.74; p = 0.006) genotypes (Fig. 3B–C, Supplementary Table 3). Baseline SPI values were not different across genotype (Supplementary Table 3). Notably, *arid1b* −/− and *arid1b* ± body sizes were not different compared to that of *arid1b* +/+ animals (Supplementary Fig. S3), indicating that differences in social preference were not likely a result of developmental delay.

4. Discussion

To test our hypothesis that variants in ASD risk genes have pleiotropic effects on sleep (Veatch et al., 2017; Doldur-Balli et al., 2022), we evaluated a high confidence ASD gene encoding a chromatin remodeling protein, *arid1b*. Notably, the SFARI Gene database categorizes *ARID1B*

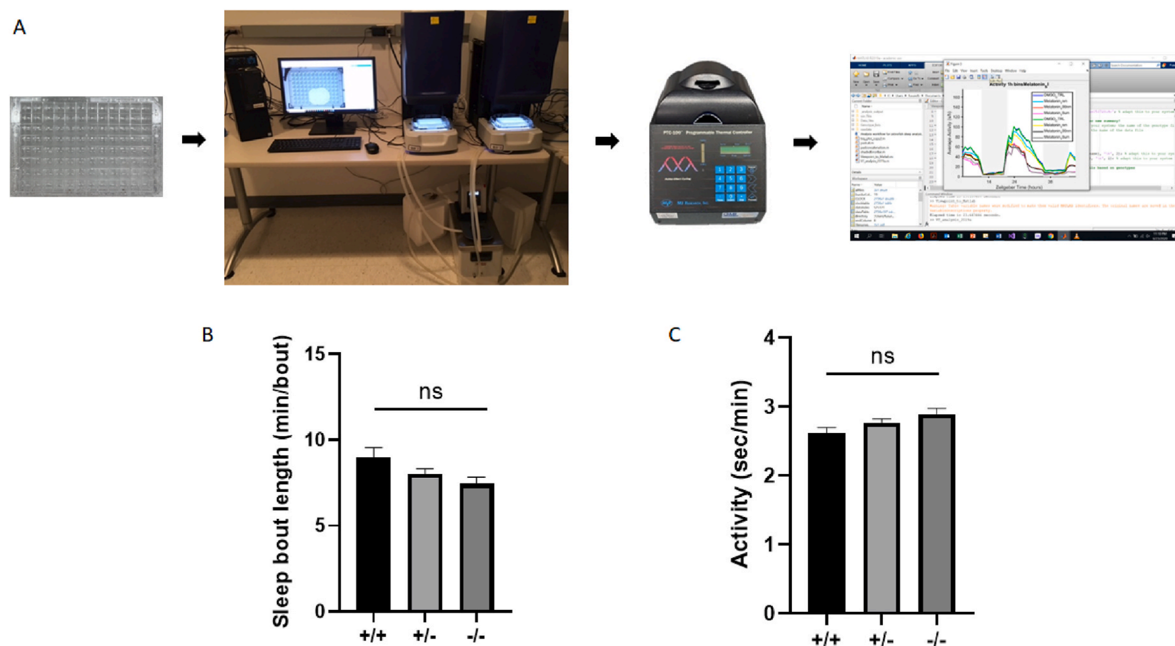

Fig. 1. ***arid1b*** **mutants showed a trend toward inability to maintain sleep bouts at night time and increased activity in daytime. A.** Schematic displaying the work flow in zebrafish sleep assays. Zebrafish larvae collected from an in-cross of heterozygous *arid1b* pairs were individually pipetted into each well of a 96 well plate. A video monitoring system and a temperature control unit are employed to run the sleep assays. DNA was extracted and genotyping was performed immediately after sleep assay was completed. Analysis was carried out to match the genotype data with the phenotype data. **B.** Comparison of sleep bout length in *arid1b* zebrafish line using data collected in dark period. There is a trend toward decreased sleep bout length in night time sleep in *arid1b* mutants. **C.** Comparison of activity in *arid1b* zebrafish line using data collected in light period. There is a trend toward hyperactivity in *arid1b* mutants during day. Data in graphs B and C are presented as mean + 95% CI for group averages. Total n = 432, *arid1b* +/+ n = 118, *arid1b* ± n = 219, *arid1b* −/− n = 95. ns. Nonsignificant after Hochberg correction.

