Genetic and evolutionary studies on imprinted genes and human behavior with special reference to Prader-Willi Syndrome

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Iiro Salminen

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Name:	**Iiro Salminen**
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Abstract

Genomic imprinting may have evolved due to an evolutionary conflict between alleles of different parental origin, carried by the mother and her offspring, which may be differently related to one's kin. Thus, genomic imprinting may be expected to highlight genes affecting regulatory mechanisms of behavior that may alter the distribution of maternal resources among offspring. The altered behavioral phenotypes shown in neurodevelopmental disorders that involve a lack of expression for one or several imprinted genes, may be further interpreted to represent extreme and dysfunctional phenotypes of human behavior. I have applied the kinship model for evolution of genomic imprinting to relevant literature on neurodevelopmental syndromes of genomic imprinting to address two questions central to understanding how genes interact with neural systems and regulate human behavior. Firstly, I propose how the evolution of genomic imprinting may be reflected in the behavioral phenotypes of the Prader-Willi- and Angelman syndromes (PWS and AS). Secondly, I ask if genetic variation of imprinted genes circulating in typical human populations might also affect non-clinical variation in human behaviors that may be partially co-regulated by imprinted genes. In chapter 2, I show that genetic variation for the maternally expressed UBE3A which is affected in both AS and PWS may also affect non-clinical variation in phenotypes of schizotypy among typically developing individuals. In chapter 3, I review evidence from relevant literature and evaluate whether phenotypes of sleep and eating in PWS and AS may be partly opposite to one another and propose hypotheses on how evolution of genomic imprinting may be reflected in the neural and behavioral phenotypes of AS and PWS. In chapter 4, I show that genetic variation of the paternally expressed SNORD116 gene, which shows a lack of expression in PWS, may also affect non-clinical variation in schizotypy among typically developing females. Finally, in chapter 5, I show that non-clinical variation in phenotypes of depression, schizotypy, autism spectrum cognition, social anxiety, sleep problems and emotional eating show significant co-variation in a population of typical individuals. The pattern of co-variation shown may reflect influences of genetic regulatory mechanisms involved in hypothalamic neural pathways, which have been shown to jointly alter the phenotypes of sleep, feeding and behavior. Behavioral phenotypes which are co-regulated by hypothalamic pathways may also be affected by variation of imprinted genes as several paternally expressed imprinted genes have also been shown to exert effects on hypothalamic pathways. In summary, I show that

paternally and maternally expressed imprinted genes may exert partly opposite effects on human behaviors that may alter phenotypes affecting the distribution of maternal resources among offspring. These behavioral alterations may further reveal genetic and neural mechanisms affecting human behaviors and may thus hold further implications for mental health and well-being both in clinical settings and among healthy individuals.

Keywords: Genomic imprinting; hypothalamus; psychotic disorders; evolutionary trade-off; intragenomic conflict

Table of Contents

List of Tables

List of Figures

Chapter 1. Introduction

1.1. Evolution of genomic imprinting and the kinship theory of intragenomic conflict.

In genomic imprinting, a specific gene is expressed predominantly from a single parental allele, while the other allele is silenced by an epigenetic mechanism. However, the nature of the epigenetic mechanism may also vary from a parental bias in expression or limiting the expression of the other parental allele in specific tissues or developmental phases to fully silencing the imprinted allele. As highlighted by both mouse models and the outcomes in genetic disorders involving dysfunctions in regulation of imprinted loci, many, though not all imprinted genes appear to be arranged in clusters of several jointly controlled genes, where the silencing of the imprinted parental allele is determined by the epigenetic state of an imprinting center [1]. These imprinting centers are typically formed from CpG islands which are large repetitive genomic segments identified by a particularly high density of G-C nucleotide pairs. In G-C nucleotide pairs, the addition of a methyl group to cytosine may act as an epigenetic signal affecting the expression of nearby transcripts. As is also further shown by the phenotypes of mouse models and a multi-locus imprinting disorder involving a deletion for the ZFP57 gene, the epigenetic marks that retain a signal for the parental origin of the allele may also be regulated by specific zinc-finger proteins [2,3]. Other epigenetic mechanisms including RNA interference and translational interference resulting from an alternate reading frame expressed from the antisense strand also appear to be employed in genomic imprinting [4–6].

Approximately 150 – 300 mammalian genes are subject to genomic imprinting, the variation in exact numbers of imprinted genes numbers being due to both lineage-specific losses and gains of imprinting and tissue-specific forms of imprinted gene expression [7,8]. Genome comparisons between extant mammalian taxa indicate that genomic imprinting evolved in tandem with the evolution of lactation and placentation, as marsupials only show imprinted gene expression for a comparably limited set of loci as compared to placental mammals, while the egg-laying monotremes show biallelic expression and conserved genomic arrangements for all known orthologs of imprinted loci [9,10]. The relatively low number of imprinted genes in mammalian genomes

appears therefore puzzling [11], when considering genomic imprinting is regulated via DNA methylation, which is pervasively distributed throughout mammalian genomes [12] and that imprinted genes have also been shown to affect phenotypes in embryonic development, lactation and behavioral phenotypes mediating maternal care [13,14], all of which may be considered central to the evolution of placental mammals. Finally, deletions of imprinted genes expressed from alleles of different parental origin appear to be associated with directly opposite phenotypes [15], which may appear redundant or even counter-intuitive to improving the fitness of the individual. Such opposite effects are highlighted by the phenotypes shown with mouse models for deletions of the Igf2 and Igf2r genes: Igf2 is expressed from the paternal allele in mice and the phenotype of the deletion is characterized by a severe growth restriction during embryonic development, which therefore implies that Igf2 promotes fetal growth during embryonic development. Notably, Igf2 has also been shown to regulate the diffusion of nutrients between the placenta and the embryo [16,17]. In contrast, Igf2r is expressed from the maternally inherited allele and the deletion shows a phenotype of prenatal overgrowth. Interestingly, Igf2r appears to be an alternative receptor of Igf2 that may compete with the Igf1r receptor and also regulate the protein turnover of Igf2 [18]. Paternally and maternally expressed genes may also exert opposite effects on behavioral phenotypes that affect the solicitation of maternal care, as is also highlighted by the phenotypical effects on ultrasonic vocalisations, which mice pups emit upon isolation to signal their need for maternal care: Deletions for the paternally expressed Peg3 and Magel2 have been associated with a reduction for producing ultrasonic vocalisations upon separation from the mother [19,20], while the deletion of the maternally expressed Ube3a has been associated with an increase in the amount of ultrasonic vocalisations produced when the pups were separated from their mother [21].

The best supported model for the evolution of genomic imprinting, referred as the kinship model, predicts that these opposite phenotypical effects of maternally and paternally expressed genes may reflect evolutionary conflicts in allocation of maternal resources between her offspring. Among mammals, the offspring is heavily dependent on maternal resources during embryonic development, infancy and juvenile periods and are thus adapted to solicit for nutrition and maternal care while the mother is equally adapted to recognizing the needs of her offspring. However, as all offspring are equally related to their mother, but may have different fathers, the alleles of different parental

origin may be expected to disagree on the distribution of maternal resources amongst the offspring: ‘More-selfish’ alleles that impose greater demands on maternal resources represent an increased cost to kin, ultimately affecting the mother’s lifetime fitness, while ‘less-selfish’ alleles that limit demands on maternal resources may present a fitness cost to the father, as the kin within the same brood and the future offspring of the mother may not share the same father. As maternally inherited selfish alleles would impose a fitness cost to the mother and her kin, and vice versa for paternally inherited selfless alleles, imprinted gene expression may evolve at a specific locus affecting the distribution of maternal resources, as the locus attains an epigenetic marker allowing the allele to be silenced based on the parental origin of the allele [11,22]. The remaining allele may evolve to be expressed at a higher rate to compensate for the silenced allele and imprinted genes exerting opposite effects on overlapping phenotypes may also evolve to exert the largest possible effect on the selected phenotype. Thus, the genomic conflict may eventually stall with minimal changes to the phenotype, which is balanced by nearly equal effects from different imprinted loci. However, the initially stalled genomic conflict may continue to spread to other loci, ultimately limited only by the rarity of genomic rearrangements and necessary complexity of the imprinting mechanisms. However, *de novo* mutations affecting the expression for one or several imprinted genes may reveal the full extent of the conflict, as is demonstrated by extreme phenotypes shown with syndromes that result from dysfunctions of genomic imprinting, which show extreme and dysfunctional alterations of human behaviors and further show a high prevalence of psychiatric disorders. The kinship model thus provides readily testable hypotheses for research of genomic imprinting and human behavior, as it may predicted that maternally and paternally expressed genes exert opposite effects for behavioral phenotypes that alter the distribution of maternal resources and hence, neurogenetic disorders involving dysfunctions in expression of either maternally or paternally expressed genes may also be expected to show partly opposite behavioral alterations to one another.

1.2. Evolution of genomic imprinting may be reflected in the altered behavioral phenotype of PWS.

Prader-Willi Syndrome (PWS) is a neurogenetic syndrome resulting from a lack of expression for ~20 paternally expressed genes and regulatory RNAs typically due to *de novo* germline mutations which include large paternally inherited deletions within the

15q11-q13 chromosome region, maternal asymmetrical disomy for chromosome 15 or an imprinting defect which renders both copies of the chromosome 15 to an epigenetic state resembling the maternally inherited chromosome [23]. Thus, PWS may be understood to result from an imbalance in genomic imprinting and may be expected to display phenotypes that represent extreme, dysfunctional alterations of behavioral phenotypes that maternally expressed genes would favor. PWS involves a distinct pattern of neural and physiological maladaptive development, accompanied by a behavioral phenotype typical to the syndrome [24,25]: In infancy, the syndrome is characterized by hypotonia, hypersomnia and failure to thrive, followed by weight gain and gradual rise in interest towards food preceding the onset of severe hyperphagia by 8 to 9 years of age. The hyperphagia is associated with constant lack of satiety with food-seeking behaviors and individuals with PWS typically require strict supervision for daily food intake [23,26]. The syndrome is accompanied by delayed motor development and mild to severe intellectual disability [23,27] while behavioral problems including irritability, stubbornness, and insistence on routines are shown at significantly higher rates compared to other individuals with intellectual disabilities of varied causes [28] PWS shows a ~ 26 % prevalence for autism spectrum disorders (ASDs), which is elevated as compared to typical human populations but comparable to the prevalence of ASDs among intellectual disorders of varied causes [29]. Psychiatric illness is also highly prevalent in PWS [30], the maternal uniparental disomy (mUPD) genotype in particular appears to show ~ 60 % lifetime prevalence for psychotic disorders, particularly cycloid psychoses with paranoid ideation, as highlighted in several studies of large PWS cohorts [30–34]. Due to the unique genomic configuration associated with this genotype, it has been further theorized that the doubled dosage of the maternally expressed UBE3A may contribute towards more severe outcomes of psychiatric illness among these PWS subjects [34,35]. The central features of PWS including hyperphagia, hypersomnia and multiple endocrine abnormalities have been attributed to hypothalamic dysfunction, as the hypothalamus jointly regulates feeding, biorhythms and endocrine pathways, and may thus represent a mechanism to alter behavioral phenotypes affecting the distribution of maternal resources among offspring [25].

Mouse models for SNORD116, MAGEL2 and NDN deletions further implicate multiple neural mechanisms for how lack of expression for paternally expressed genes in the hypothalamus may be involved in the development of the PWS phenotype [36–41].

In the context of the kinship model of imprinting, it has been further posited that the behavioral phenotype of PWS might represent a maladaptive extreme for phenotypes affecting the timing of weaning and the subsequent transitions to supplemental foods and independent foraging [42,43]. In primates, the lifetime fitness of the mother is heavily affected by the length of interbirth intervals. Hence, maternally expressed genes are expected to favour an earlier transition from weaning to supplemental foods and to independent foraging to both reduce maternal costs and facilitate shorter interbirth intervals, while paternally expressed genes are expected promote behaviors that favour the prolongation of both weaning and the subsequent transition to independent feeding [42]. In PWS, the gradual development of hyperphagia appears to coincide with nutritional transitions in typical human development, firstly with a transition from suckling to supplemental foods which is associated with increased appetite and secondly, the development of food-seeking behaviors and lack of satiety, which occurs during the age where children would typically transition towards independent foraging and family foods, thus lessening the burden placed on the mother. [42,44]. The phenotypes of feeding and behavior shown in both PWS and mouse models of genomic imprinting thus show that evolution of genomic imprinting may also exert effects on genetic regulation of hypothalamic circuits and understanding the nature of these effects may also further highlight how genes interact with neural circuits to shape behaviors and development.

