Circadian Abnormalities in Mouse Models of Smith–Magenis Syndrome: Evidence for Involvement of *RAI1*

Melanie Lacaria[1], **Wenli Gu**[1], and **James R Lupski**[1,2,3,*]

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas

[2]Department of Pediatrics, Baylor College of Medicine, Houston, Texas

[3]Texas Children's Hospital, Houston, Texas

Abstract

Smith–Magenis syndrome (SMS; OMIM 182290) is a genomic disorder characterized by multiple congenital anomalies, intellectual disability, behavioral abnormalities, and disordered sleep resulting froman ~3.7 Mb deletion copy number variant (CNV) on chromosome 17p11.2 or from point mutations in the gene *RAI1*. The reciprocal duplication of this region results in another genomic disorder, Potocki–Lupski syndrome (PTLS; OMIM 610883), characterized by autism, intellectual disability, and congenital anomalies. We previously used chromosome-engineering and gene targeting to generate mouse models for PTLS (*Dp(11)17/+*), and SMS due to either deletion CNV or gene knock-out (*Df(11)17–2/+* and *Rai1*$^{+/-}$, respectively) and we observed phenotypes in these mouse models consistent with their associated human syndromes. To investigate the contribution of individual genes to the circadian phenotypes observed in SMS, we now report the analysis of free-running period lengths in *Rai1*$^{+/-}$ and *Df(11)17–2/+*mice, as well as in mice deficient for another known circadian gene mapping within the commonly deleted/duplicated region, *Dexras1*, and we compare these results to those previously observed in *Dp(11)17/+* mice. Reduced free-running period lengths were seen in *Df(11)17–2/+*, *Rai1*$^{+/-}$, and *Dexras1*$^{-/-}$, but not *Dexras1*$^{+/-}$ mice, suggesting that *Rai1* may be the primary gene underlying the circadian defects in SMS. However, we cannot rule out the possibility that *cis* effects between multiple haploinsufficient genes in the SMS critical interval (e.g., *RAI1* and *DEXRAS1*) either exacerbate the circadian phenotypes observed in SMS patients with deletions or increase their penetrance in certain environments. This study also confirms a previous report of abnormal circadian function in *Dexras1*$^{-/-}$ mice.

*Correspondence to: James R. Lupski M.D., Ph.D., D.Sc. (hon), Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030. jlupski@bcm.edu.

Keywords

Dexras1; sleep disorder; CNV; PTLS; genomic disorder

INTRODUCTION

Smith–Magenis syndrome (SMS; OMIM 182290) and Potocki– Lupski syndrome (PTLS; MIM 610883) are two distinct prototypical genomic disorders characterized by multiple congenital anomalies, intellectual disability, and behavioral abnormalities usually resulting from a 3.7 Mb deletion (SMS) or reciprocal duplication (PTLS) copy number variants (CNVs) on chromosome 17p11.2 [Greenberg et al., 1991; Potocki et al., 2000a, 2007]. Many of the pleiotropic features of SMS can also result from haploinsufficiency of a single gene located within the SMS critical region, *retinoic acid induced 1* (*RAI1*), as determined by the identification of non-deletion SMS patients presenting most of the same phenotypes, and harboring heterozygous loss of function point mutations in *RAI1* [Slager et al., 2003; Bi et al., 2004, 2006].

Disordered sleep is one of the most penetrant and clinically problematic features of SMS. These patients have difficulty falling asleep, difficulty staying asleep, frequent nighttime arousals, early waking, reduced rapid eye movement (REM) sleep, and decreased total sleep time beginning in early childhood [Greenberg et al., 1996; Smith et al., 1998]. The sleep disorder seen in SMS appears to be due to an underlying defect in circadian functioning, which has been extensively studied in patients; SMS patients have inverted melatonin cycling, with elevated melatonin secretion from the pineal gland peaking at~12:00 p.m. (compared to~3:30 a.m. in age-matched controls) [Potocki et al., 2000b; De Leersnyder et al., 2001a,b; Carpizo et al., 2006; Gropman et al., 2006; Boone et al., 2011]. In several cases, successful treatment of this inverted circadian rhythm of melatonin has been achieved by administering β_1-adrenergic antagonists during the day to block the abnormally phased melatonin release, followed with an evening dose of melatonin, resulting in improved nighttime sleep, decreased daytime drowsiness, and improvement in overall behavior [De Leersnyder et al., 2001b; Carpizo et al., 2006]. Interestingly, one out of the 19 SMS patients tested for urinary excretion of 6-sulphatoxymelatonin (aMT6s; the major metabolite of melatonin) by Potocki et al. [2000b], did not demonstrate the abnormal diurnal melatonin profile, despite displaying sleep disturbance; this patient had a large, uncommon, recurrent deletion of ~5 Mb. Another subsequent case report identified an additional patient with a large uncommon, nonrecurrent deletion of ~5.5 Mb, sleep disturbance, and normal melatonin circadian rhythm, suggesting that the altered melatonin profile alone may not explain the sleep disturbance seen in SMS [Boudreau et al., 2009].

