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Behavioural characteristics of the Prader–Willi syndrome related biallelic *Snord116* mouse model

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ABSTRACT

Prader–Willi syndrome (PWS) is the predominant genetic cause of obesity in humans and is associated with several behavioural phenotypes such as altered motoric function, reduced activity, and learning disabilities. It can include mood instability and, in some cases, psychotic episodes. Recently, the *Snord116* gene has been associated with the development of PWS, however, it's contribution to the behavioural aspects of the disease are unknown. Here we show that male and female mice lacking *Snord116* on both alleles exhibit normal motor behaviours and exploration but do display task-dependent alterations to locomotion and anxiety-related behaviours. Sociability is well developed in *Snord116* deficient mice as are social recognition memory, spatial working memory, and fear-associated behaviours. No sex-specific effects were found. In conclusion, the biallelic *Snord116* deficiency mouse model exhibits particular endophenotypes with some relevance to PWS, suggesting partial face validity for the syndrome.

1. Introduction

SNORD116, which is also known as *HBII-85*, is a non-coding ribonucleic acid (RNA) molecule. It plays a role in the modification of other small nuclear RNAs and is often referred to as a guide RNA or a small nucleolar RNA (snoRNA), as it is located in the nucleus of eukaryotic cells. Cavaille and co-workers discovered that *SNORD116* in wild type-like mice is exclusively expressed in the brain and that it maps to chromosome 15q11–q13 in humans (Cavaille et al., 2000). This region and micro-deletions to the *SNORD116* snoRNA cluster have been associated with the Prader–Willi syndrome (PWS) including the typical hyperphagia and obesity ((Sahoo et al., 2008; de Smith et al., 2009) but see also (Runte et al., 2005)). In line with this, *SNORD116* is absent from the brain of patients with PWS and work utilising *Snord116* knockout mice has suggested that the snoRNA *Snord116* gene cluster is a critical element in PWS formation (Ding et al., 2008; Sahoo et al., 2008; de Smith et al., 2009).

PWS is one of the most common genetic obesity disorders and is associated with a variety of symptoms including behavioural alterations such as delayed motor and language development, excessive eating and gradual development of morbid obesity (from early childhood onwards). Furthermore, affected patients can develop cognitive disabilities as well as temper tantrums and compulsive behaviour later in life (Cassidy et al., 2012). Some of the more the specific characteristics of human PWS are of short stature, low muscle tone, poor suckling reflex, incomplete sexual development, cognitive impairments and extreme and insatiable appetite, which can lead to excessive food consumption and consequently morbid obesity (Cassidy et al., 2012). Furthermore, PWS patients can suffer from compulsive behaviours (e.g. skin-picking), psychiatric symptoms, motor function deficiencies, and enhanced levels of anxiety (Feurer et al., 1998; Reddy and Pfeiffer, 2007).

The PWS locus is subject to parent-of-origin imprinting. The maternal allele of the gene(s) of interest is imprinted and thus silenced via epigenetic mechanisms whereas the paternal allele is mutant and therefore non-functional (human: (Cassidy et al., 2012) mouse: (Ding et al., 2008)). If the mutant allele is maternally derived, individuals do not develop PWS but the related Angelman syndrome (Saitoh et al., 1997).

The mouse PWS locus is highly homologous to the one in humans. Mouse models for *Snord116* deficiency show similar symptoms to humans suffering from PWS. Skyrabin and co-workers describe that a

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deletion in *Snord116* snoRNA (or more precisely, the so-called *MBII-85* snoRNA cluster on one allele) results in postnatal growth retardation (Skryabin et al., 2007). Mice with no parental copy of the *Snord116* snoRNA cluster were significantly smaller on postnatal day 10 than wild type-like siblings. The postnatal growth retardation was evident across six generations and independent of the genetic background. The differences in growth dynamics continued into adulthood, but contrary to humans with PWS these *Snord116* deficient mice do not develop an obese phenotype. Interestingly, there appears to be a moderate effect of sex with female knockout mice developing a less pronounced phenotype than males (Skryabin et al., 2007). No weight differences were detected during embryonic development or late gestation, which suggest that poor sucking behaviour of knockout offspring might be responsible for early growth retardation. Postnatal lethality of knockout mice was dependent on the genetic background and relatively low (i.e. around 15% in mice on 129SvJxC57BL/6J background). Fertility and the expression of other snoRNA genes (*MBII-436*, *MBII-13*, and *MBII-52*) as well as other genes with relevance to PWS (i.e. *Necdin*, *Magel2*, *Mkrn3*, *Frat3*, and *Snurf-Snrpn*) were not significantly altered suggesting that deletion of the *MBII-85* snoRNA cluster does not affect imprinting of neighbouring genes (Skryabin et al., 2007).

Another study investigated the effects of a paternally derived deletion of *Snord116* in male and female mice (Ding et al., 2008). The knockout mice also exhibited growth delay in the first three postnatal weeks (no lethality) but exhibited normal fertility and lifespan. Furthermore, at 3 months of age, knockout mice developed hyperphagia but stayed lean on normal and high fat diets. These mice also showed normal energy homeostasis maintenance. Behavioural testing of 2–6 months old male mice revealed a defect in motor learning but not in baseline motor coordination or balance (i.e. tested in the accelerod test). Muscle tone and strength were unaltered in *Snord116* knockout mice as were locomotion and exploration in the open field test. Furthermore, knockout mice had no deficits in working memory and spatial memory in two versions of the Y-maze test and showed normal pain sensitivity in the hot plate test. In contrast, *Snord116* deficient mice displayed increased anxiety and locomotion in the elevated plus maze and also developed hyperphagia, elevated levels of plasma ghrelin and altered metabolism in adulthood, although energy homeostasis regulation was normal (Ding et al., 2008).