1.3. Are the altered behavioral phenotypes of Angelman syndrome (AS) also affected by genomic imprinting?

The cluster of imprinted genes within the 15q11-q13 chromosome region also hosts two maternally expressed genes, ATP10A and the E3 ubiquitin ligase gene UBE3A, which has a causative role in AS, as short deletions within the UBE3A, in addition to large deletions within 15q11-q13 chromosome region, paternal uniparental disomies and imprinting defects have also been shown to cause AS [45]. Interestingly, AS has also been noted to show several behavioral phenotypes partly opposite to the phenotypes shown in PWS [44,46]. Firstly, in contrast to the stubborn and irritable behaviors shown in PWS [28], individuals with AS show easily excitable and happy demeanor, which has also been hypothesized to be an extreme, dysfunctional form of positive affect signaling, a set of behaviors which may have evolved to regulate social resources, particularly between the mother and her child [47]. AS has also been

estimated to show a prevalence ~ 40 – 80 % for ASDs [48–51], and while the severe language and developmental delays associated with AS may account for some of the overlap between AS and ASDs, the subset of AS children diagnosed with ASDs also appears to show a lack of reciprocal social interactions, preferring repetitive forms of play with objects as opposed interactions with others [50]. Secondly, as opposed to the hypersomnia shown in PWS, individuals with AS have also been found to consistently sleep less than typically developing peers; children with AS sleep ~ 6 hours in a day on average and show consistent difficulties in falling asleep [44,52]. Mouse models of AS also show that UBE3A may take part in regulation of biorhythms by affecting the protein turnover of the circadian transcription factor BMAL1 in the hypothalamus [53–55] and it may be interesting to consider if this interaction may have affected the evolution of the UBE3A imprinting mechanism. Thirdly, as opposed to the highly indiscriminate form of hyperphagia shown in PWS, individuals with AS may show particular preferences for foods which resemble complementary foods (reviewed in [44]). The neural development of AS is typically accompanied by severe intellectual disability, speech and learning impairments, ataxia and a stiff, jerky gait [45]. *In vitro* studies and mouse models of AS show that UBE3A is involved in the formation of ubiquitin chains on that designate specific proteins as targets for proteasomal recycling [56,57], and mouse models which show impairments in synaptic plasticity, learning and memory imply potential neural mechanisms for the behavioral phenotypes shown in AS [58–60].

The paternal copy of UBE3A is silenced only in mature neurons and the paternal expression of the UBE3A antisense transcript is necessary for the imprinting mechanism [4]. Further mouse model studies have recently shown that UBE3A is expressed from both copies during early embryonic brain development yet mice with paternal deletions of UBE3A showed no significant behavioral differences to wildtype mice despite lower protein levels of UBE3A, thus showing that AS is primarily characterized by impairments in late embryonic brain development [61,62]. A boundary element between SNORD116 and SNORD115 that bears an epigenetic mark on the paternally inherited allele functions as a termination site for the antisense transcript of UBE3A in biallelic expression. However, in neurogenesis, regulatory proteins at the boundary element are removed, allowing transcription of the antisense transcript to proceed through SNORD115 and the antisense sequences of UBE3A [63]. Since maternal expression of UBE3A has been shown to fully compensate for the loss of paternal UBE3A expression

during neurogenesis [64], the epigenetic mechanism within the border element may have evolved either to allow for transcription of SNORD115 in neurons or due to intragenomic conflict, to limit the expression of UBE3A as each parental allele evolves towards their favoured level of expression [8]. The neural functions of UBE3A and SNORD115 are not fully understood and their imprinted expression in the brain may be at least partly intertwined with their underlying functions. Thus, research of AS and PWS also represents an opportunity to understand firstly, how UBE3A may shape synaptic plasticity and learning and secondly, if UBE3A might also interact with hypothalamic traits via genetic regulation of biorhythms.

1.4. Do the behavioral phenotypes of AS and PWS reflect opposite imbalances in imprinted gene expression?

The behavioral phenotypes of AS and PWS appear opposite in several respects discussed here, however, lack of expression for different genes does not readily explain the opposite behavioral phenotypes shown between AS and PWS. The two syndromes may be better characterized in the context of the kinship model of imprinting, with AS reflecting extreme phenotypes of high resource demands mediated by an imbalance towards the expression of paternally expressed genes, while phenotypes shown in PWS would be expected to reflect low resource demands due to an imbalance towards expression maternally expressed imprinted genes. In this thesis we have reviewed the evidence for how an opposite imbalance in expression imprinted genes may be reflected in the opposite behavioral phenotypes of PWS and AS to resolve questions central to understanding how genomic imprinting may alter phenotypes of human behavior in neurogenetic syndromes, and further applied the knowledge to study how genomic imprinting might affect non-clinical variation in human behaviors.

Firstly, AS and PWS appear to show partly differing vulnerabilities for psychiatric disorders with AS showing a high prevalence for ASDs and PWS showing evidence for an elevated prevalence of both ASDs and psychotic disorders. Yet the role of UBE3A, which is characterized by loss of expression in AS and doubled dosage in the mUPD genotype of PWS, remains unclear. Interestingly, large cohort studies have also shown that interstitial duplications of the 15q11-q13 chromosome region are associated with an increased risk for developmental delay, multiple congenital anomalies and ASDs, but only maternally inherited duplications were uniquely associated with an increased risk of

schizophrenia [65,66]. In addition, a duplication of UBE3A has been reported to be associated with learning difficulties and psychiatric phenotypes including depression and anxiety when inherited maternally [67]. Thus, evidence from several independent sources appears to indicate that increased expression of UBE3A and relative imbalances in expression of maternally and paternally expressed imprinted genes may predispose individuals towards the development of psychotic disorders. Our work provides an additional line of evidence linking UBE3A with psychosis by characterizing whether genetic variation of UBE3A might be associated with differences in non-clinical variation of autism spectrum cognition, schizotypy or perhaps both.

Secondly, AS and PWS may be expected to show opposite phenotypes for feeding and sleeping due to the opposite imbalances in expression of imprinted genes and how central these phenotypes are to distribution of maternal resources among the offspring. However, both PWS and AS appear to show phenotypes of hyperphagia [24,68] and only AS has shown conclusive evidence for shortened sleep duration and increased sleep latency [52] while the sleep phenotypes associated with PWS primarily involve increased somnolence and sleep-disordered breathing [25,43]. Thus, it is unclear if the sleep phenotypes of AS and PWS are fully opposite of one another. Hence, it is imperative to fully characterize which feeding and sleeping behaviors show opposite phenotypes between PWS and AS and to also review relevant mouse models for the genetic and neural mechanisms of sleep and hyperphagia in PWS and AS to develop further hypotheses on how genomic imprinting might regulate the phenotypes of feeding and sleep in the offspring.

Thirdly, psychiatric illness with depressive symptoms is also prevalent with the 15q11-q13 chromosomal deletion genotype of PWS [30,35] while the behavioral phenotype of PWS also involves increased irritability and stubbornness and mood fluctuations [28]. Therefore, lack of expression for paternally expressed imprinted genes, in addition to the doubled dosage of UBE3A, might also mediate increased risk of psychiatric illness in PWS. Our research also addresses whether genetic variation for the paternally expressed SNORD116 gene might also mediate differences in non-clinical variation of schizotypy or autism spectrum cognition, which may also highlight novel neural mechanisms affecting paranoid ideation. Finally, altered phenotypes in regulation of sleep, feeding, mood and social behaviors appear central to PWS which also involves hypothalamic dysfunction. While animal models imply that hypothalamic circuits show

pleiotropic effects in regulation of sleep and feeding [69], it is unclear whether the hypothalamic dysfunction of PWS might also contribute to the behavioral phenotype or the increased vulnerability to psychiatric illness shown with PWS. If hypothalamic pathways may jointly alter feeding, sleep and behavior, we might expect that mouse models and other neurodevelopmental syndromes involving hypothalamic dysfunction to also show consistent alterations in behavior. Given that genetic variation affecting hypothalamic function in non-clinical ranges may also be circulating among typical individuals, we might also expect that behavioral phenotypes affected by hypothalamic function may also show covariation in typical human populations.

1.5. Thesis objectives and chapters

As showcased by the extreme, maladaptive behavioral phenotypes associated with neurogenetic syndromes involving lack of expression for imprinted genes and the directly opposite phenotypes and functions shown with animal models for imprinted genes expressed from alleles of different parental origin, genomic imprinting may highlight genes and neural circuits poised to regulate social behaviors, mood, sleeping and feeding. The phenotypes of these behaviors are shaped in part by the tug-of-war between mothers and their offspring as each sibling vies for a larger share of the maternal resources. Might the genetic variation of imprinted genes also contribute to underlying vulnerabilities to mental disorders and maladaptive behaviors among typically developing individuals? We have focused primarily on the imprinted genes involved in the Prader-Willi and Angelman syndromes to develop and test models on how imprinted genes and their evolution might affect complex behavioral and psychological traits in four independent studies among typical human populations. Below, the approaches taken in each chapter are introduced and discussed briefly.

In chapter 2, we review relevant research on whether genetic variation of UBE3A is associated with either autism spectrum disorders or psychotic disorders, both of which show greatly increased prevalence within PWS and AS. Since UBE3A may be mediating risks for underlying vulnerabilities for either psychotic disorders or autism spectrum disorders, we may also ask if small-effect polymorphisms of UBE3A circulating among populations of typically developing individuals would be associated with non-clinical variation in phenotypes of schizotypy or autism spectrum cognition.

In chapter 3, we review existing literature on phenotypes of sleeping and feeding in AS and PWS, which appear to show opposite phenotypes in relevant literature and may be expected to affect the distribution of maternal resources, particularly during infancy when the child relies exclusively on the mother for nutrition. We also review phenotypes of feeding and sleeping shown in mouse models of AS and PWS to develop hypotheses on how opposite effects might arise from lack of expression for different genes. This work also carries greater implications on how genes interact with neural systems that regulate feeding and sleeping, particularly in the hypothalamus which jointly regulates these phenotypes and the dysfunction of which has also been theorised to be involved in the development of PWS.

In chapter 4, we focus on the paternally expressed SNORD116 small nucleolar RNA gene which shows a lack of expression in PWS and has been highlighted in particular by a small number of subjects with microdeletions including only the SNORD116 locus, displaying behavioral phenotypes that resemble the full behavioral phenotype of PWS. Furthermore, mouse models of PWS have also shown lack of expression for SNORD116 may be associated with hyperphagia and sleep and behavioral phenotypes resembling PWS [37,70–72]. As PWS also shows increased prevalence for both autism spectrum conditions and psychotic disorders [29,30,33], we may also expect that small-effect polymorphisms of SNORD116 circulating among typical subjects may also be mediating for non-clinical variation in these phenotypes. Furthermore, whether lack of expression for SNORD116 might also affect schizotypical cognition in PWS is also of importance as it cannot be distinguished if the increased prevalence of psychotic disorders shown for the mUPD genotype of PWS can be attributed solely to increased dosage of UBE3A or to a combined effect also involving a lack of expression for one or several paternally expressed genes.

In chapter 5, we ask whether hypothalamic dysfunction may jointly affect phenotypes of feeding, sleeping, mood and social behaviors which all show altered phenotypes in PWS. To this end, we review relevant literature on neurogenetic syndromes involving hypothalamic dysfunctions and mouse models of imprinted genes that involve effects on neural development of hypothalamic brain regions. If hypothalamic brain regions may jointly alter the phenotypes affected in PWS, one may also expect the full or specific a subset of these phenotypes to show co-variation with one another due to overlapping effects from multiple co-regulated hypothalamic

mechanisms affecting a diverse suite of human behaviors. Thus, we also collected questionnaire data on non-clinical variation of phenotypes relevant to the full behavioral phenotype of PWS, including mood, sleep problems, social anxiety, appetite and both schizotypy and autism spectrum cognition to verify if such phenotypes might show co-variation with one another among typical subjects. Finally, we review the findings of these studies and discuss the conclusions drawn in contexts relevant to further research on how imprinted genes may interact with neural pathways altering human behaviors that may affect the distribution of maternal resources.

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Chapter 2. Genetic variation of UBE3A is associated with schizotypy in a population of typical individuals.

2.1. Abstract

The maternally expressed imprinted gene UBE3A has been implicated in autism, schizophrenia and psychosis. The phenotype of Angelman syndrome, caused by loss of UBE3A expression, involves autism spectrum traits, while Prader-Willi syndrome, where the genotype of maternal disomy increases dosage of UBE3A, shows high penetrance for the development of psychosis. Maternal duplications of the 15q11-q13 chromosome region that overlap the imprinted region also show an association with schizophrenia, further implying a connection between increased dosage of UBE3A and the development of schizophrenia and psychosis. We phenotyped a large population of typical individuals for autism spectrum and schizotypal traits and genotyped them for a set of SNPs in UBE3A. Genetic variation of rs732739, an intronic SNP tagging a large haplotype spanning nearly the entire range of UBE3A, was significantly associated with variation in total schizotypy. Our results provide an independent line of evidence, connecting the imprinted UBE3A gene to the schizophrenia spectrum.

Keywords: Angelman syndrome, Prader-Willi syndrome, Autism spectrum disorder, Schizophrenia, 15q11-q13 duplication, Genomic imprinting.