Alterations of the circadian system in SMS are likely due in large part to abnormal functioning of *RAI1*, as SMS patients with point mutations in this gene and suspected normal expression of the surrounding genes in the SMS critical interval have also been identified with both sleep disturbance and altered melatonin rhythms [Girirajan et al., 2005; Boone et al., 2011]. However, there are other candidate genes within the SMS critical interval that have known association with circadian function, including *DEXRAS1* and

COPS3. The exact function of the *RAI1* gene is not known, but it is known to be highly expressed in the brain, and it is thought to encode a transcriptional regulator [Bi et al., 2004, 2007]. *COPS3* encodes subunit 3 of the COP9 signalosome, which is conserved from plants to humans and is closely related to the 26S proteasome regulatory complex that has previously been associated with control of the rate-limiting step in melatonin metabolism by *N*-acetyltransferase [Potocki et al., 1998; Wei et al., 1998; Chamovitz and Glickman, 2002]. Previous investigation into *COPS3* functioning in SMS patient lymphoblastoid cell lines did not find any defect in COP9 signalosome assembly, expression, or kinase function; however, that study could not rule out the potential effects of *COPS3* hemizygosity during development [Elsea et al., 1999].

DEXRAS1 regulates responsiveness of the circadian clock to both photic and nonphotic stimuli, and is rhythmically expressed in the suprachiasmatic nucleus (SCN) of the hypothalamus [Takahashi et al., 2003; Cheng et al., 2004, 2006]. Previous studies have demonstrated that *Dexras1*$^{-/-}$ mice have increased responsiveness to nonphotic stimuli (access to running wheel) compared to wild-type controls [Cheng et al., 2004]. However, a subsequent study challenging this hypothesis, by directly opposing photic and non-photic zeitgebers 180° out of phase, did not find a difference in nonphotic phase shifting in *Dexras1*$^{-/-}$ mice compared to wild-type control animals [Dallmann and Mrosovsky, 2007]. Furthermore, although Cheng et al. [2004], reported a shortening of tau (τ, or period length) in *Dexras1*$^{-/-}$ mice housed in constant darkness (D/D), a follow-up study did not observe a similar effect when the mice had *ad libitum* access to a running wheel [Dallmann and Mrosovsky, 2007]. Overall, both studies, although contradictory in select findings, suggest a complex circadian phenotype in *Dexras1*$^{-/-}$ mice that warrants further study. Furthermore, the specific circadian phenotype of *Dexras1*$^{+/-}$ mice, which may more closely model the haploinsufficient dosage seen in SMS, has not been examined to date. Whether, or to what extent, *DEXRAS1* and the other candidate genes interact to produce the circadian phenotypes seen in SMS is also not yet known. The cumulative effect of reduced dosage of multiple haploinsufficient genes within the SMS region, a phenomenon referred to as *cis* genetics, could also potentially contribute to the penetrance or variable expression of the full phenotype [Lupski et al., 2011].

The circadian phenotype of PTLS patients has not been extensively studied, and parents did not specifically report sleep disturbances in their children with PTLS in a study of the natural history of the disease [Potocki et al., 2007]. However, a 24-hr sleep study in a cohort of PTLS patients documented multiple nocturnal awakenings and sleep-disordered breathing characterized by obstructive sleep apnea, resulting in significant oxygen desaturation and hypercarbia in the absence of substantial airway obstruction (as determined by otolaryngologic examination) [Potocki et al., 2007]. Furthermore, abnormal EEG findings during sleep were identified in subjects with the common duplication [Potocki et al., 2007]. If the genes responsible for the sleep disorder observed in SMS are highly dosage-sensitive, then it is plausible that over-expression, as in the case of PTLS, might also convey a circadian phenotype. In this study we utilize previously generated chromosome-engineered mouse models of SMS (*Df(11)17–2/+*), as well as knock-out mice for the circadian candidate gene *DEXRAS1* (*Dexras1*$^{+/-}$ and *Dexras1*$^{-/-}$) and the main dosage-sensitive gene

in SMS/PTLS, *RAI1* (*Rai1*$^{+/-}$). We then compared these results to previously published data for a mouse model of PTLS (*Dp(11)17/+*) to investigate the circadian phenotypes in SMS and PTLS.

MATERIALS AND METHODS

We used the Mini Mitter circadian rhythm wheel-running system (Respironics, Murraysville, PA) along with VitalView and ActiView software to analyze free-running period lengths and wheel-running activity levels in adult (2–4 months) female *Dp(11)17/+*, *Rai1*$^{+/-}$, *Df(11)17–2/+*, *Dexras1*$^{+/-}$, and *Dexras1*$^{-/-}$ mice on a congenic (>10 generations) C57BL/6J^{Tyr}Brd background. *Dexras1*$^{-/-}$ mice were made available by H.Y. Cheng (Columbus, OH) and backcrossed to our C57BL/6J^{Tyr}Brd background to eliminate background effects between strains. Mice were housed individually in cages with running wheels under standard light/dark (L/D; 12 hr/ 12 hr) cycle for two weeks in order to acclimatize the mice to the circadian cages and to evaluate their circadian rhythms with external photic (light) cues. Food and water were given *ad libitum*. We then changed the light cycle to a dark/dark (D/D; 12 hr/12 hr) cycle for an additional 2 weeks to analyze the free-running period length. Tau (τ; period length) values were calculated from actigram graphs generated from activity data (pattern, timing, and number of wheel turns) throughout the day. Wild-type littermates for each mutant strain were studied to evaluate any potential cage-specific differences between wild-type animals. ActiView software was utilized to determine total activity levels during light/dark and dark/dark cycles, and VitalView software was utilized to calculate average daily wheel running activity. Data were analyzed using ANOVA and significant values were followed-up with Bonferroni- Holm post hoc analyses.