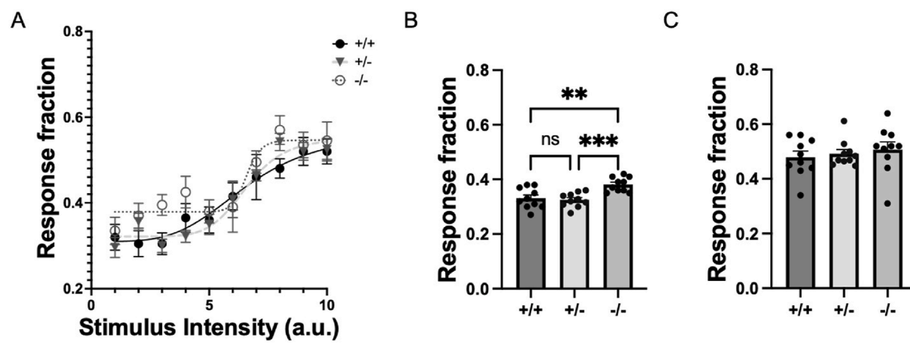

Fig. 2. ***arid1b*−/− larvae are hyper-responsive to low stimulus intensities during the night. A.** Stimulus-response curves for *arid1b*+/+, +/−, *and* −/− larvae from arousal threshold assay. **B.** *arid1b*−/− larvae show significantly increased ($F_{(2,27)} = 10.10$, $p = 0.0005$, by one-way ANOVA) responsiveness to lower intensity stimuli (<5 a.u.) compared to *arid1b*+/+ ($p = 0.0031$, by Tukey's multiple comparisons test) and +/− ($p = 0.0010$, by Tukey's multiple comparisons test). **C.** No significant differences were observed among groups at higher intensity stimuli (>5 a.u.). Asterisks indicate significance following p-value adjustment using Tukey's multiple comparisons test. $^{**}p < 0.01$, $^{***}p < 0.001$. Data are presented as mean ± 95% CI for group averages across each of 10 trials (n = 10 trials for 44 *arid1b*+/+, 94 *arid1b* +/−, and 45 *arid1b* −/− larvae).