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2.2. Introduction

The imprinted gene UBE3A, encoding an E3 ubiquitin ligase protein, is expressed only from the maternal copy in neurons [1]. The silencing of the paternally inherited copy is mediated by a long RNA transcript, which also contains a sequence complementary to UBE3A (UBE3A antisense transcript, UBE3A-ATS) and initiates from

the unmethylated, paternally inherited copy of the 15q11-q13 imprinting center near the SNURF-SNRPN gene. In the maternally inherited copy, the corresponding imprinting center is methylated, preventing the expression of the UBE3A-ATS and the subsequent paternally expressed genes imprinted genes (reviewed in [2]). The E3 ubiquitin ligases co-operate with specific E2 ubiquitin ligase proteins to identify a range of target proteins, which are subsequently marked for degradation, activation or relocation in the cell [3]. The gene is expressed in multiple alternatively spliced mRNAs and three different protein isoforms [4], which have functions in neuron differentiation and development, as suggested by mouse models. Thus, UBE3A affects neuron development and function as well as behavioral and psychological phenotypes via multiple different mechanisms, involving both direct and indirect interactions with specific target proteins (reviewed in [5]).

UBE3A is located in a ~ 2Mb imprinted domain in the 15q11-q13 chromosome region, which is flanked by several chromosomal break points [6]. The 15q11-q13 chromosome region is particularly well-known for a ‘sister pair’ of neurodevelopmental syndromes, which involve lack of expression for paternally expressed genes in Prader-Willi Syndrome (PWS) and a lack of expression for the maternally expressed UBE3A gene in Angelman Syndrome (AS), respectively [7,8]. The two syndromes show distinct behavioral phenotypes: AS subjects typically show a sociable and happy disposition with frequent laughter [8], while autism spectrum disorders (ASD) and behaviors typical of the autism spectrum are also highly prevalent [9]. In comparison, PWS involves rapid mood fluctuations and considerable negative affect [10], with psychiatric disorders including depression, bipolar disorder and most prominently, affective psychoses being highly prevalent, especially for the genotype of maternal disomy [11]. Given that AS involves loss of expression of UBE3A [1,12], while PWS due to uniparental disomy involves overexpression of this gene [13,14], UBE3A may be linked to such alterations of social behavior in the context of autism spectrum and psychotic-affective conditions.

Lack of expression for UBE3A has been implicated in risk for ASDs from two lines of evidence. Firstly, AS involves a high prevalence of ASDs, with estimates ranging from ~40 to 80% of affected individuals [9,15,16]. Behaviors relevant to the autism spectrum in AS include deficits in social interactions, repetitive and stereotypical behaviors, as well as deficits in communication, partly attributable to developmental delay with near or complete lack of speech. A recent study showed that AS individuals

diagnosed with autism spectrum traits in early childhood showed later improvements in both expressive and receptive language skills, while the levels of autism spectrum traits did not change [17]. Thus, the behavioral phenotype of AS reflects the broad characteristics typical of ASDs involving developmental delay, which indicates a connection between lack of expression for UBE3A and the development of autism spectrum traits.

Secondly, several studies have investigated associations of markers within UBE3A with idiopathic autism, with mixed results. Cook et al. [18] found that several microsatellite markers in the genetic region of UBE3A, including D15S122 (chr15:25434850–25435145, repeating sequence AC), located at the 5′ untranslated region of UBE3A, did not show any patterns of preferential transmission in autism families. Nurmi et al. [19] studied a population of families where at least two children had a diagnosis of autism and found that the marker D15S122 showed a pattern of preferential transmission among autism families. The microsatellite marker was analyzed as 12 different alleles with lengths ranging from 137-bp to 163-bp, with the 145-bp, 147-bp and 149-bp alleles covering the majority of the variation. A 155-bp allele showed a significant maternal effect in transmission to affected siblings, while a 147-bp allele showed a significant negative association with autism. A later study by the same group, using six intronic SNP markers within UBE3A did not find any significant pattern of preferential transmission in autism families [20]. Guffanti et al. [21] found that an allele of the microsatellite marker D15S122 showed preferential under transmission to affected children, partially replicating the earlier results of Nurmi et al. [19]. Here, the microsatellite marker was analyzed as four classes of different alleles designated as 222-bp, 224-bp, 226-bp and other lengths, with the three main alleles covering the majority of the variation. Two other studies using SNP markers within UBE3A did not find any significant associations between UBE3A and ASDs [22,23]. While these studies suffer from limited sample sizes and a lack of statistical power, their results suggest that genetic variation affecting UBE3A, most notably rare haplotypes tagged by the marker D15S122, may be associated with an increased risk for ASDs.

In contrast to the link between lack of expression for UBE3A and AS, increased dosage of UBE3A has been associated with psychotic-affective spectrum conditions from several independent lines of evidence. Firstly, PWS individuals with the genotype of maternal disomy show approximately 2- to 3-fold higher expression of UBE3A, as shown

in studies with cDNA microarrays from cell lines of individuals with different genotypes of PWS [13,14]; as noted above, PWS subjects with maternal disomy also show a significantly increased risk for psychotic and bipolar disorders as compared to individuals with deletions [11]. Both case studies and several independent studies with large cohorts (references in ([24], see supplementary table) have shown that individuals with the genotype of maternal disomy show a high prevalence of affective psychoses, involving mainly paranoid ideation and auditory hallucinations [25–28]. Associations between the genotype of maternal disomy, with two maternally inherited copies of UBE3A and development of psychosis in PWS, suggest that increased dosage of UBE3A may contribute to the development of psychotic-affective phenotypes and conditions.

Secondly, similarly to the genotype of maternal disomy in PWS, maternal duplications of the 15q11-q13 chromosome region involve a ~1.5- to 3-fold increase in expression of UBE3A as shown by studies with mRNA derived from post-mortem brain tissues and cell lines [29,30]. Studies of large cohorts of individuals with schizophrenia have indicated that only maternal duplications (and not paternal duplications) of the 15q11-q13 region increase the risk of schizophrenia. Ingason et al. [31] genotyped cohorts of schizophrenia patients from several European countries and found significant overrepresentation of maternal duplications among the schizophrenia group as compared to the controls. Similarly, a more comprehensive follow-up study including large cohorts from both ASDs and developmental delay, as well as schizophrenia, found that only maternal duplications mediated an increased risk for schizophrenia, while both paternal and maternal duplications were found to increase the risk for ASDs [32]. The duplications of the schizophrenia patients did not show consistent patterns with regard to the size of the duplication, but the imprinted region between break point 2 and break point 3, which contains UBE3A, was common to all duplications associated with schizophrenia. As only maternal duplications of the region showed a consistent association with schizophrenia, the authors of both studies suggested that maternally expressed genes, and in particular UBE3A, are likely to be involved in mediating the risk for schizophrenia [31,32].

Thirdly, Noor et al. [33] found that a microduplication encompassing only UBE3A had segregated for several generations in a family line, and that maternal inheritance of the duplication was associated with developmental delay, anxiety, depression, and schizophrenia in family members. Furthermore, cell line experiments with a patient's

fibroblasts showed approximately twofold expression levels of UBE3A as compared to controls, with no effect on the expression of surrounding genes, more directly implicating increased expression of UBE3A in connection with depression and schizophrenia.

Duplications of the entire 15q11-q13 chromosome region have also been shown to be associated with ASDs, which has been suggested to indicate a link between increased dosage of UBE3A and autism [34,35]. Isles et al. [32] analyzed large cohorts of individuals with ASD and developmental delay from several previously published studies along with new data and found that both paternal and maternal duplications overlapping with the region between breakpoints 2 and 3, or alternatively with breakpoints 1 and 3 in the 15q11-q13 chromosome region, showed significant associations with a phenotype involving ASD with developmental delay and multiple congenital anomalies, with ~ 50% penetrance for maternal and ~20% penetrance for paternal duplications. Given that these duplications overlap with both imprinted and non-imprinted domains, several different genes in the chromosome region may also be involved in mediating the risk for ASDs.

Several mouse model studies of UBE3A show notable effects of varying expression of this gene in neuron development and function, whereby deletions involve opposite social phenotypes as compared to increased dosage [36–42]. In particular, model mice with a lack of expression for UBE3A showed significantly increased amounts of ultrasonic vocalization as compared to controls and prolonged social interest in an interaction test with a novel mouse of same sex, as compared to controls [43,44]. In comparison, two studies found that transgenic mice with two additional copies of UBE3A showed a reduced amount of ultrasonic vocalizations during interactions with a same-sex pair as compared to controls [45,46] and did not show a typical preference for interaction with a caged mouse of same sex [46]. These results suggest that opposite imbalances in dosage of UBE3A may be linked to opposite alterations in aspects of social behavior.

A consistent pattern across studies thus indicates that increased dosage of UBE3A, as shown with both maternal disomy in PWS and maternal 15q11-q13 duplications, may be mediating the risk for schizophrenia, and related psychotic-affective conditions, in clinical populations. In contrast, lack of expression for UBE3A may be linked with ASDs, as implicated by the high prevalence of ASDs in AS, which involves a

lack of expression for UBE3A in neurons. Furthermore, haplotypes of UBE3A may mediate an increased risk of ASDs. As both psychotic-affective spectrum conditions and ASDs can be considered as genetic and neuropsychiatric continuums grading into typical functioning and social behavior [47–49], it can be further postulated that genetic variation in UBE3A may affect phenotypes of autism spectrum traits or schizotypy in non-clinical populations. We have characterized variation in both autism spectrum traits as well as schizophrenia spectrum traits in a large population of typically developing individuals and genotyped them for a set of SNPs in the UBE3A genetic region. We hypothesized that genetic variation in UBE3A may thus be associated with variation in schizotypal traits, autism spectrum traits or both, due to effects on mRNA levels, post-transcriptional regulation, or activity of the protein, and other processes.

2.3. Methods

The study was approved by the ethics boards at both the University of Alberta (Pro00015728) and Simon Fraser University (2010s0554), with all participants providing prior written informed consent. We collected questionnaire and DNA data from 507 undergraduate students (285 females and 222 males) of Caucasian ancestry. Forms and levels of schizotypal traits were quantified with the Schizotypal Personality Questionnaire - Brief Revised (SPQ-BR) [50]. The questionnaire consists of 32 items using a 5-point Likert scale ranging from ‘strongly disagree’ to ‘strongly agree’. The questions are further divided across seven subscales of personality traits and social behavior which include 1. Ideas of Reference, 2. Constricted Affect, 3. Eccentric Behavior, 4. Social Anxiety, 5. Magical Thinking, 6. Odd Speech and 7. Unusual Perceptions, and sum together to total schizotypy. The Autism Spectrum Quotient (AQ) [51] was used to quantify the extent that individuals endorsed personality traits and behavior associated with the autistic spectrum. The questionnaire is comprised of 50 items and assesses personality traits and social behavior across five domains of 1. Social skills, 2. Attention Switching, 3. Attention to Detail, 4. Communication and 5. Imagination and sum into the total AQ score.

A set of six SNPs was initially chosen to characterize the genetic variation of UBE3A. In particular, rs732739 (chr15:25434520, C/T, in hg38, 2013) is located within the first intron of UBE3A, 330-bp downstream from the starting site of D15122, a microsatellite marker, which has been previously shown to display patterns of

preferential transmission in autism families [19,21]. The marker is also in partial linkage disequilibrium (D′ = 1.0, r2 = 0.54, in CEU 1000genomes) with rs189782611 (chr15:25434997, C/G) located within D15S122. We used both D' and r2 to measure LD; D′ indicates how often an allele of a given marker is inherited together with the associated allele of the other marker, while r2 is also affected by the allele frequencies. Therefore, a pair of genetic markers showing high D′ with low r2 do not correspond to the same underlying genetic variation. Two SNPs, rs11630723 (chr15:25442741, A/T) and rs11161178 (chr15:25444144 A/G), were selected to target the promoter region of UBE3A, while rs1041933 (chr15:25409602, C/T) rs17115577 (chr15:25422613, A/C) and rs7176461 (chr15:25419872, A/C) were selected to target genetic variation in the untranslated region 5′ of UBE3A. As rs11630723 and rs7176461 were reported to be in complete LD with other genetic markers in our study, based on data from individuals of Caucasian descent (rs11161178 and 11630723, r2 = 1.0, D′ = 1.0; rs732739 and rs7176461 r2 = 1.0, D′ = 1.0), rs11630723 and rs7176461 were not included in the full analysis; in each case, we analyze data and report results from the markers with larger sample sizes. Unadjusted p-values for all six SNPs are provided in supplementary files. As genetic variation may differ between populations, our analyses were limited to students of Caucasian descent, based on demographic data collected with the questionnaires. The SNPs in our study population showed nearly identical allele frequencies as compared to previously reported results with populations of Caucasian descent.