We used only one gender to control for any potential innate gender differences in the circadian phenotypes observed in this study that may be present in rodents. Inhuman patients with SMS, a meta-analysis of 105 cases (including 44 males and 61 females) did not reveal any gender difference in the presence of sleep disturbance or any abnormal circadian phenotype examined [Edelman et al., 2007]. For this reason, the use of female mice versus male mice is not likely to affect the results. For practical reasons, the maintenance of deletion mouse strains was carried out using male mice due to frequent loss of pregnancies/ pups when deletion females were used in breeding cages. In order to obtain a sufficient number of mice for this study, we therefore utilized female mice.

RESULTS

The circadian rhythms of adult female mice entrained to a 12/12 hr L/D cycle were evaluated as an indicator of potential sleep abnormalities. Female mice were used for this study in order to control for gender influences on sleep and to reduce the total number of animals used. Once the mice were acclimated to the circadian cages and a standard 12/12 L/D light cycle (after a 2-week period), they were challenged with 12/12 D/D cycle to determine their free-running period length, or tau (τ). Running wheel actigrams for each animal were generated and utilized to calculate τ values for each animal; two representative actigrams for each genotype are shown in Figure 1. Next, τ values were calculated,

averaged, and compared to those of the respective wild-type littermates for each genotype, as well as to other mutant strains (Table I; Fig. 2).

We found that the free-running period length was significantly shortened in *Df(11)17–2/+*, *Rai1+/−*, and *Dexras1−/−* mice compared to wild-type as determined by ANOVA($F = 12.67$, $P = 1.59 \times 10^{-11}$) followed by post hoc analyses (Fig. 2). There was no significant difference between *Dexras1+/−* mice and their wild-type littermates. We next compared these deletion (*Df(11)17–2/+*) or gene haploinsufficiency (*Rai1+/−*, *Dexras1+/−*, and *Dexras1−/−*) animal models with a previous study of a duplication animal model; the latter work was published as part of a study examining behavior in an animal model for PTLS since such patients manifest many behavioral characteristics of autism spectrum disorder [Lacaria et al., 2012a].

A previous report identified circadian abnormalities in the mouse model harboring the deletion (*Df(11)17*) CNV reciprocal to *Dp(11)17*, but did not identify any significant differences in *Dp (11)17/+*mice compared to wild-type mice [Walz et al., 2004]. We performed a subsequent study and found that female *Dp(11)17/+* mice did have a shortened period length compared to their wild-type littermates [Lacaria et al., 2012b]. The initial study examined male mice on a mixed (C57BL/6J/129S5/SvEv) background, which may explain the lack of observable difference in the *Dp(11)17/+* animals. Alternatively, the gender difference may account for the differing results, as gender differences have previously been observed in circadian phenotypes in mice [Iwahana et al., 2008]. Similar to this previous study, we also found a shortened period length in deletion mice, although we analyzed mice with a shorter deletion (595 kb) compared to the ~2 Mb deletion strain studied by Walz et al. [2004]. It is important to note that both of these deletion strains have only one copy of *CopS3*, *Dexras1*, and *Rai1* [Yan et al., 2007].

Of further interest, we also found that female *Dexras1−/−* mice on a congenic (N > 12) C57BL/6J[Tyr]Brd background have significantly shortened period length compared to wild-type mice, and our results for period length are closer in raw value as well as in significance to those in the original report by Cheng et al. (Table I). In contrast to our current study, both of the previous studies of these mice were performed on male mice, which may account for some phenotypic differences described between our report and these previous studies [Cheng et al., 2004; Dallmann and Mrosovsky, 2007]. Importantly, the number of mice utilized in this study (n = 24) is much greater than those used in either of the previous studies (n = 12 for both), [Cheng et al., 2004; Dallmann and Mrosovsky, 2007] and our results are consistent with the report of Cheng et al. [2004]. As noted above, our analysis of *Dexras1+/−* mice, which may more closely model the genetic haploinsufficiency in SMS, did not identify any abnormal shift in free-running period length (Fig. 1B). However, *Rai1+/−* mice, which have not previously been analyzed for circadian rhythm abnormalities, did demonstrate a significant shortening in period length compared to wild-type mice (Fig. 2).

We also analyzed total activity levels for each of the genotypes, that is, *Df(11)17–2/+*, *Rai1+/−*, *Dexras1+/−*, and *Dexras1−/−* mice, and found no significant difference in wheel running activity between L/D and D/D phases for any of the genotypes analyzed (*P* > 0.05 for all). However, significant changes were observed in average L/D and D/D values

between genotypes (F = 7.31, $P = 2.25 \times 10^{-6}$, and F = 8.52, $P = 2.74 \times 10^{-7}$, for L/D and D/D, respectively). Similar to the results of Cheng et al. [2004] and Dallmann and Mrosovsky [2007], we observed no difference in wheel running activity between $Dexras1^{-/-}$ mice and their wild-type littermates (Table I, Fig. 3). As expected, there was also no difference in wheel running activity for $Dexras1^{+/-}$ mice. We performed regressional analysis to determine whether period length is negatively correlated with activity level in $Dexras1^{-/-}$ mice, as observed by Cheng et al. [2004]. However, we did not observe any significant correlation or difference between $Dexras1^{-/-}$ and wild-type mice (Supplemental eFig. 1—see Supporting Information Online). The reason that our observation differed from those reported is not known, but it may potentially be explained by strain background differences (Cheng et al. studied mice on an N5 C57BL/6J background, and our study used a congenic N > 12 albino C57BL/6J^{Tyr}Brd background), or by subtle environmental differences that may have influenced either photic or nonphotic circadian inputs.