As *Snord116* is a paternally imprinted gene, most studies assume a simple pattern of imprinting (i.e. expression of paternally inherited copy but silencing of maternal copy). However, more complex patterns of imprinted genes exist, which depend on genetic information derived from both parents (Wolf et al., 2008). Thus, we evaluate here for the first time a novel homozygous mouse model for *Snord116* for its face validity (Takao et al., 2007). For this, we carried out a comprehensive battery of behavioural paradigms with relevance to PSW symptoms in biallelic *Snord116* deficient mice. Mice were tested for motor coordination and muscle strength, balance, locomotion and exploration, and anxiety behaviour, as well as cognitive domains (i.e. spatial and recognition memory as well as fear-associated memory).

2. Materials and methods

2.1. Animals

In order to determine the behavioural consequences of a complete germline deletion of the *Snord116* cluster in mice, we crossed floxed *Snord116* mice (*Snord116*$^{lox/lox}$) (Ding et al., 2008) with a germline oocyte-specific Cre-line (Schwenk et al., 1995). The resultant heterozygous *Snord116* knockout mice were crossed to generate homozygous mice (*Snord116*$^{-/-}$ or SNORD KO). All mice were on a pure C57BL/6J background. The successful deletion of the *Snord116* gene was then confirmed by PCR and in situ hybridisation of brain sections from *Snord116* KO mice and wild type-like (WT) controls. In short, fresh frozen brains were sectioned at 30 μm thickness and thaw-mounted on Superfrost Plus® glass microscope slides (Lomb Scientific Pty Ltd., NSW 2229, Australia). In situ hybridisation was performed, as previously described (Parker and Herzog, 1999). Briefly, matching hypothalamic sections of deletion and control mice were hybridised with candidate mRNAs, which were labelled with [^{35}S] thio-dATP (Amersham Pharmacia Biotech, Buckinghamshire, UK) using terminal deoxynucleotidyltransferase (Roche, Mannheim, Germany). Silver grain densities of labelled mRNAs were analysed and compared using ImageJ software (US National Institutes of Health). DNA oligonucleotides used included those complementary to the mRNAs of mouse *Snord116* 5′-GTTCAGCTTTTCCAAGGA ATGTTTGACTGGGAATCATCATAGATCC-3′.

WT as well as biallelic *Snord116* deficient mice of both sexes (data were pooled across sex as no main effects of 'sex' were found: N = 12–17 per genotype) were transported to the Garvan Institute of Medical Research (Garvan) at 17–20 weeks of age, where they were group-housed in Polysulfone cages (1144B: Techniplast, Rydalmere, Australia) equipped with some tissues for nesting. Mice were kept under a 12:12 h light:dark schedule [light phase:white light (illumination: 124 lx) — dark phase: red light (illumination: < 2 lx)] for at least 2 weeks of habituation before behavioural testing started. Food and water were provided ad libitum, except where specified. Adult A/J mice from Animal Resources Centre (Canning Vale, Australia) were used as standard opponents for the social preference test.

Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Behavioural phenotyping

All experiments were carried out at least 1 h after onset of the light phase and completed within the first 6 h of the light phase. At the conclusion of each test trial, the test device was cleaned with 70% ethanol solution. Test order was as follows: open field, elevated plus maze, motor function tests, social preference test, Y-maze, and fear conditioning (inter-test interval of at least 48 h) (see also Table 1).

2.2.1. Open field test (OF)

In this test, the conflict between the drive to explore a new environment and a natural aversion to illuminated open areas is used to examine both anxiety and motor activity (Crawley, 1985). Mice were tested in an automated, photobeam-controlled OF, 43.2 × 43.2 cm (MedAssociates Inc., Vermont, USA). The arena was divided into a central and a peripheral zone (central zone photobeam coordinates 3/3, 3/13, 13/3, 13/13 (Long et al., 2012)). Mice were placed in a corner of the arena (illumination level: 20 lx) and were allowed to explore the arena for the following 30 min, while their activity was measured automatically (software settings: box size: 4; ambulatory trigger: 2; resting delay: 1500 ms). Measures of anxiety include the time spent in the central area of the open field and distance travelled in the centre as a percentage of overall distance travelled. Distance travelled, time spent '*resting*' (no photobeam-detectable movement), and small motor movements (photobeam breaks without ambulation, i.e. only 1 beam break within 1.5 s) were recorded as measures of motor activity and overall activity. Vertical activity (*rearing*) was used as a measure of exploration.

Table 1
Test biography: test age [d] ± 3 days and test order of control (WT) and *Snord116* knockout mice (SNORD KO) are shown (N = 12–17 per genotype).

Test age [d]	Behavioural paradigm
130	Open field (OF)
131	Elevated plus maze (EPM)
135	Motor function (pole test and wire hang test)
137	Social preference test (SPT)
140	Y-maze test (YM)
144	Contextual and cued fear conditioning (FC)