Fluorophore-labelled primers for SNPs rs732739, rs1041933, rs17115577, rs1716461, rs11630723 and rs11161178 were used in TaqMan genotyping using a Roche Light-Cycler 96 Real-Time PCR machine. Fluorescence data were analysed under Endpoint Genotyping with the LightCycler 96 Software v. 1.1.0.1320 (2011) and genotyping success varied between 96.8 to 99.0%. Each of the markers was in Hardy-Weinberg equilibrium, as measured by the Fisher Exact test (rs1041933, p = 0.07; rs17115577, p = 1; rs732739, p = 0.21; rs7176461, p = 0.34; rs11630723, p = 0.39; rs11161178, p = 0.45).

Under the simplest model of imprinting, only the maternally inherited allele would be expected to affect the expression of a maternally expressed imprinted gene. Thus, at an imprinted locus with the alleles A and a, Aa and aA heterozygotes, differing by parental origin, would be expected to show distinct phenotypes. However, as our data

does not include genotypes of family trios, and the silencing of paternal copy of UBE3A is not mediated by methylation of the promoter area [2], we are unable to determine the distinct genotypes of the heterozygous individuals. We have conducted an additional analysis relevant to this issue, by comparing the phenotypic variation of the two homozygous groups, showing the effects of both alleles unambiguously. T-tests and ANOVAs for phenotypic AQ and SPQ differences were calculated for each SNP under four genetic models: 1. Codominant, (common homozygotes versus heterozygotes versus rare homozygotes), 2. Dominant, (common homozygotes versus heterozygotes + rare homozygotes), 3. Recessive (common homozygotes + heterozygotes versus rare homozygotes) and 4. Homozygotes-only (common homozygotes versus rare homozygotes) in R v. 3.5.1. (2018). As the genetic models are not independent of each other, corrections for multiple testing were based on tests on four genetic markers, adjusted separately for each of our initial hypotheses on schizotypy and autism spectrum traits. Further adjustments accounting for tests on both AQ and SPQ scales and tests on different genetic models were also performed (data not shown). False Discovery Rate (FDR) adjustments using the 0.05 level of significance were performed with the p.adjust feature in the R stats package (v. 3.5.1.)

2.4. Results

The total schizotypy score showed significant differences between individuals with different genotypes of rs732739 under the codominant (rs732739 unadjusted $p = 0.018$), and the recessive model (rs732739 unadjusted $p = 0.0055$) as shown in Table 1. In addition, the total schizotypy score showed significant differences in our additional analysis, comparing the two homozygote genotypes (unadjusted $p = 0.0057$, see supplementary Table 2 in publication). After FDR adjustment for tests on four genetic markers, only the results with the recessive and homozygotes-only models for rs732739 remained significant. These results also remained significant after twofold adjustments for tests using both total AQ and total SPQ-BR scores. Comparison of the mean SPQ-BR scores indicates that the rare homozygote (TT) individuals on average had about 18% lower SPQ-BR scores than individuals with other genotypes. The total AQ scores did not show significant results on any of the markers, but the subscale of social skills showed nominally significant differences (unadjusted $p = 0.03$) with the dominant model on rs11161178 (see supplementary Table 1 in publication). On SPQ-BR subscales,

rs732739 showed nominally significant differences on the subscales of ideas of reference, constricted affect, eccentric behavior, interpersonal features, and disorganized features, on codominant, imprinted and recessive models (see supplementary Table 1 in publication), but none of the associations between genetic markers and AQ or SPQ-BR subscales remained significant after FDR adjustments.

Table 1. AQ and SPQ phenotypes for genotypes of the four UBE3A SNPs with dominant-recessive genotype models, with ANOVA tests between genetic variance of the UBE3A SNPs and total AQ and SPQ-BR scores. Mean ± s.d. for Autism Quotient (AQ) and Schizotypal Personality Questionnaire - Brief Revised (SPQ-BR) scores for each genotype of the four UBE3A SNP markers are shown. Unadjusted p-values (p) for ANOVA tests on three genetic models (Codominant, Dominant and Recessive) are shown first, while p-values corrected by a false discovery rate of 0.05 (pfdr) are also provided. The FDR correction was based on tests on four different markers, adjusted separately for each genetic model and phenotypic variable (degrees of freedom for each test: 4, see methods for reasoning).

SNP	**Genotype 1 (n)**	**Genotype 2 (n)**	**Genotype 3 (n)**	**Cod (p/p_{fdr})**	**Dom (p/p_{fdr})**	**Rec (p/p_{fdr})**
Rs732739	CC (381)	CT (111)	TT (8)			
AQ	16.77 ± 5.41	17.08 ± 5.56	13.50 ± 4.90	0.198 / 0.402	0.898 / 0.898	0.085 / 0.17
SPQ-BR	86.10 ± 15.58	87.00 ± 16.47	70.63 ± 15.09	0.018 / 0.072	0.903 / 0.922	0.006 / 0.02
Rs1041933	TT (284)	CT (187)	CC (27)			
AQ	16.93 ± 5.58	16.80 ± 5.22	14.96 ± 5.58	0.201 / 0.402	0.467 / 0.801	0.076 / 0.17
SPQ-BR	86.63 ± 14.80	86.50 ± 15.94	82.63 ± 14.80	0.495 / 0.660	0.922 / 0.922	0.245 / 0.327
Rs17115577	CC (446)	AC (45)	AA (4)			
AQ	16.87 ± 5.50	17.08 ± 5.56	13.50 ± 4.90	0.198 / 0.402	0.898 / 0.898	0.085 / 0.17

SPQ-BR	86.10 ± 15.58	87.00 ± 16.47	70.63 ± 15.09	0.018 / 0.072	0.903 / 0.922	0.006 / 0.02
Rs11161178	GG (302)	172 (AG)	28 (AA)			
AQ	16.65 ± 5.44	17.13 ± 5.57	15.54 ± 5.15	0.313 / 0.417	0.601 / 0.801	0.226 / 0.301
SPQ-BR	85.61 ± 15.87	87.27 ± 16.17	82.11 ± 13.67	0.226 / 0.452	0.515 / 0.922	0.184 / 0.327

2.5. Discussion

Our primary results are twofold. First, rs732739, an intronic marker within UBE3A, shows a significant FDR-adjusted association with total schizotypy, and nominally significant associations with several subscales, under codominant and recessive models. These results indicate that genetic variation of UBE3A segregating among non-clinical populations affects levels of schizotypy within the range of typical variation. This finding fits with previously discussed evidence showing that UBE3A appears to mediate the risk for development of psychosis in PWS [25–28] and the risk for schizophrenia associated with maternal 15q11-q13 duplications [31,32]. Our results show nominally significant associations with the subscales of interpersonal and disorganized schizotypy, with the former reflecting constricted affect and paranoid ideation in social contexts, and the latter reflecting endorsement of statements regarding odd speech and eccentric behaviors. The phenotypes of psychosis in PWS similarly involve, most commonly, paranoid ideation in the forms of second-person hallucinations and persecutory delusions, as well as mood swings, confusion and obsessive rituals [25,27], which resemble disorganized aspects of schizophrenia spectrum disorders. Thus, our results with the maternally expressed UBE3A, which shows about twofold increase in expression levels with the genotype of maternal disomy in PWS [13,14] appear consistent with previous clinical findings as regards to psychiatric traits mediated by UBE3A.

Second, we note that rs732739, the marker associated with total schizotypy in our study, is linked (rs189782611, D′ = 1.0, r2 = 0.54, located within 330-bp) with a genetic marker located within D15S122, a microsatellite marker associated with autism in two transmission disequilibrium studies [19,21]. However, as neither our study nor any previously published study included genotyping of the D15S122 microsatellite along with rs732739, it is not possible with available data to determine if a particular allele of rs732739 is associated with a D15S122 microsatellite allele of a certain size. Future work could usefully address this issue.

A large region of the UBE3A gene interacts with the UBE3A antisense transcript, which as previously mentioned, regulates the silencing of the paternal copy of UBE3A in neurons [2]. As shown by a mouse model study in which a mutation in the maternally inherited copy of Ube3a increased the expression of the Ube3a-ATS as compared to controls [52], the paternally and maternally inherited alleles may interact with each other directly. More recently, a cell model study found that a border element consisting of the IPW and PWAR1 genes is necessary for the imprinting of the paternal allele in nerve cells, as removal of this region led to increased expression of UBE3A-ATS in AS-derived nerve cells, without repressing the expression of UBE3A from the paternally inherited allele [53]. Thus, genetic variation of regions overlapping the antisense transcript may affect the expression of UBE3A via the paternally inherited allele in some manner. Such a mechanism may have implications for tests of the hypothesis that increased expression of UBE3A is expected to mediate the expression of schizotypy, while reduced expression of UBE3A influences the expression of autism spectrum traits.

The genetic marker showing our main result, rs732739, is strongly linked with genetic markers within the UBE3A promoter region (D′ = 1.0 r2 = 0.947 with rs72697799, located approximately 1000-bp upstream of exon 1, at chr15:25440750) As previously discussed, UBE3A is alternatively spliced in several tissue types, including neurons [3,4] and the longer forms of UBE3A mRNA include 5′-UTR sequences comprising of the first exons, which are not translated to the amino acid sequence in any of the three different isoforms of UBE3A. While the specific purpose of mechanisms regulating alternative splicing and translation of UBE3A is not known in humans[3,5], other 5′-UTR sequences are known to include ribosomal binding sites and mRNA secondary structures, which further regulate translation events [54]. Thus, both D15S122 and rs732739 may tag genetic variation linked to promoter and 5′-UTR regions of UBE3A that regulates

expression. The SNP rs732739 indeed represents a highly-significant expression QTL for muscle tissues [55], indicating that the marker is functionally associated with expression-level variation in UBE3A. Further studies are needed on mechanisms whereby rs732739 and linked regions may mediate brain development and function via regulation of gene expression, alternative splicing, protein interactions or other mechanisms, with regard to both autism and psychotic-affective spectrum disorders.

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Chapter 3. Baby food and bedtime: evidence for opposite phenotypes from different genetic and epigenetic alterations in Prader-Willi and Angelman syndromes.

3.1. Abstract

Prader-Willi and Angelman syndromes are often referred to as a sister pair of neurodevelopmental disorders, resulting from different genetic and epigenetic alterations to the same chromosomal region, 15q11-q13. Some of the primary phenotypes of the two syndromes have been suggested to be opposites to one another, but this hypothesis has yet to be tested comprehensively, and it remains unclear how opposite effects could be produced by changes to different genes in one syndrome compared to the other. We evaluated the evidence for opposite effects on sleep and eating phenotypes in PWS and AS, and developed physiological-genetic models that represent hypothesized causes of these diametric differences. Sleep latency (time to fall asleep) shows opposite deviations from controls in PWS and AS, with shorter latency in PWS by meta-analysis, and longer latency in AS from previous studies. These differences can be accounted for by the effects of variable gene dosages of UBE3A and MAGEL2, interacting with clock genes, and leading to acceleration (in PWS) or deceleration (in AS) of circadian rhythms. PWS and AS also show evidence of opposite alterations in hyperphagic food selectivity (underselective vs overselective respectively), with more-paternally biased subtypes of AS apparently involving increased preference for complementary foods ('baby foods'); hedonic reward from eating may also be increased in AS and decreased in PWS. These differences can be explained in part under a model whereby hyperphagia and food selectivity are mediated by effects of the genes SNORD-116, UBE3A, MAGEL2, with outcomes depending upon the genotypic cause of AS. The diametric variation observed in sleep and eating phenotypes in PWS and AS is consistent with predictions from the kinship theory of imprinting, reflecting extremes of higher resource demand in AS, and lower demand in PWS, with special emphasis on social-attentional demands and attachment associated with bedtime, and feeding demands associated with mother-provided complementary foods compared to offspring-foraged family-type foods. These results have important implications for the genetic and epigenetic bases of human variation and disorders related to sleeping, eating, and child development.

This chapter was adapted for the thesis from a journal article published by Salminen et al. (2019)

Salminen I, Crespi B, Mokkonen M. Baby food and bedtime: Evidence for opposite phenotypes from different genetic and epigenetic alterations in Prader-Willi and Angelman syndromes. Sage Open Medicine, 2019. 7.

3.2. Introduction

Prader–Willi syndrome (PWS) and Angelman syndrome (AS) are often referred to as a sister pair of genetic disorders, both resulting in cognitive and neurological impairments, along with unique physiological and behavioral phenotypes [1]. Similarly, both AS and PWS show a low degree of comorbidity with autism spectrum disorders (ASDs) as compared to the general population [2–5]. However, expression levels of several traits related to early childhood development, such as birth weight, interest toward suckling and somnolence have been suggested to be opposite between the syndromes [6]. The behavioral phenotypes of the syndromes have also been noted to contrast each other, as individuals with AS typically show a sociable disposition, with constant smiling and laughter, while individual PWS tends to show considerable negative affect, with frequent temper tantrums and obsessive–compulsive behavior [1,7]. While both syndromes are due to multiple types of different mutations in the 15q11-q13 chromosome region, the specific nature of each mutation is critical to the epigenetic alterations involved in each syndrome as shown in Figures 1. and 2. and Table 1. AS is due to the absence of the maternal copy of the 15q11-q13 chromosome region (the UBE3A gene in particular), while PWS is due to the absence of the paternal copy of the same chromosome region. The resulting genetic and epigenetic alterations lead to losses of expression for separate sets of genes in each syndrome, which presents an apparent paradox. Are the phenotypes of these syndromes truly opposite of each other and, if so, what underlying factors could explain the opposite nature of the phenotypes despite the different nature of the genetic alterations involved in each syndrome?