Both $Df(11)17–2/+$ mice and $Rai1^{+/-}$ mice had decreased wheel running activity compared to their respective wild-type littermates, which may be linked to their underlying metabolic phenotypes [Burns et al., 2010; Lacaria et al., 2012a]. $Dp(11)17/+$ mice also had decreased overall activity, which was not observed in a previous analysis of activity levels in $Dp(11)17/+$ mice [Lacaria et al., 2012a]. The results from this current study were obtained over a much longer sampling period (1 month vs. 30 min), which may explain the improved detection of this phenotype.

DISCUSSION

Although the changes in τ identified in this study were small in absolute time shift (ranging from 0.103 to 0.235 hr), they were highly significant. The observed highly significant change in period length can be relevant; when analyzing a phenotype that is tightly regulated, even a small change from the mean may be an indication of an underlying pathology, and the downstream impact of these changes can be detrimental to the health of the animal.

The results of this study reveal that mice that are heterozygous for a mutation in $Rai1$ display decreased free-running period length and decreased wheel-running activity, supporting a role for this gene in the etiology of the sleep disorder observed in SMS. In addition, although the number of human subjects studied to date is small (n = 2 and n = 7), studies in SMS patients with $RAI1$ point mutations revealed apparent circadian abnormalities [Girirajan et al., 2005; Boone et al., 2011]. Little is known about the precise function(s) of RAI1, although retinoic acid signaling has been shown to regulate cortical synchrony during sleep via the retinoic acid receptor beta, which determines the contribution of delta oscillations (a hallmark of slow wave sleep depth and consolidation) to sleep EEG [Maret et al., 2005]. It is therefore plausible that $RAI1$, which has also been shown to be regulated by retinoic acid signaling *in vitro*, may function in a similar or related pathway to influence circadian/sleep phenotypes. Furthermore, given that RAI1 may function as a transcriptional regulator the phenotypic consequences of circadian abnormality may be mediated by a downstream gene.

Indeed, a recent study investigating the molecular function of *RAI1* determined that RAI1 regulates the master circadian rhythm gene, *CLOCK*, as well as other key circadian genes, including *PER2*, *CRY1*, and *BMAL*, among others [Williams et al., 2012]. Our results also suggest that *RAI1* is the predominant gene regulating the circadian phenotypes in SMS, as mice harboring a chromosomal deletion of the SMS region containing *Rai1* as well the other main candidate for circadian function, *Dexras1*, did not have a more severe phenotype than heterozygous *Rai1* knock-out mice. If negative *cis* effects between these two genes were present, we might anticipate that haploinsufficiency of both genes, as in the deletion strain, could amplify the circadian defect. Alternatively, *cis* interactions between these genes may cause a modification or neutralization of the phenotype, resulting in a milder phenotype, similar to what was observed in the *Df(11)17–2/+* mice. Therefore, we also cannot rule out the possibility that *cis* interactions between *DEXRAS1*, *RAI1*, and other gene(s) in the SMS/PTLS critical region are required for the full circadian defect observed in SMS, and future studies could investigate potential epistatic interactions between gene(s) in this region.

Previous studies have examined the free-running period of *Dexras1$^{-/-}$* mice and these studies report different observations. Cheng et al. reported a period length of 23.28 ± 0.11 hr ($n = 6$), which was significantly shorter ($P = 0.008$) than wild-types with a period length of 23.71 ± 0.08 hr ($n = 6$) [Cheng et al., 2004], whereas Dallmann and Mrosovsky [2007] did not see any significant differences between these genotypes (23.3 ± 0.1 for *Dexras1$^{-/-}$* compared to 23.4 ± 0.1 in wild-types; n = 12 for each group). Our results confirm previous hypotheses that Dexras1 influences circadian period length in mice. However, this gene is not required for the maintenance of circadian rhythms, as all of the *Dexras1$^{-/-}$* mice were able to maintain a basic rhythmicity in both the presence and absence of light. This study implicates Dexras1 in the regulation of core clock mechanisms, which may be occurring through enhancement of nonphotic input effects (i.e., wheel running activity). It is not known if haploinsufficiency of Dexras1 is causative of any circadian phenotypes in humans. These studies do not rule out this possibility, as homozygous knock-out mouse models can sometimes more closely reflect the phenotypes seen in humans with heterozygous mutations due to overlapping gene function or genetic redundancy in rodents that is not present in humans [Barbaric et al., 2007]. Alternatively, differences in the perturbation of gene function required to maintain proper physiological function may differ between these two species. As such, we cannot be certain of the role of Dexras1 in patients with SMS following this study. Future experiments should take a more sophisticated approach to dissect the specific circadian defects, as *Dexras1$^{+/-}$* mice may have abnormal phenotypes when specific photic and nonphotic inputs are altered or challenged, when photic response curves are examined, or when MAPK signaling or melatonin metabolism are evaluated, even though they do not have an overall reduced free-running period length under the conditions examined herein.

As expected, we did not observe any significant differences in wheel running activity between L/D and D/D conditions for *Df(11) 17–2/+*, *Dp(11)17/+*, *Rai1$^{+/-}$*, *Dexras1$^{+/-}$*, or *Dexras1$^{-/-}$* mice, indicating that activity is not solely subjected to photic regulation, and that similar periods of total wakefulness and activity are maintained in the absence of photic signals, regardless of period length. Nonphotic regulation likely plays a key role in the

regulation of the circadian rhythms in these mice. Although we did identify reduced total activity for *Rai1*$^{+/-}$ and *Df(11)17–2/+*mice, this may be linked to an underlying metabolic phenotype rather than a circadian phenotype, as both strains are significantly overweight, and also manifest metabolic syndrome-like phenotypes [Bi et al., 2005; Walz et al., 2006; Burns et al., 2010; Lacaria et al., 2012a]. Alternatively, this reduction in total activity could be an indication of an increased amount of time spent eating food or foraging around the cage. These possibilities cannot be distinguished with this experimental design. These findings warrant further investigation into the underlying mechanism, whether metabolic or circadian in origin.