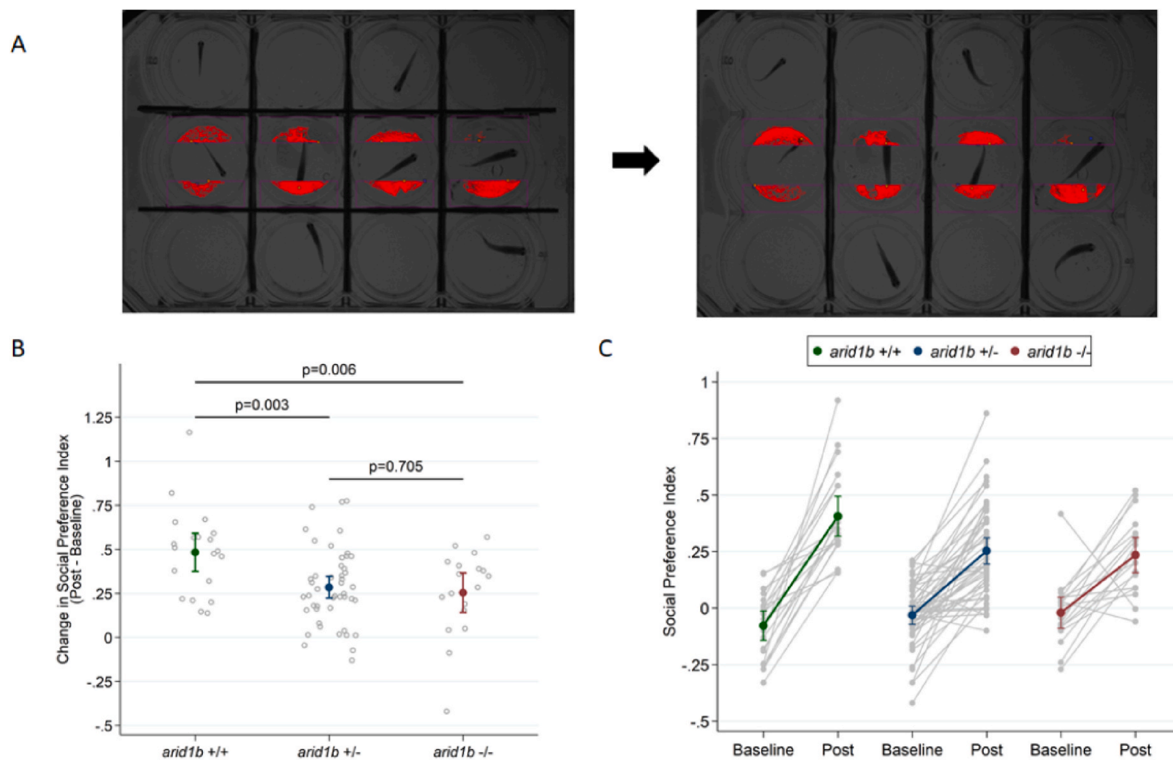

Fig. 3. ***arid1b* mutants display impaired social preference for the social cue. A.** Image showing the experimental set-up to collect baseline and post-baseline social preference data. Dividers are taken out following habituation-I (5 min) and baseline (10 min) periods to perform habituation-II (5 min) and post-baseline (10 min) recordings. Data is captured from the virtually defined two rectangular zones on the middle well. **B.** Comparison of change in SPI across 3 genotypes. A significant difference in the time spent in interaction with the social cue, a zebrafish which is at the same age and size with the experimental animal, among *arid1b* genotypes ($F_{(2, 81)} = 5.40$; $p = 0.0063$) was observed. $^{**}p < 0.01$. **C.** Graphs showing SPI difference between baseline and post-baseline recordings in wild type, heterozygous and homozygous *arid1b* zebrafish. Heterozygous and homozygous animals spent less time in interaction with the social cue compared to the wild type controls. Data in graph B is presented as mean + 95% CI for group averages. Dots indicate individuals and red data represent mean ± 95% CI. Total n = 85, *arid1b* +/+ n = 20, *arid1b* ± n = 47, *arid1b* −/− n = 18. n: number of animals. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

as one of only 232 such genes (https://gene.sfari.org/, release: 2022 Q4, updated Feb 1, 2023) (SFARI 2023). High confidence ASD risk genes are overrepresented in 3 broad categories based on known function: synaptic, chromatin remodeling and transcriptional (De Rubeis et al., 2014). Dysfunction in genes involved in chromatin remodeling may contribute to neurodevelopmental conditions as this process is important for neurogenesis and neural differentiation (De Rubeis et al., 2014). Chromatin remodeling machinery may influence sleep as it is evidenced to be involved in sleep circuit formation with previous studies observing that loss of other chromatin remodeling genes in files results in disrupted both social behavior and circadian rhythms (Gong et al., 2021). Our study assessed multiple behaviors in fish with *arid1b* gene mutations and observed that the most robust effects were related to increased arousability during sleep in homozygous mutants, and reduced social interaction.

The finding that loss of *arid1b* results in increased awakenings in response to low intensity stimuli indicates reduced sleep depth. Lighter sleep would then be expected to result in inability to maintain sleep bouts, consistent with our observation of a nominally significant decrease in bout length during nighttime sleep in *arid1b* homozygous mutant animals. This suggests sleep maintenance problems in mutants although this effect did not survive multiplicity correction. Notably, we

performed sleep/wake assays in a highly controlled environment without any sleep interrupters. In humans (or animals), sleeping in a less controlled environment, *ARID1B* mutation may increase vulnerability to external challenges (Devnani and Hegde 2015). Arousal threshold assays are not currently part of routine assessments in ASD models (Hoffman et al., 2016; Ruzzo et al., 2019); our data suggest that future studies of sleep in models of ASD should incorporate arousability measures. Assessment of ASD risk genes to detect whether these genes are expressed in a cyclical pattern might be an additional parameter in these studies. Furthermore, epileptic seizures might be present in individuals with ASD (van der Sluijs et al., 2019). It is possible to experience disturbed sleep due to epileptic seizures in these cases (Besag 2018). Response of the mutants to seizure-inducing drugs (such as pentylenetetrazol) should be screened to determine co-occurrence of sleep disturbance and epilepsy (Hoffman et al., 2016; Copping and Silverman 2021).

In humans with *ARID1B* haploinsufficiency, sleep disturbances may also reflect sleep apnea (van der Sluijs et al., 2019; Kruizinga et al., 2020). Notably, lower arousal thresholds in humans are evidenced to be a physiological risk factor that is predictive of obstructive sleep apnea [OSA] (Owens et al., 2015). Our findings of increased arousability in fish with loss of *arid1b* suggest a lower arousal threshold is not solely due to sleep-related breathing disruption. For example, increased arousability may also relate to sensory integration issues that are common in individuals with ASD and other neurodevelopmental conditions (Russo et al., 2010). This evidence could be useful to informing more effective treatment options for sleep apnea in individuals with sensory sensitivities who may require more personalized strategies to improve adherence to therapeutic devices (e.g., positive airway pressure) (Weiss et al., 2021, McLaren-Barnett and Narang, 2022). Given the physiological differences, zebrafish could be employed as a model organism for understanding the independent effects of genetic variation related to OSA risk on other sleep phenotypes.