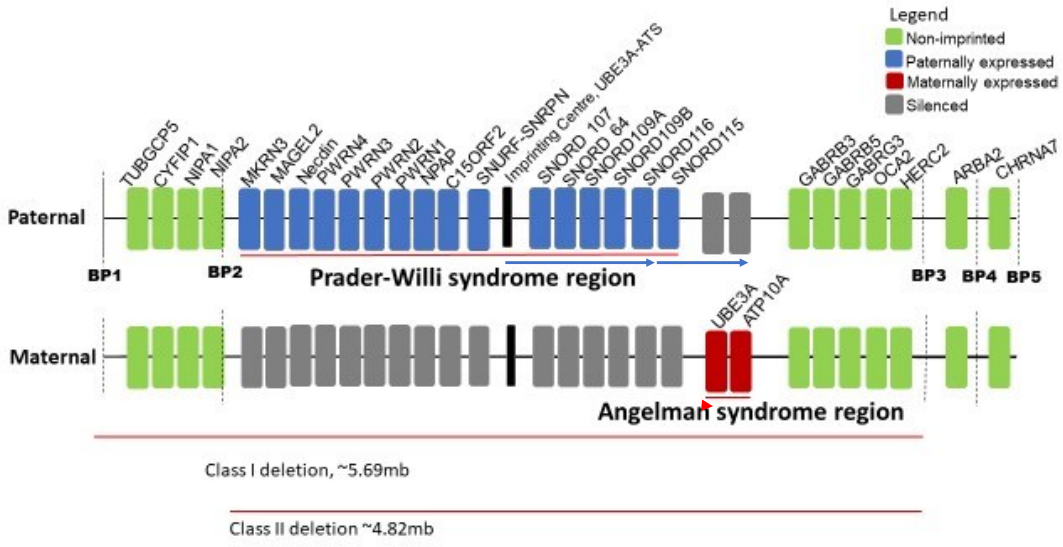


Figure 1. Paternally and maternally expressed imprinted genes the PSW-AS genomic region. Relevant genes and deletions in the 15q11-q13 chromosome region are shown on lines depicting the paternally and the maternally inherited copies of chromosome 15. The paternally expressed genes involved in PWS are marked in blue, while the maternally expressed genes involved in AS are marked in red. Genes marked in gray are silenced by an imprinting mechanism, while the genes in green are expressed from both parental copies. The blue arrow shows the region specific to a long, non-coding antisense transcript that contains a sequence complimentary to UBE3A (UBE3A-ATS). This transcript is transcribed only from the paternal allele and has been theorized to regulate the silencing of the paternal copy of UBE3A in neurons (8).

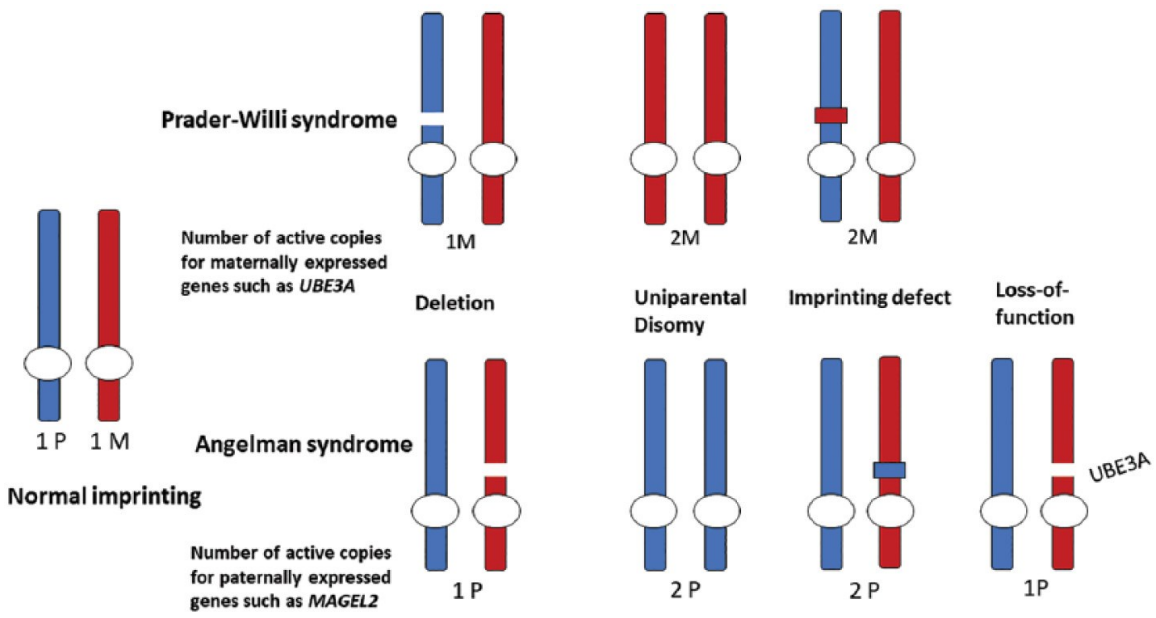


Figure 2. The different chromosomal genotypes and dosages of imprinted genes involved in AS and PWS. The different genotypes involved in PWS and AS are shown in comparison to typical development and one another. In typical development, paternally imprinted genes are expressed only from the maternally derived chromosome and vice versa. In PWS and AS, de novo germline mutations lead to a lack of expression for paternally or maternally expressed genes in the 15q11q-q13 chromosome region. However, the dosage of paternally and maternally expressed genes in the chromosome region varies depending on the genotype as shown above, with P standing for dosage of paternally expressed genes, and M for dosage of maternally expressed genes.

Table 2. Genotypical effects and frequencies of the chromosomal genotypes involved in AS and PWS. A comparison of the different mutations, their frequencies, effects on the imprinted and non-imprinted genes in the Prader-Willi and Angelman syndromes is shown. Note that while loss-of-function mutations for UBE3A show the full phenotype of Angelman syndrome, no single gene mutation has been shown to reproduce the full phenotype of Prader-Willi syndrome.

	Deletion	**Uniparental disomy**	**Imprinting defect**	**Loss-of-function mutations**
PWS	Frequency	65%–75% of affected individuals[7]	20%–30% of affected individuals [7]	1%–3% of affected individuals [7]
	Effect on imprinted genes	No expression of paternally expressed genes in the 15q11-q13 chromosome region[7]	No expression of paternally expressed genes in the 15q11-q13 chromosome region, predicted increases in dosage for maternally expressed genes [7]	No expression of paternally expressed genes in the 15q11-q13 chromosome region, predicted increases in dosage for maternally expressed genes [7]
	Effect on non-imprinted genes	One copy of the *GABRB3, GABRB5, GABRG3, OCA2* and *HERC2* genes, additional loss of *TUBGC5, CYFIP1, NIPA1* and *NIPA2* in Class I deletions [8]	None	None
AS	Frequency	~70% of affected individuals[8]	~2% of affected individuals [8]	~2%–3% of affected individuals [8]
	Effect on imprinted genes	No expression of *UBE3A* in neurons [9]	No expression of *UBE3A* in neurons [9]	No expression of *UBE3A* in neurons [9]
	Effect on non-imprinted genes	One copy of the *GABRB3, GABRB5, GABRG3, OCA2* and *HERC2* genes, additional loss of *TUBGC5, CYFIP1, NIPA1* and *NIPA2* in Class I deletions [8]	None	None

Both PWS and AS involve a number of imprinted genes, which are expressed in a manner dependent on their parental origin. According to the kinship theory, genomic imprinting may evolve from intragenomic conflict when genes of different kin benefit from different phenotypes of the same trait [10]. In particular, genes that may increase the inclusive fitness of the mother by exerting effects that lead to more equal distribution of resources among offspring are expected to be expressed only when maternally inherited. In contrast, maternally imprinted (paternally expressed) genes are expected to exert effects that lead to increased demands imposed on the mother, increasing the fitness of the child's paternal genes. Consequently, the phenotype of PWS has been

argued to reflect extreme, pathological development of phenotypes associated with fitness benefits to the mother [6]. Thus, as children with PWS fail to express paternally derived genes, the resulting phenotype displays low birth weight, sleepiness and low activity, poor sucking ability and a failure to thrive [7], followed by gradual development of a voracious appetite, which coincides with the timing of early adrenarche, around the age of 8–9 years [11,12]. As maternally and paternally derived genes tend to disagree over the allocation of maternal resources, it has been further argued that suckling presents a cost to the mother's inclusive fitness through nutritional value and conversely the transition to family and self-foraged foods after weaning presents comparably reduced maternal costs [6,13].

In contrast to PWS, subjects with AS fail to express maternally derived genes and show comparatively high birth weight [14] and have been argued to sleep less than their peers [15]. In the context of the kinship theory and parent–offspring conflict, the behavioral phenotype of AS has been argued to reflect an extreme pathological development of phenotypes related to affect signaling. Smiling and laughter are hypothesized as signals of positive affection, which have fitness benefits to the child, sending the signal, and fitness costs to the mother, receiving the signal and providing increased parental attention [16,17].

Based on the lines of evidence and theory described above, core phenotypes of PWS and AS have been considered to result from disruptions of genetic conflict, where the disappearance of one side of opposing developmental influences leads to an extreme response disadvantageous to both the mother's and the child's inclusive fitness. However, the opposite nature of the phenotypes of these two syndromes has never been evaluated in any detail.

In this article, we focus on reviewing two aspects of the behavioral phenotypes of PWS and AS, sleeping and eating behavior. First, we provide relevant background on the genetic and epigenetic causes of PWS and AS, focusing on the different dosages of paternally and maternally expressed genes in each syndrome. Second, we use currently available research to determine the degree to which the observed phenotypes of sleep and eating behavior in PWS and AS are opposite to one another and in what respects. Third, we review current research on mouse models of PWS and AS to assess the roles of imprinted genes in sleep and behavior in the two syndromes and to develop genetic

and physiological hypotheses to explain how the different genetic alterations in the two syndromes could produce the observed phenotypes. Finally, we evaluate whether the phenotypic patterns observed in PWS compared to AS, and our hypotheses, are consistent with the kinship theory of genomic imprinting.

3.3. Methods

Comprehensive searches of the literature were conducted for each aspect of the review, with both review and research articles utilized. The search was conducted with the Web of Science database using both term- and reference-based search strategies. Search terms included Prader-Willi and Angelman, coupled with relevant terms including gene names and general terms related to traits and characteristics including obesity, hyperphagia, sleep, sleep pressure, duration, latency, REM, apnea, daytime sleepiness, somnolence, circadian, melatonin, food-related behavior, food preferences, food refusal, eating, feeding, homeostatic feeding hedonic feeding, serotonin and dopamine.

Literature search for meta-analysis of sleep latency and duration in PWS

We searched both PubMed and Web of Science databases for peer-reviewed scientific articles up to May 2018. Search terms were “Sleep AND Prader-Willi,” which brought up 208 articles at PubMed and 371 articles at Web of Science, which were all screened manually. The search was further supplemented by manual searching strategies such as articles cited in the literature relevant to PWS. Studies were included in the meta-analysis based on the following criteria: 1. the study included a measurement of sleep onset latency or sleep duration on PWS individuals and a comparison to a control group of typically developing individuals; 2. sufficient data were available for estimating mean and standard deviation of the relevant sleep parameters. The application of these criteria yielded a total of eight studies for sleep onset latency as well as seven studies for sleep duration. The selection process is detailed in full with a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist provided in the Supplementary File provided in the publication.

Statistical procedures

As a few of the studies involved in our analyses only provided ranges and medians for the relevant sleep traits, the mean and the standard deviation of these parameters were estimated based on the procedures described in Hozo et al. [19]. The meta-analysis was conducted with the R software (version 3.5.0 “Joy in playing,” [20]), using the metafor package [21]. We chose a fixed-effect model for our approach and Hedge’s G was used as a measurement of the effect size. Heterogeneity among the studies was measured using the Q test, while publication bias was evaluated using a weighted regression test with multiplicative dispersion to test for funnel plot asymmetry between the estimate of effect size and within-study standard error.