Overall, the results of this study further implicate one or more genes in this genomic interval in the control of circadian clock regulation. Specifically, we found that *Rai1* is likely the main gene responsible for the shortened period length in *Df(11)17–2/+* mice, as the deletion strain did not display a more severe circadian phenotype than mice harboring only one copy of *Rai1* but two copies of the genes in the surrounding genomic interval.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

References

Barbaric I, Miller G, Dear TN. Appearances can be deceiving: Phenotypes of knockout mice. Brief Funct Genomic Proteomic. 2007; 6:91–103. [PubMed: 17584761]

Bi W, Saifi GM, Shaw CJ, Walz K, Fonseca P, Wilson M, Potocki L, Lupski JR. Mutations of RAI1, a PHD-containing protein, in nondeletion patients with Smith-Magenis syndrome. Hum Genet. 2004; 115:515–524. [PubMed: 15565467]

Bi W, Ohyama T, Nakamura H, Yan J, Visvanathan J, Justice MJ, Lupski JR. Inactivation of Rai1 in mice recapitulates phenotypes observed in chromosome engineered mouse models for Smith-Magenis syndrome. Hum Mol Genet. 2005; 14:983–995. [PubMed: 15746153]

Bi W, Saifi GM, Girirajan S, Shi X, Szomju B, Firth H, Magenis RE, Potocki L, Elsea SH, Lupski JR. RAI1 point mutations, CAG repeat variation, and SNP analysis in non-deletion Smith-Magenis syndrome. Am J Med Genet Part A. 2006; 140A:2454–2463. [PubMed: 17041942]

Bi W, Yan J, Shi X, Yuva-Paylor LA, Antalffy BA, Goldman A, Yoo JW, Noebels JL, Armstrong DL, Paylor R, Lupski JR. Rai1 deficiency in mice causes learning impairment and motor dysfunction, whereas Rai1 heterozygous mice display minimal behavioral phenotypes. Hum Mol Genet. 2007; 16:1802–1813. [PubMed: 17517686]

Boone PM, Reiter RJ, Glaze DG, Tan DX, Lupski JR, Potocki L. Abnormal circadian rhythm of melatonin in Smith-Magenis syndrome patients with RAI1 point mutations. AmJ Med Genet Part A. 2011; 155A:2024– 2027. [PubMed: 21739587]

Boudreau EA, Johnson KP, Jackman AR, Blancato J, Huizing M, Bendavid C, Jones M, Chandrasekharappa SC, Lewy AJ, Smith AC, Magenis RE. Review of disrupted sleep patterns in Smith-Magenis syndrome and normal melatonin secretion in a patient with an atypical interstitial 17p11.2 deletion. Am J Med Genet Part A. 2009; 149A:1382–1391. [PubMed: 19530184]

Burns B, Schmidt K, Williams SR, Kim S, Girirajan S, Elsea SH. Rai1 haploinsufficiency causes reduced Bdnf expression resulting in hyperphagia, obesity and altered fat distribution in mice and humans with no evidence of metabolic syndrome. Hum Mol Genet. 2010; 19:4026–4042. [PubMed: 20663924]

Carpizo R, Martinez A, Mediavilla D, Gonzalez M, Abad A, Sanchez-Barcelo EJ. Smith-Magenis syndrome: A case report of improved sleep after treatment with beta1-adrenergic antagonists and melatonin. J Pediatr. 2006; 149:409–411. [PubMed: 16939758]

Chamovitz DA, Glickman M. The COP9 signalosome. Curr Biol. 2002; 12:R232. [PubMed: 11937035]

Cheng HY, Obrietan K, Cain SW, Lee BY, Agostino PV, Joza NA, Harrington ME, Ralph MR, Penninger JM. Dexras1 potentiates photic and suppresses nonphotic responses of the circadian clock. Neuron. 2004; 43:715–728. [PubMed: 15339652]

Cheng HY, Dziema H, Papp J, Mathur DP, Koletar M, Ralph MR, Penninger JM, Obrietan K. The molecular gatekeeper Dexras1 sculpts the photic responsiveness of the mammalian circadian clock. J Neurosci. 2006; 26:12984–12995. [PubMed: 17167088]

Dallmann R, Mrosovsky N. Non-photic phase resetting of Dexras1 deficient mice: A more complicated story. Behav Brain Res. 2007; 180:197–202. [PubMed: 17459494]

De Leersnyder H, De Blois MC, Claustrat B, Romana S, Albrecht U, Von Kleist-Retzow JC, Delobel B, Viot G, Lyonnet S, Vekemans M, Munnich A. Inversion of the circadian rhythm of melatonin in the Smith- Magenis syndrome. J Pediatr. 2001a; 139:111–116. [PubMed: 11445803]

De Leersnyder H, de Blois MC, Vekemans M, Sidi D, Villain E, Kindermans C, Munnich A. beta(1)-adrenergic antagonists improve sleep and behavioural disturbances in a circadian disorder, Smith-Magenis syndrome. J Med Genet. 2001b; 38:586–590. [PubMed: 11546826]