We also found that social preference was strongly impaired in *arid1b* heterozygous and homozygous larvae. This result from studies of heterozygous mutants is in line with previous findings from mouse model studies in which social interaction and social novelty preference were tested in *Arid1b* haploinsufficient animals (Celen et al., 2017; Jung et al., 2017; Shibutani et al., 2017; Moffat et al., 2022). Notably, homozygous *Arid1b* mutations are embryonic lethal in mouse (Sim et al., 2014). Zebrafish models of both heterozygous and homozygous *arid1b* mutants are viable, which is another advantage of using zebrafish in modelling ASD. It is interesting that we detected a social impairment phenotype in heterozygous mutants but observed increased arousability only in homozygous mutants. This, as well as our observed group differences, indicates that effects of *arid1b* haploinsufficiency on social behavior phenotype are stronger than the effects on sleep behavior. Taken together, these data suggest that *arid1b* is independently involved in regulation of both sleep and social behavior (i.e., sleep problems do not merely reflect a mediator phenotype that increases risk for social impairment in ASD) (Veatch et al., 2017), providing a basis to support pleiotropic effects of this chromatin remodeling gene on both domains.

Previous work has demonstrated dysfunctional chromatin regulation contributes to alterations in gene expression in fast spiking cells (parvalbumin expressing inhibitory neurons) resulting in differences in formation of inhibitory brain circuitry during neurodevelopment in mouse brain leading to ASD-like pathophysiology (Shibutani et al., 2017). *CHD8* is another gene which encodes for a chromatin remodeling factor associated with ASD pathophysiology (Shibutani et al., 2017). Sleep disturbance including difficulties with falling and staying asleep and frequent awakenings has been reported in individuals carrying *CHD8* mutations (Mitchel et al., 1993; Dingemans et al., 2022). A *Drosophila* model of *CHD8/CHD7/Kismet* gene family also recapitulated sleep phenotype in humans (Coll-Tane et al., 2021). Interaction of sleep and social preference are the result of behavior of neural circuits (Chen and Hong 2018). Considering that sleep/wake switching functions through an interaction between wake and sleep prompting neuronal groups (Saper et al., 2010), it is plausible that pleiotropy of ASD risk gene variants might be a result of interaction among inhibitory circuits. Future work aimed at profiling sleep in ASD models in which genes from all 3 categories of ASD risk genes are matched may inform whether pleiotropic effects on sleep and social behavior occur in all categories of ASD risk genes. Sleep disturbance is considered as a transdiagnostic process in nervous system disorders (Harvey et al., 2011). ASD is a highly heterogeneous disorder both from a clinical and genetic perspective (Masi et al., 2017). Rather than therapies targeting individual genes and their products, efforts to discover therapeutic approaches that address a common process such as sleep may be effective. Therefore, examining the interplay between sleep and ASD is of great importance.

Potential limitations of this study include the use of two different ages (4–7 dpf and 3–4 wpf) to assess pleiotropic effects of an ASD risk gene. Zebrafish larvae at 4 days into development are generally used in sleep/wake assays (Lee et al., 2022) and the arousal threshold assay is also performed at this developmental stage (Singh et al., 2015). On the other hand, social behavior emerges at 2 weeks of age in zebrafish larvae and gradually improves, becoming a robust behavior by 3 weeks of age (Dreosti et al., 2015). Sleep in adult zebrafish (≥89 days old) has been defined as inactivity for at least 6 s and authors were able to examine activity, sleep duration, sleep bout length, wake bout length and number of sleep/wake transitions (Yokogawa et al., 2007); however, measuring sleep beyond early larval stages would result in lower throughput studies as there are technological limits to the number of animals that can be evaluated. In addition, to our knowledge, a protocol to assess sleep in juvenile zebrafish (i.e., developmental stages between larval and adult) has not yet been developed.

Although we did not detect a significant difference in body sizes of animals across 3 genotypes in our social behavior studies, growth delay has been reported in a knockdown model of *arid1b* in zebrafish (Liu et al., 2020). Short stature and neurodevelopmental delays are suggested to be associated with individuals carrying *ARID1B* mutations (Smith et al., 2016). We attempted to reduce potential effects related to delayed growth by using a social cue (a wild type larva) at the same age and size with the experimental larva in each session.

Despite these potential limitations, this work provides additional support for the use of zebrafish as a vertebrate model system to study the intersection between sleep and ASD. With the substantial advantages of high throughput phenotyping and ease of creating mutants, zebrafish are a powerful tool to help tease apart the complicated mechanisms underlying the expression of sleep disturbances in individuals with ASD.

CRediT authorship contribution statement

Fusun Doldur-Balli: Methodology, Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Amber J. Zimmerman:** Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Brendan T. Keenan:** Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Zoe Y. Shetty:** Methodology. **Struan F.A. Grant:** Writing – review & editing. **Christoph Seiler:** Resources, Writing – review & editing. **Olivia J. Veatch:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing, Supervision. **Allan I. Pack:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition, All authors read and approved the final manuscript.

Declaration of competing interest

Declarations of interest: None.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.nbscr.2023.100096.

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