3.4. Results

Genetic, genomic and epigenetic causes of PWS and AS

The 15q11-q13 chromosome region contains a number of both paternally and maternally imprinted genes as shown in Figure 1. AS has been shown to be caused by a lack of expression for the maternally derived copy of UBE3A [21,22]. More specifically, the paternal copy of UBE3A is uniquely silenced in neurons and so, deletions or epigenetic alterations affecting the maternal copy of the gene lead to a complete lack of UBE3A expression in neurons [23]. By contrast, no single mutation of a gene has been shown to display the full phenotype of PWS, although lack of expression for the SNORD116 snoRNA has been shown to produce several central aspects of the phenotype of PWS, indicating an important role in the development of the disorder [24].

As both PWS and AS are ultimately due to de novo mutations of the same chromosome region, it follows that deletions involve a loss of one parental copy of the 15q11-q13 region, while in uniparental disomy both chromosomes are derived from the same parent. The imprinting mechanisms involved in both PWS and AS are further controlled by the imprinting center of the 15q11-q13 chromosome region, which is defined by the shortest mutations known to produce the full phenotype of each syndrome. The paternal copy of the imprinting center is transcribed as a part of a long, non-coding antisense transcript, which spans across the imprinting center and the SNURF-SNRPN gene to the end of the opposite strand of UBE3A and is thought to

regulate the silencing of the paternal copy of UBE3A in neurons [25]. In contrast, the maternal copy of the imprinting center is typically methylated, preventing the expression of the antisense transcript [26]. Thus, imprinting defects result from either microdeletions which span the shortest region of overlap for each syndrome or varied epigenetic causes. These genotypes demonstrate either a fully paternal or a fully maternal methylation pattern despite the presence of both parental alleles, as is also shown in Figure 2.

The different mechanisms behind the genotypes of the two syndromes lead to the corresponding differences in dosage for the imprinted genes involved, as also shown in Figure 2 and Table 1. Maternal uniparental disomy (matUPD) in PWS involves two maternally expressed copies of UBE3A and ATP10A, and conversely the genotype of paternal uniparental disomy (patUPD) in AS involves two copies of the paternally expressed genes in the locus. Deletions involve additional losses of non-imprinted genes which may result in haploinsufficiency for a varying number of genes depending on the size of the deletion (see Table 1).

Sleep phenotypes in PWS and AS:

Sleep phenotypes and the regulation of circadian rhythms

Sleep phenotypes are notably altered in both AS and PWS and have been well studied in both. By way of background, the sleep–wake cycle is regulated by two major processes: first, homeostatic sleep pressure which is defined as the gradual accumulation of sleep factors (i.e. peptides, hormones and neurotransmitters) promoting sleep in the brain, which slowly inhibit the function of wake-promoting neural pathways [27]. Second, circadian rhythms can be viewed as a network between the internal time-keeping mechanisms based on the self-maintaining feedback loop of the core time-keeping genes Per, Cry, CLOCK and BMAL1 and their target genes, which in turn mediate circadian timing in physiological processes such as sleep, feeding and energy balance [28]. In vertebrates, circadian rhythms form a hierarchical network between the suprachiasmatic nucleus (SCN) and a number of peripheral circadian clocks in different tissues and cells [29]. The secretion of melatonin from the pineal gland is controlled by a projection from the SCN [30]. However, the circadian integration of sleep, activity and feeding further depends on a reciprocal connection between the SCN and the arcuate nucleus (ARC) [31]. Recent work has also revealed that there may be a higher than

expected number of imprinted genes expressed in the hypothalamus [32]. Given the crucial role of the hypothalamus in the regulation of sleep, it can be seen that imprinted genes may similarly influence the regulation of sleep via the hypothalamus [33]. As a portion of these imprinted genes are also central to the genotypes of the PWS and AS [7,22], the sleep phenotypes of AS and PWS may also be affected due to lack of expression.

The quality of sleep has been traditionally measured with subjective observations, but a more precise study of the sleep phenotype and structure can be conducted with a combination of electrophysiological recordings (i.e. polysomnography). While research on sleep relies on the simultaneous measurement of electrical brain activity (i.e. electroencephalography (EEG)) and physiological parameters such as heart rate and respiration, these are only used as biomarkers for the underlying neural and physiological states [34]. The aforementioned states of sleep can be broadly divided into rapid eye movement (REM) sleep, defined by a high degree of brain activity and its counterpart, and non-rapid eye movement (NREM) sleep, conversely defined by a lack of such activity. NREM sleep is maintained by the neural circuitry controlling homeostatic sleep pressure, while REM sleep is promoted by the subdorsolateral nucleus [27]. Together, the two opposing processes produce the typical cycle of NREM and REM sleep states [34].

A range of qualitative and quantitative sleep phenotypes are altered in both PWS and AS [6,15,35,36]. In this section, we describe research on sleep phenotypes that have been studied in both AS and PWS, to address whether or not they exhibit opposite or similar features, and how these features are associated with the genetic and epigenetic alterations that underlie the two syndromes. In particular, we will discuss six main sleep phenotypes in the context of both syndromes:

1. Sleep onset latency and difficulties with falling asleep: sleep onset latency can be defined as the quantification of time from sleep attempt to initiation, and difficulties with falling asleep refer to any occurrence where the subject experiences difficulties with falling asleep in the evening, regardless of the underlying cause.

2. Sleep duration, which refers to the total time spent asleep during a 24-h period.

3. Sleep efficiency, commonly defined as a part of an overnight sleep study, calculated as the time spent asleep compared to the time spent in bed during the measurement period.

4. Sleep architecture, referring to the durations and total percentages of the various sleep stages such as REM sleep and slow-wave sleep, commonly measured during an overnight sleep study with a polysomnographic recording.

5. Daytime sleepiness, referring to increased sleepiness during the day, quantified either with a subjective estimate as a part of a caretaker survey or with a study measuring the subject's proneness to falling asleep during the day (e.g. the multiple sleep latency test (MSLT)).

6. Associations of sleep traits with melatonin, a hormone involved in the regulation of the sleep–wake cycle. Namely, melatonin secretion from the pineal gland is controlled directly by the suprachiasmatic nucleus [30], so the secretion of melatonin may be altered in AS and PWS, influencing their sleep phenotypes.

The overall features of sleep phenotypes in AS and PWS

Extreme sleep disturbances, abnormal sleep–wake cycles and diminished need for sleep are typically described as features of the sleep phenotype in AS [15,35]. The abnormal sleep–wake cycle refers to the common observation that individuals with AS often have difficulties in initiating and maintaining sleep and sleep less than their age-matched peers as a result. The feature is especially prominent in childhood but continues to improve toward adulthood with a moderate prevalence [37,38]. While the lack of sleep is often described to "not affect the alertness or activity level of the subject or even their personal quality of life" [15], daytime sleepiness has also been reported in a number of studies [37,39,40]. However, a recent meta-analysis later inferred that daytime sleepiness was not significantly affected as compared to controls in AS [35]. Nevertheless, as the sleep phenotype of AS is highly disturbed, the genotype of the syndrome may also be involved with the regulation of sleep.

The sleep phenotype of PWS is characterized most broadly by excessive daytime sleepiness (EDS), apparent problems with organization of REM sleep patterns [41] and obstructive sleep apnea (OSA) [42]. While daytime sleepiness is often attributed

to OSA, daytime sleepiness in PWS is often described to be unrelated to the quantity and quality of nocturnal sleep [43].

As noted above, previous studies have shown that sleep phenotypes are prominently altered in both PWS and AS. However, no study to date has systematically compared these syndromes with regard to their sleep phenotypes.

Sleep onset latency and difficulties with falling asleep

Extreme variation of sleep onset emerges as a prominent phenotype in numerous studies concerning the sleep phenotypes of both AS and PWS. Several questionnaire studies concerning sleep and sleep-related behaviors have addressed difficulties with falling asleep and bedtime resistance (behavioral avoidance of bedtime), while a number of physiological overnight sleep studies (polysomnography) have further quantified the trait as sleep onset latency, defined as the time spent between settling down in bed and falling asleep, based on the detection of the first stage of sleep in the subject's electroencephalogram pattern.

The notion of prolonged sleep onset latency in AS is supported by a recent meta-analysis of sleep studies, which showed a moderate but significant effect size for difficulties with falling asleep across relevant questionnaire studies concerning AS subjects [35]. In closer detail, all three questionnaire studies reviewed for the meta-analysis state that about 50%–60% of AS subjects are reported to show frequent difficulties with falling asleep [37,38,40] indicating that the sleep phenotype of AS may involve increased sleep onset latency as compared to typically developing individuals. Similarly, a recent meta-analysis of relevant overnight sleep studies indicated that sleep onset latency is significantly increased in AS across the relevant studies [35]. In contrast, a study comparing sleep problems in groups of infants with different neurogenetic disorders found that infants with AS showed marginally shortened sleep onset latency, as compared to the control group of typically developing infants [44]. However, the authors mention that the AS group also showed atypically longer night wakings as compared to the control group and showed a high amount of variation for night waking frequency (a range of 0–10 awakenings in a night) which suggests that problems with maintaining and initiating sleep may emerge in early infancy in AS.

Numerous behavioral studies have characterized behaviors with further connections to bedtime and falling asleep. First, behaviors indicating high sensitivity to the sleep environment, characterized by regular complaints on feeling uncomfortable in bed, were estimated as significantly more common among AS subjects as compared to the control subjects [35]. Second, fear or anxiety regarding sleep has been shown to be higher in AS than in matched controls, by meta-analysis [35]. Third, regular use of both medical and non-medical sleep aids (such as light in bedroom or security objects) has also been estimated to be significantly higher among AS subjects compared to typically developing controls [35]. Finally, several studies note a particular tendency for bedtime resistance and insistence on particular bedtime routines among individuals with AS. A questionnaire study on sleep problems on individuals with AS notes that reluctance to go to bed was reported in a significantly higher proportion of AS subjects (approximately 60% of individuals under the age of 15 years) compared to typically developing controls [37]. Insistence on particular bedtime routines has similarly been reported in 63% of the subjects [38]. Furthermore, a small-scale trial study found that behavioral treatment for children with AS, including regular sleep schedules and adequate parent–child bedtime interactions, led to shortening of sleep onset latency and the children also showed significant improvements in bedtime behavior and were able to fall asleep independently, as opposed to baseline results prior to treatment [45]. A questionnaire study on parental stress and the sleep habits of children with AS found that the sleep time variability of the child was positively correlated with parental stress. The children with AS also showed a higher level of concerning sleep habits including bedtime resistance, sleep anxiety, night waking and difficulties in falling asleep, compared to a group of typically developing children [46]. In summary, the increased bedtime resistance, insistence of particular bedtime routines and wide use of both medical and non-medical sleeping aids suggest that the increased sleep onset latency of AS subjects may be caused by their greater restlessness around bedtime.

In PWS, numerous sleep studies show opposite results in comparison to AS, with questionnaire studies showing a lack of problems related to sleep onset, while overnight sleep studies offer mixed support for shortened sleep onset latency. In particular, an early questionnaire study on sleep and behavioral problems in PWS found that none of the PWS subjects were reported to suffer from regular sleep settling problems [47].

Similarly, a later questionnaire study on sleep and behavioral problems in PWS found that only 1 of the 79 subjects was reported to have a regular sleep settling problem [48]

As overnight sleep studies in PWS provide conflicting evidence on sleep onset latency, we conducted a meta-analysis of the relevant studies [41,44,48–54] to further quantify sleep onset latency in PWS, as compared to typically developing individuals.

We chose a fixed-effect model for our approach, as a relatively small number of studies fit with our inclusion criteria and fixed-effect models are known to perform better when the number of studies included is low [20]. Furthermore, the measured trait was defined in a similar fashion across the studies, and as all of the studies involve a comparison between PWS individuals and age-matched controls, we would expect the true effect of the condition on sleep onset latency to be rather similar across the studies.

Table 3. Meta-analysis of sleep onset latency in PWS, based on 10 studies as also shown in Figure 3. The effect size for each study was measured with Hedge's G and the test statistic was converted to a p-value with a left-tailed test.

Subjects (PWS)	Controls	Overall effect (M)	Error variance (VM)	Standard error (SEM)	Test statistic (Z)	p (left-tailed test)	Confidence Interval
125	172	-0.6226	0.0157	0.1251	-4.9746	0.00001	0.2453

Our findings with the meta-analysis of sleep onset latency in PWS were threefold. First, we found that sleep onset latency in PWS shows a small but significant negative effect in comparison to typically developing individuals, indicating evidence of reduced sleep onset latency in PWS. Second, our analysis indicates that there is significant heterogeneity across the studies. This finding mirrors the recent large-scale meta-analysis on sleep traits in AS [35] and likely reflect the variation across the relevant studies with other factors potentially influencing sleep traits, such as age, and medication and OSA. The scale of the effects shown in each study and the overall effect across the studies is also shown in Figure 3. Finally, we conducted an analysis of publication bias by fitting the relevant studies into a funnel plot and found no significant evidence of publication bias with respect to sleep onset latency.