Edelman EA, Girirajan S, Finucane B, Patel PI, Lupski JR, Smith AC, Elsea SH. Gender, genotype, and phenotype differences in Smith- Magenis syndrome: a meta-analysis of 105 cases. Clin Genet. 2007; 71:540–550. [PubMed: 17539903]

Elsea SH, Mykytyn K, Ferrell K, Coulter KL, Das P, Dubiel W, Patel PI, Metherall JE. Hemizygosity for the COP9 signalosome subunit gene, SGN3, in the Smith-Magenis syndrome. Am J Med Genet. 1999; 87:342–348. [PubMed: 10588842]

Girirajan S, Elsas LJ II, Devriendt K, Elsea SH. RAI1 variations in Smith-Magenis syndrome patients without 17p11.2 deletions. J Med Genet. 2005; 42:820–828. [PubMed: 15788730]

Greenberg F, Guzzetta V, Montes de Oca-Luna R, Magenis RE, Smith AC, Richter SF, Kondo I, Dobyns WB, Patel PI, Lupski JR. Molecular analysis of the Smith-Magenis syndrome: A possible contiguous-gene syndrome associatedwithdel(17)(p11.2). AmJHumGenet. 1991; 49:1207–1218.

Greenberg F, Lewis RA, Potocki L, Glaze D, Parke J, Killian J, Murphy MA, Williamson D, Brown F, Dutton R, McCluggage C, Friedman E, Sulek M, Lupski JR. Multi-disciplinary clinical study of Smith-Magenis syndrome (deletion 17p11.2). Am J Med Genet. 1996; 62:247–254. [PubMed: 8882782]

Gropman AL, Duncan WC, Smith AC. Neurologic and developmental features of the Smith-Magenis syndrome (del 17p11.2). Pediatr Neurol. 2006; 34:337–350. [PubMed: 16647992]

Iwahana E, Karatsoreos I, Shibata S, Silver R. Gonadectomy reveals sex differences in circadian rhythms and suprachiasmatic nucleus androgen receptors in mice. Horm Behav. 2008; 53:422–430. [PubMed: 18164002]

Lacaria M, Saha PK, Potocki L, Bi W, Yan J, Girirajan S, Burns B, Elsea SH, Walz K, Chan L, Lupski JR, Gu W. A duplication CNV that conveys traits reciprocal to metabolic syndrome and protects against diet induced obesity in mice and men. PLoS Genet. 2012a; 8:e1002713.10.1371/journal.pgen.1002713 [PubMed: 22654670]

Lacaria M, Spencer C, Gu W, Paylor R, Lupski JR. Enriched rearing improves behavioral responses of an animalmodel forCNV-based autisticlike traits. Hum Mol Genet. 2012b; 21:3083–3096.10.1093/hmg/dds124 [PubMed: 22492990]

Lupski JR, Belmont JW, Boerwinkle E, Gibbs RA. Clan genomics and the complex architecture of human disease. Cell. 2011; 147:32–43. [PubMed: 21962505]

Maret S, Franken P, Dauvilliers Y, Ghyselinck NB, Chambon P, Tafti M. Retinoic acid signaling affects cortical synchrony during sleep. Science. 2005; 310:111–113. [PubMed: 16210540]

Potocki L, Chen KS, Lupski JR. Subunit 3 of the COP9 signal transduction complex is conserved from plants to humans and maps within the Smith–Magenis syndrome critical region in 17p11.2. Genomics. 1998; 57:180–182. [PubMed: 10191102]

Potocki L, Chen KS, Park SS, Osterholm DE, Withers MA, Kimonis V, Summers AM, Meschino WS, Anyane-Yeboa K, Kashork CD, Shaffer LG, Lupski JR. Molecular mechanism for duplication 17p11.2- the homologous recombination reciprocal of the Smith-Magenis microdeletion. Nat Genet. 2000a; 24:84–87. [PubMed: 10615134]

Potocki L, Glaze D, Tan DX, Park SS, Kashork CD, Shaffer LG, Reiter RJ, Lupski JR. Circadian rhythm abnormalities of melatonin in Smith-Magenis syndrome. J Med Genet. 2000b; 37:428–433. [PubMed: 10851253]

Potocki L, Bi W, Treadwell-Deering D, Carvalho CM, Eifert A, Friedman EM, Glaze D, Krull K, Lee JA, Lewis RA, Mendoza-Londono R, Robbins-Furman P, Shaw C, Shi X, Weissenberger G, Withers M, Yatsenko SA, Zackai EH, Stankiewicz P, Lupski JR. Characterization of Potocki-Lupski syndrome (dup(17)(p11.2p11.2)) and delineation of a dosage-sensitive critical interval that can convey an autism phenotype. AmJHum Genet. 2007; 80:633–649.

Slager RE, Newton TL, Vlangos CN, Finucane B, Elsea SH. Mutations inRAI1 associated withSmith-Magenis syndrome. NatGenet. 2003; 33:466–468.