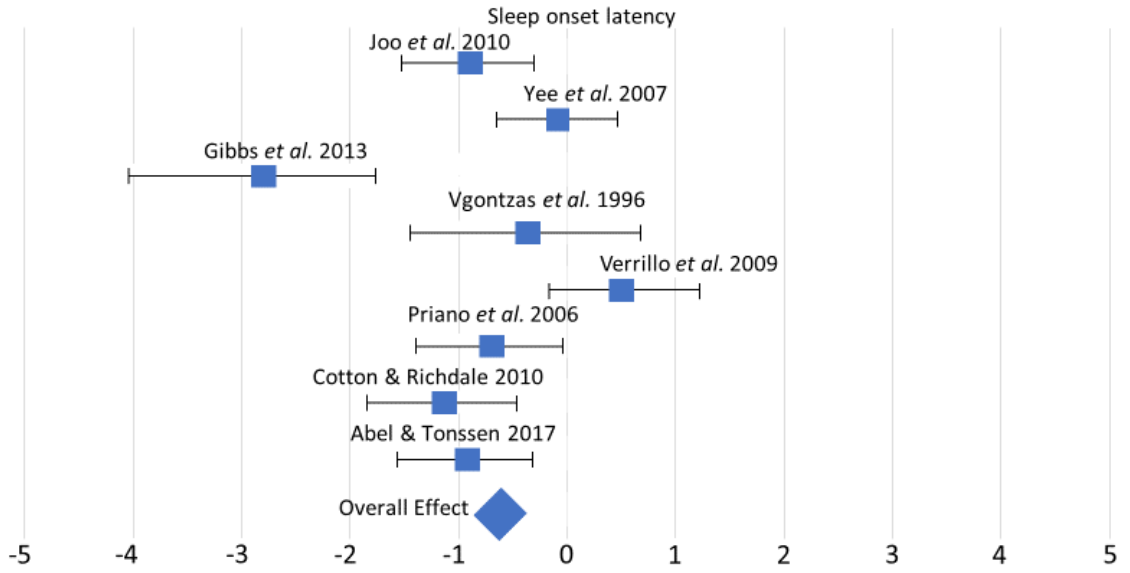


Figure 3. The effect of PWS on sleep onset latency, shown as deviation from typical development. The effect of PWS in relation to sleep onset latency. The effect size shown in each study is measured with Hedge's G, along with upper and lower limits of the effect sizes. The overall effect is the weighted average of the different effect sizes.

In summary, there is a diametric pattern to sleep onset latency in AS compared to PWS individuals. Both questionnaire studies and overnight sleep studies offer support for the notion of increased sleep onset latency in AS. In comparison, the opposite pattern is shown in PWS, as both questionnaire studies and overnight sleep studies support the notion of reduced sleep onset latency in relation to typically developing individuals.

Sleep duration in AS and PWS

Extreme phenotypes of sleep duration have been characterized in both AS and PWS, and, in direct comparison, infants with AS showed a significantly shortened sleep duration as compared to typically developing controls, while the PWS group showed a longer sleep duration, though only approaching significance compared to controls [38,40].

Questionnaire studies on sleep problems in AS have featured questions on whether the caretaker felt that the child slept less than children of their age, and this item has been endorsed consistently across studies with a prevalence of about 40%–50%

among AS patients [38,40]. Furthermore, a questionnaire study on sleep problems in AS found that a significantly greater percentage of the AS subjects compared to the control group of typically developing children was reported to regularly sleep less than 8 h in a day [37]. Similarly, a recent meta-analysis of currently available overnight sleep studies shows a significantly shortened sleep duration (of about 7 h) in AS individuals, as compared to controls [35].

Sleep duration in PWS has been primarily with overnight sleep studies, while questionnaires have focused mainly on sleep problems [47,48]. A recent sleep study by Ghergan et al. [55] found that approximately 58% of adult PWS subjects were reported to sleep more than 9 h in day. While 8% of the participants were found to sleep over 11 h in a 24-h sleep recording session, the mean sleep duration of PWS subjects was below 8 h. As earlier overnight sleep studies have provided similarly conflicting results on sleep duration, we conducted a meta-analysis of all previous overnight sleep studies which featured a comparison between PWS subjects and a typically developing control group.

A total of seven studies fulfilled our criteria [44,49–54]. First, our analysis with a fixed-effect model indicated that sleep duration in PWS subjects did not show a significant effect in either direction as compared to typically developing individuals. Second, our analysis indicated significant heterogeneity across the studies. Finally, the funnel plot analysis did not indicate a significant publication bias with respect to sleep duration.

In summary, relevant studies have shown support for significantly shortened sleep duration in AS, but an opposite pattern of increased sleep duration cannot be seen in studies with PWS. Our analysis of currently available studies indicates that despite relatively high self-reported levels of sleepiness and long sleep durations in PWS, sleep duration in PWS as a whole does not differ significantly from typically developing subjects.

Sleep efficiency in AS and PWS

Sleep efficiency reflects the time spent awake during the night, which is affected by both sleep latency and wakefulness after sleep onset and can be induced by factors such as apnea or restlessness. In AS, four overnight sleep studies have included measurement of sleep efficiency and were later compiled into a meta-analysis, which

showed that sleep efficiency is significantly reduced in AS compared to control subjects [35]. A particular overnight sleep study also notes that AS subjects showed higher wakefulness after sleep onset compared to patients with varied intellectual disabilities [56].

In PWS, sleep efficiency has been especially studied in connection to apnea. OSA has been assessed as a co-occurring condition with PWS, with a meta-analysis reporting an 80% prevalence among 224 patients from a total of 14 studies [42]. Apnea in PWS has traditionally been associated with obesity and, in support of this notion, the meta-analysis study also reported that greater body mass index (BMI) was positively correlated with OSA. However, a later collaborative study of a large cohort of PWS patients found no correlation between obstructive apnea; high BMI and facial dysmorphic features or hypotonia were instead suggested to play a role in the occurrence of OSA [57].

While OSA could be expected to affect the sleep efficiency significantly, results from other overnight sleep studies in PWS are somewhat inconclusive. Two overnight sleep studies have reported a reduced sleep efficiency as compared to normative values [52,58], while three other studies found no significant difference in sleep efficiency between the PWS and control groups or normative values [51,53,59].

In summary, sleep efficiency is reduced in both PWS and AS; however, current studies suggest that the underlying reasons are different. In AS, longer sleep latency and wakefulness after sleep onset are likely to affect sleep efficiency negatively, whereas the high prevalence of obstructive apnea is likely to affect sleep efficiency in PWS. While obesity has been shown to correlate positively with apnea, other factors may also affect sleep efficiency in PWS.

Variation in sleep architecture

Several polysomnographic studies have noted differences in sleep structure in AS and PWS patients. First, a significantly reduced overall amount of REM sleep as compared to control subjects has been found in AS [56,60]. Similarly, a meta-analysis of overnight sleep studies in PWS found that 55% of PWS patients analyzed in a total of 20 sleep studies showed a reduced percentage (REM% < 20) of REM sleep [36]. While two

later sleep studies found that overall REM sleep percentage did not differ significantly from normative values [58,59], an overnight sleep study comparing PWS subjects and typically developing individuals found that PWS subjects had a significantly reduced overall REM sleep percentage as compared to controls [52].

Second, an opposite pattern between PWS and AS is suggested with regard to the timing of onset for REM sleep. Typically, the onset of REM sleep first occurs approximately 90 min after the onset of sleep [34]. However, AS patients show a considerably increased REM latency, though a comparison with a control group only approached statistical significance [56,60]. Conversely, in PWS REM sleep is often present during sleep onset. A meta-analysis of sleep studies in PWS found that 27% of PWS subjects showed the presence of REM during sleep onset at night, while a further 34% showed an onset of REM during a daytime nap. Similarly, a shortened REM latency (>70 min) was reported in 17% of PWS subjects [36]. Intrusions of REM sleep during daytime naps have similarly been found in later studies concerning PWS [55,59]. Fragmentation of sleep architecture is also evident in PWS, as multiple sleep studies have reported either a significant increase in sleep stage shifts [52] or an increased amount of REM sleep periods [41,53].

In summary, reduced overall amount of REM sleep has been shown in both AS and PWS, while an opposite pattern is suggested in REM latency, with reduced REM latency in PWS and increased REM latency in AS. As the hypothalamus is known to regulate sleep through both neural mechanisms [34] and circadian rhythmicity, [30] the opposite dysregulations of REM onset could also be indicative of disrupted regulation of sleep onset in PWS and AS.

Daytime sleepiness

EDS is a common concern with PWS and has been studied in both questionnaire and overnight sleep studies, while several questionnaire studies have also addressed the condition in AS. First, daytime sleepiness among AS subjects has been reported in several questionnaire studies. A recent meta-analysis of these studies showed a small but significant effect size, indicating a higher prevalence of daytime sleepiness in AS compared to controls [35]. Second, two questionnaire studies have addressed daytime sleepiness in connection to behavioral problems in PWS. A questionnaire study on sleep

and behavioral problems noted that daytime sleepiness was reported in PWS subjects with significantly greater prevalence (about 35%) compared to a control group, and that daytime sleepiness was correlated positively with behavioral problems [47]. A later questionnaire study similarly found that daytime sleepiness is shown with about 35% of PWS subjects, but no significant correlations between sleep problems and behavioral problems were found [48]. A recent large-scale sleep study found that about 50% of PWS subjects showed a questionnaire result indicative of daytime sleepiness, while about 65% of the subjects reported sleepiness as a problem [55].

Several sleep studies have also quantified daytime sleepiness in PWS with MSLTs [36]. Commonly used as a quantitative measure of EDS and narcolepsy, MSLT measures the onset of sleep during multiple 20-min daytime nap periods. A mean sleep onset time lower than 5 min is usually considered to indicate that the condition is affecting the patient's daytime activities. A meta-analysis on sleep studies in PWS lists a total population of 72 patients across eight different studies, with 40% of the subjects showing an MSLT result indicative of being severely affected [36]. Daytime sleepiness in PWS is commonly attributed to a deficiency of sleep due to OSA, which is also common concern in PWS [42]. However, while apnea has been suggested as a co-occurring condition in PWS and apnea has also been shown to correlate positively with the BMI among PWS subjects, support for the causation between EDS and OSA in PWS is not entirely conclusive. Three studies have indeed reported a positive correlation between apnea or BMI and a measure of sleepiness with PWS subjects [42], but a number of conflicting studies found no significant correlations between BMI or reports of apnea and sleepiness in PWS [47,55,59].

In summary, daytime sleepiness shows a significantly increased prevalence as compared to controls in both PWS and AS. In PWS, the trait may be partially attributable to OSA, but the underlying causes are likely to be multifactorial. In AS, the condition is likely to be connected to reduced sleep duration and frequent waking at night.

Associations with melatonin secretion

Circadian rhythms regulate sleep patterns, and the secretion of melatonin is directly regulated by the circadian period in the SCN [30]. As a disruption of sleep patterns is evident in both AS and PWS, an imbalance in the secretion of melatonin may

be affecting the sleep phenotypes of the two syndromes. Melatonin and its associations to sleep phenotype have been studied in AS using sleep studies and treatment trials. A study on circadian rhythms and sleep traits in AS reported that circadian sleep disorders were diagnosed with a prevalence of about 50% among AS subjects, and they found that nighttime serum levels of melatonin were significantly reduced in AS subjects as compared to a control group [61]. A later overnight sleep study similarly found that AS subjects showed a significant delay in the pattern of nighttime melatonin secretion as compared to the control group [62]. Furthermore, treatment trials among AS subjects have shown that treatment with melatonin improves sleep duration and reduces sleep latency [62,63].

While melatonin and its connection to sleep traits have not been studied as extensively in PWS as in AS, a study on endocrinal traits found that melatonin levels of PWS subjects did not differ significantly from typical controls [64]. A later study on morning melatonin levels in PWS subjects similarly reported that serum melatonin levels did not differ from the control group [65].

In conclusion, current evidence supports a reduction in nighttime melatonin secretion in AS. However, an opposite pattern is not seen in PWS, as melatonin secretion has not been shown to differ significantly from controls in currently available studies.

Effect of genotype and gene dosage on sleep phenotypes in AS and PWS

Given the different dosages of paternally and maternally expressed genes between the different genetic subtypes of PWS and AS (as shown in Figure 2), variation in sleep phenotypes between the genetic subtypes could be expected in both syndromes. However, several studies of sleep phenotypes in both AS and PWS have independently reported that no significant associations between genetic subtype and specific sleep phenotypes were found in their results [37,48,52,55,58,59,66]. The only exception is that the paternal UPD genotype of AS has been associated with an increased tendency toward expressive sleeping disturbances, such as nightmares [38] though a later questionnaire study was unable to replicate this result [40]. The lack of variation in sleep phenotypes within each syndrome may indicate that the mechanisms for the alterations of sleep phenotypes apparent in each syndrome are not dependent on

gene dosage effects, but rather the lack of expression for the imprinted genes in both syndromes.