Smith AC, Dykens E, Greenberg F. Sleep disturbance in Smith- Magenis syndrome (del 17p11.2). Am J Med Genet. 1998; 81:186–191. [PubMed: 9613860]

Takahashi H, Umeda N, Tsutsumi Y, Fukumura R, Ohkaze H, Sujino M, van der Horst G, Yasui A, Inouye ST, Fujimori A, Ohhata T, Araki R, Abe M. Mouse dexamethasone-induced RASprotein 1 gene is expressed in a circadian rhythmic manner in the suprachiasmatic nucleus. Brain Res Mol Brain Res. 2003; 110:1–6. [PubMed: 12573527]

Walz K, Spencer C, Kaasik K, Lee CC, Lupski JR, Paylor R. Behavioral characterization of mouse models for Smith-Magenis syndrome and dup (17)(p11.2p11.2). Hum Mol Genet. 2004; 13:367–378. [PubMed: 14709593]

Walz K, Paylor R, Yan J, Bi W, Lupski JR. Rai1 duplication causes physical and behavioral phenotypes in a mouse model of dup(17) (p11.2p11.2). J Clin Invest. 2006; 116:3035–3041. [PubMed: 17024248]

Wei N, Tsuge T, Serino G, Dohmae N, Takio K, Matsui M, Deng XW. The COP9 complex is conserved between plants and mammals and is related to the 26S proteasome regulatory complex. Curr Biol. 1998; 8:919–922. [PubMed: 9707402]

Williams SR, Zies D, Mullegama SV, Grotewiel MS, Elsea SH. Smith- Magenis syndrome results in disruption of CLOCK gene transcription and reveals an integral role for RAI1 in the maintenance of circadian rhythmicity. Am J Hum Genet. 2012; 90:941–949. [PubMed: 22578325]

Yan J, Bi W, Lupski JR. Penetrance of craniofacial anomalies in mouse models of Smith-Magenis syndrome is modified by genomic sequence surrounding Rai1: Not all null alleles are alike. AmJHumGenet. 2007; 80:518–525.
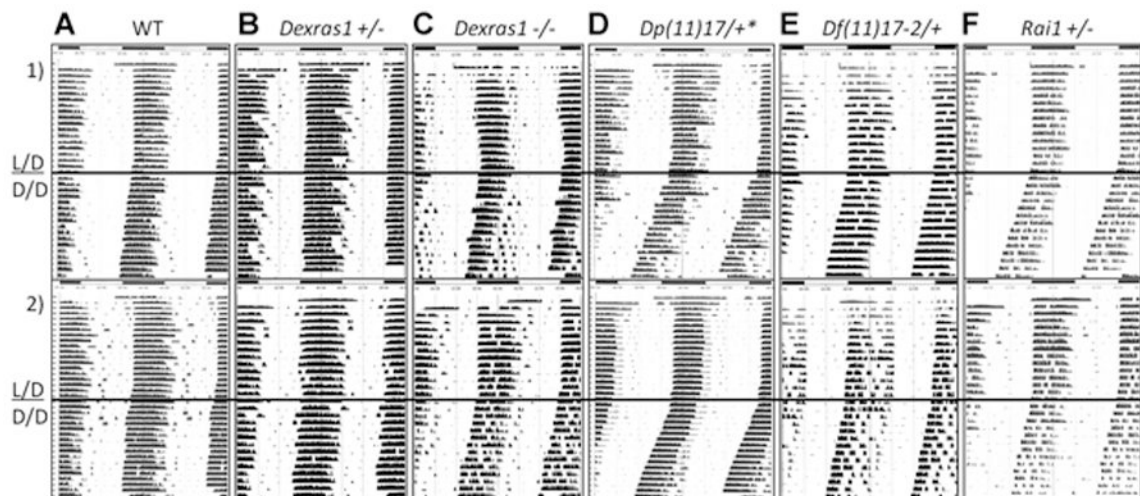
FIG. 1.

Representative actigrams from the different mouse strains examined in this study. Actigrams are double-plotted, with the same activity information both below and to the right. Dark bars above indicate periods of darkness and light bars indicate when the lights were on during standard light/dark (L/D) conditioning. Periods of constant darkness are indicated below the dark horizontal bar in the center of the actigrams (D/D). Tau values were calculated as the slope of the period during this condition. Rows (1) and (2) represent examples of two different mice from the same genotype. Genotypes are indicated above and labeled as follows: column (A) wild-type, (B) *Dexras1*$^{+/-}$, n = 22, (C) *Dexras1*$^{-/-}$, n = 24, (D) *Dp(11)17/+*, n = 22, (E) *Df(11)17–2/+*, n = 15, (F) *Rai1*$^{+/-}$, n = 9. *Previously described data [Lacaria et al., 2012b].
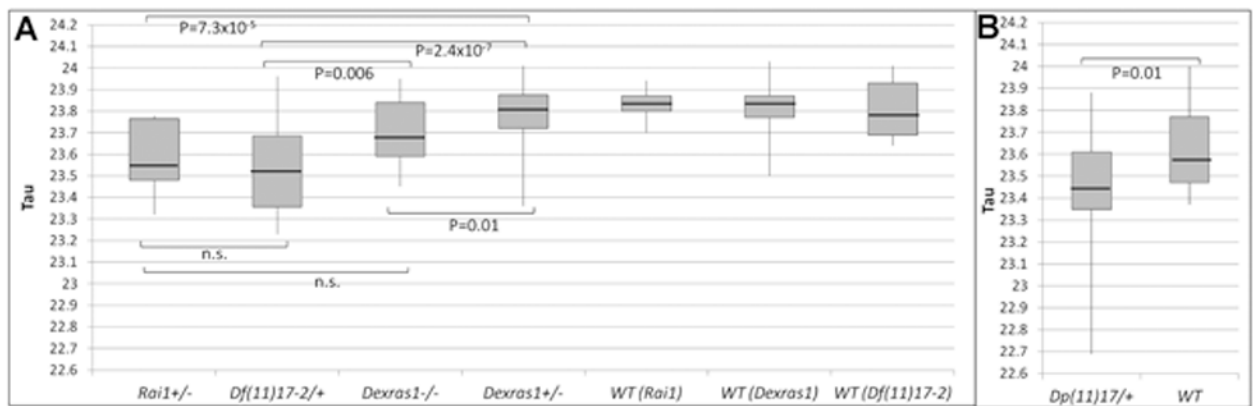
FIG. 2.
Graphical representation of tau values and comparison between mutants. Boxplots displaying the median, variability, and range of tau values are given for the following mutants: (A) $Rai1^{+/-}$, n = 9, $Df(11)17–2/+$, n = 15, $Dexras1^{-/-}$, n = 24, and $Dexras1^{+/-}$, n = 22, (B) $Dp(11)17/+$, n = 22, compared to their respective wild-type littermates (n = 14 for $Rai1^{+/-}$, n = 13 for $Df(11)17/+$, n = 18 for $Dexras1+/^-$, and n = 29 for $Dp(11)17/+$ littermates). n.s. = not significant.
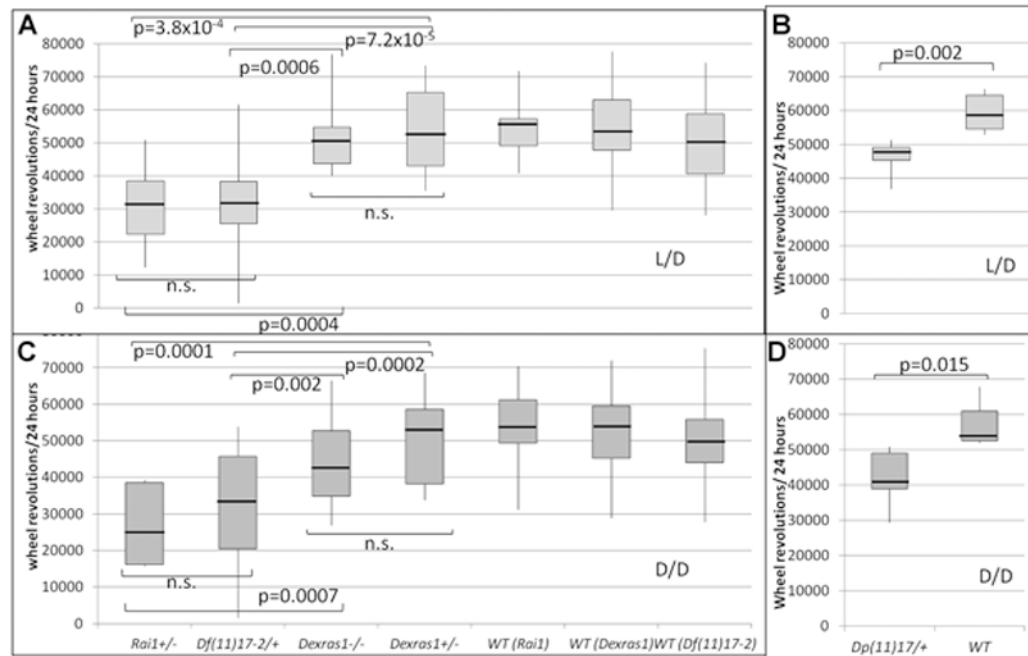
FIG. 3.
Daily wheel-running activity for mutants and their wild-type littermates. Boxplots displaying the median, variability, and range of activity levels for light/dark (L/D) (A,B) and dark/dark (D/D) (C,D) light cycles. Genotypes are written across the *x*-axis, with wild-type littermates for each mutant genotype displayed individually. *P*-values for comparisons between individual mutant genotypes are indicated. *Df (11)17–2/+* (n = 15), *Rai1*$^{+/-}$(n = 9), *Dexras1*$^{+/-}$ (n = 22), *Dexras1*$^{-/-}$ (n = 24), *Dp(11)17/+* (n = 22), wild-type littermates (n = 18 for *Dexras1*$^{+/-}$, n = 29 for *Dp(11)17/+*, n = 13 for *Df(11)17/+*, and n = 14 for *Rai1*$^{+/-}$ littermates).

TABLE I

A Comparison of Tau (τ) Values Representing Free-Running Period Length and Total Wheel Running Activity for the Different Strains Examined in This Study

Genotype	τ (hr) ± SEM	N	P-value	Wheel revolutions per 24 hr			
				L/D ± SEM	P-value	D/D ± SEM	P-value
WT	23.62 + 0.041	29		59,534 + 3,034		56,747 + 3,697	
Dp(11)17/+	23.46 + 0.043[a]	22	**0.01**	46,246 + 2,053	**0.002**	41,650 + 1,900	**0.015**
WT	23.81 + 0.019	18		50,485 + 3,436		47,721 + 3,682	
Dexras1+/−	23.79 + 0.018	22	0.576	51,267 + 2,574	0.852	48,971 + 2,626	0.775
Dexras1−/−	23.71 + 0.030	24	**0.003**	47,956 + 2,856	0.567	42,254 + 2,753	0.225
WT	23.76 + 0.029	13		49,593 + 3,625		48,807 + 3,165	
Df(11)17−2/+	23.55 + 0.055	15	**0.003**	30,088 + 4,336	**0.005**	28,956 + 4,430	**0.002**
WT	23.82 + 0.019	14		54,416 + 1,057		53,715 + 1,454	
RAI1+/−	23.59 + 0.053	9	**0.00009**	30,893 + 4,566	**0.0005**	26,258 + 1,379	**0.0003**

P values comparing mutant versus wild-type littermates were calculated using Bonferroni–Holm post hoc analyses following ANOVA, and significant values are highlighted in bold.

[a]Previously described data [Lacaria, 2012b].