Genetic bases of sleep phenotypes and circadian rhythms in PWS and AS

Recent mouse model studies have highlighted the roles of imprinted genes in the regulation of circadian rhythms and sleep [33]. Several mouse models of both PWS and AS have shown that imprinted genes take part in the regulation of circadian rhythms. Circadian clock mechanisms regulate the daily cycle of activity and rest through a transcriptional feedback loop of the core clock genes, which in turn coordinate the daily oscillation of thousands of diurnally regulated genes [67]. Diurnally regulated genes also show periodic changes in methylation, which further corresponds to rhythmic changes in expression patterns of these genes [68,69]. The transcriptional feedback loop of the core circadian clock is composed of the activators Clock and BMAL1 (ARNTL in human), which act as transcription factors for both other clock genes and diurnally regulated genes, and the repressors, Period (Per1, Per2 and Per3) and Cryptochrome (Cry1, Cry2), which regulate the activators, eventually suppressing their own transcription. The expression of the core clock genes follows a roughly 24-h circadian rhythm, which, by convention, is indicated as circadian time (CT), where CT 0 stands for the beginning of the subjective day and CT 12 stands for the beginning of the subjective night [28]. The rhythmic expression of the core clock genes shows a 3- to 9-h delay between the master pacemaker in the SCN and peripheral tissues [29]. In the mouse SCN, the relative level of gene expression for Bmal1 starts to rise after the subjective midday (CT 8) and peaks during the subjective evening (CT 12). The CLOCK and BMAL1 proteins accumulate in the nucleus and in turn activate the transcription of the Per and Cry genes around the midpoint of subjective night (CT 18), further reaching the relative peak of their expression around subjective midday. The PER and CRY proteins accumulate over time and interact with CLOCK and BMAL1, effectively repressing their own transcription [28]. The relative protein levels of both the repressors and the activators are further regulated by ubiquitination mechanisms, and genetic alterations to these mechanisms have been characterized to alter the length of the circadian period and phenotypes of sleep [28]. The circadian rhythm of gene expression has been characterized in humans via the blood transcriptome, where the expression of the core clock genes follows a different

phase, with transcription of Per genes reaching its relative peak during the subjective night, whereas the expression of Arntl (Bmal1) peaks during the day [70].

Sleep and regulation of circadian rhythms in mouse models of AS

Several studies have highlighted the role of UBE3A in the regulation of sleep and circadian rhythms, suggesting that epigenetic mechanisms involving this gene could provide specific cues to the central circadian clock in the SCN. Circadian rhythmicity has been studied in the mouse models of AS with somewhat conflicting results, due to the nature of the Ube3a imprinting mechanism in the SCN. A mouse model study by Ehlen et al. [71] found that Ube3a imprinting is uniquely relaxed in the SCN, allowing the AS mouse model to largely maintain its circadian rhythmicity. The model mice showed no significant differences in their ability to maintain the circadian rhythms in constant darkness or aberrant lighting in comparison to controls. However, the model mice would skip a rest period typical to the wild-type mice, and they showed a blunted response to sleep deprivation, with a significant decrease in the overall amount of REM sleep during a 24-h period. The authors concluded that Ube3a regulates the accumulation of sleep pressure, the homeostatic mechanism in the regulation of sleep.

Contrary to these results, another mice model study by Shi et al. [72] found that maternal deletion of Ube3a lengthens the circadian period. In similarity to Ehlen et al. [71], circadian rhythmicity was measured with running wheel activity in varied lighting and the AS model mice were found to show a significantly longer circadian period in constant darkness compared to controls, while the lengthened circadian period was even more pronounced in the mouse model with a larger deletion spanning from Ube3a to Gabrb3, which is similar to the Class 1 deletion of AS. Since Bmal1 has been identified as a target protein of the UBE3A ubiquitin ligase [73], the authors further suggested that Ube3a may regulate the turnover of Bmal1, and so the deficiency for expression of Ube3a would in turn lead to an excess of activators in the nucleus as opposed to the repressors. This imbalance would further lengthen the 24-h circadian period. As the secretion of melatonin is controlled by the input of the SCN [30] lengthening of the circadian rhythm would offer a direct explanation for the reduced melatonin levels and increased sleep onset latency in AS.

Molecular studies of the expression of clock genes in AS model mice have similarly produced contrasting results. Ehlen et al. [71] initially reported that the model mice showed no significant differences in the expression levels of the clock genes or the protein levels of Per2 in SCN tissues, as compared to controls. Similarly, Jones et al. [74] ascertained the expression of paternally derived Ube3a in the SCN by comparing protein levels of Ube3a in mice with maternal deletions, deletions of both copies and controls. As opposed to mice with deletions of both copies, Ube3a was detected uniquely in the SCN tissues of the AS model mice, implying that the imprinting of the paternal copy must be relaxed in the SCN. In contrast, Shi et al. [72] ascertained the expression of the clock genes with a Per2:LUC reporter gene and compared the rhythmicity of Per2 expression between SCN slices and spleen and lung tissues. The lack of expression for Ube3a was found to significantly increase the length of the luminescence period in SCN tissues but not in the lung or spleen tissues, indicating that lack of expression for Ube3a may alter feedback loops of core clock genes. Furthermore, treatment with topotecan, a topoisomerase inhibitor, which may inhibit the gene silencing mechanisms, significantly shortened the luminescence period of Per2 in SCN slices of the model mice but not in the controls or other tissues, indicating that Ube3a expression from the paternal copy may have shortened the circadian period in the treated SCN slices, while the treatment leaves the circadian period unaltered in other tissues, where Ube3a is expressed from both parental copies [72].

Current mouse model studies may thus indicate that circadian rhythmicity is mosaic in AS: imprinting of the paternal copy is relaxed in the peripheral tissues and the SCN, while other brain regions and neurons experience a lengthened circadian period. While the secretion of melatonin depends on an indirect projection from the SCN [30], the circadian integration of activity and rest further depends on a reciprocal connection between the SCN and the ARC [31]. Reduced levels of melatonin secretion and a relatively high incidence of circadian sleep disorders have been shown in studies concerning AS [61,62]. However, it remains unclear if the mosaic nature of circadian rhythmicity can fully explain the alterations to the sleep phenotypes of AS.

Sleep in mouse models of PWS

Recent mouse model studies have also linked the genotype of PWS to circadian rhythmicity. To date, two of the maternally imprinted genes affected in the syndrome,

MAGEL2 and SNORD116, have been shown to be involved in the regulation of circadian rhythms [75,76]. A study of a PWS mouse model found that Magel2 shows a pattern of circadian expression in the SCN and that loss of Magel2 expression confers a phenotype of fragmented activity and rest [75]. The length of the circadian period in constant darkness did not differ significantly from controls. However, the model mice showed significantly less nighttime activity compared to controls and a fragmentation for periods of activity and rest, evident as a significantly greater number of bouts of activity and a significantly shortened average bout duration. The expression of Magel2 in the SCN was shown to peak late in the day, with a marked decrease in levels of expression during the subjective night. The fragmentation of activity and rest and the circadian pattern of gene expression in the SCN thus indicate that Magel2 may be regulating the circadian clock mechanism in the SCN.

Further molecular studies have shown that the expression of Magel2 may further regulate the transcriptional feedback loop of the circadian clock mechanism [77]. In a cell-based model, the co-expression of Magel2 with Clock and Bmal1 was shown to repress the transcription of Per2, as compared to the level of expression with only Clock and Bmal1 present. The effect was intermediate in strength, compared to the more repressive Cry which is known to regulate the negative feedback loop of the circadian clock mechanism. Several possible mechanisms for the repressing effect of Magel2 were investigated, and Magel2 was shown to promote the cytoplasmic accumulation of Clock. While Clock is expressed continuously in the SCN, the subcellular localization of the Clock protein shows circadian variation. Clock is primarily cytoplasmic by itself and starts to accumulate in the nucleus only as the relative levels of gene expression of Bmal1 start to rise. Therefore, Magel2 may further regulate a programmed delay in the circadian feedback loop period through post-translational modification of the Clock protein [77]. As the expression of diurnally regulated genes is regulated by the nuclear accumulation of both Clock and Bmal1 [28], the lack of a programmed delay in the circadian period due to lack of expression for Magel2 could offer a direct mechanism for the dysregulation of sleep and activity evident in PWS.

Loss of expression for Snord116 has also been shown to alter the expression of diurnally regulated genes. Powell et al. [78] identified dysregulation of about 6000 diurnally regulated genes in the mouse cortex, including dysregulation of the pacemaker genes such as Cry1, Clock, Per2 and Mtor as well as increased expression of Ube3a,

which regulates the oscillatory pattern of Bmal1 via ubiquitination [73]. Coulson et al. [68] further showed that loss of expression for Snord116 led to a pattern of shifted diurnal methylation characterized by losses during the light phase and increased diurnal methylation during the dark phase. The authors suggest that the gene expression of epigenetic and circadian regulators is increased in the model mice during the light phase, which may lead to prolonged accumulation of these proteins into the dark phase, resulting in the shifted methylation pattern. As circadian rhythms regulate the daily cycle of activity and rest by further regulating the gene expression of thousands of diurnally regulated genes, the dysregulation of these gene expression patterns through a shift in the diurnal methylation cycle due to lack of expression for SNORD116 could explain the dysregulation of activity and rest which is evident in PWS.

In conclusion, recent mouse model studies of sleep and circadian rhythmicity show that both MAGEL2 and SNORD116 are involved in the regulation of circadian rhythms and diurnally regulated gene expression. However, unlike UBE3A, none of the genes have been shown to alter the length of the circadian rhythm directly. Instead, MAGEL2 may alter the regulation of diurnal gene expression by regulating the circadian feedback loop in the SCN [75,77] while SNORD116 may alter the expression of genes critical to the oscillatory pattern of the circadian rhythm, further affecting the expression of diurnally regulated genes [78,79].

Model for explaining opposite sleep phenotypes in PWS and AS

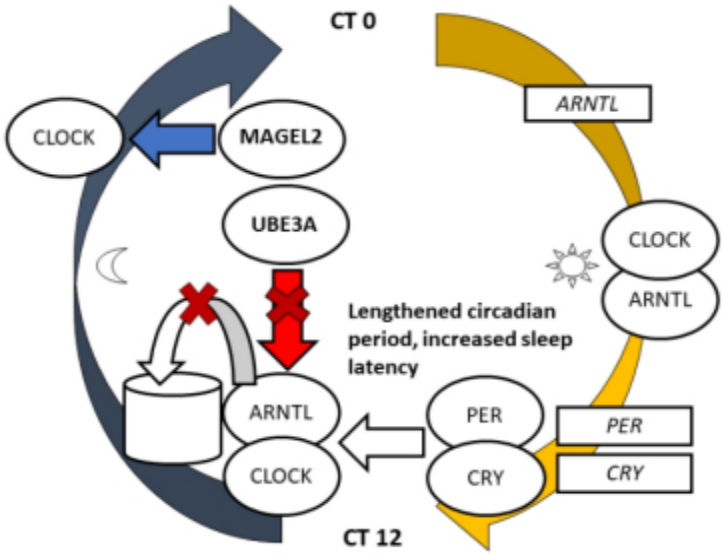
Since both AS and PWS show evidence of opposite phenotypes for sleep onset latency, and relevant mouse model studies indicate that both the maternally expressed *UBE3A* and the paternally expressed *MAGEL2* and *SNORD116* may be involved in the regulation of circadian rhythmicity in the SCN, we propose a hypothetical model for explaining the opposite sleep phenotypes based on the known interactions of these genes with the circadian clock mechanisms. First, as a baseline, assuming that the gene expression pattern of the core clock genes in peripheral tissues is delayed by approximately 6 h relative to the circadian rhythm of the SCN [28], we estimate that the transcription of *ARNTL* would reach its peak in the subjective morning, leading to nuclear accumulation of CLOCK and ARNTL proteins during the day and transcription of the *Per* and *Cry* genes reaching its peak early in the evening [70]. As the PER and CRY

proteins heterodimerize and accumulate to the nucleus, the expression of *PER* and *CRY* is suppressed completely by the midpoint of the subjective night.

Second, the maternally expressed *UBE3A*, as well as the paternally expressed *MAGEL2* and *SNORD116* genes, has been shown to interact with the clock genes in the SCN, which may alter the length of the circadian period and the rhythmic expression of diurnally regulated genes. The maternally expressed *UBE3A* regulates the turnover of BMAL1 (ARNTL) via ubiquitination. As the duration of the circadian period is determined by rhythmic variation of abundance of the core clock proteins, lack of expression for *UBE3A* and the reduced turnover of BMAL1 may lengthen the circadian period [72]. Our model shows that the imbalance in the protein levels of BMAL1 (ARNTL) may also alter the timing of sleep onset at the subjective evening in AS. In contrast, the paternally expressed *MAGEL2* may promote the cytoplasmic accumulation of CLOCK or regulate the expression of *PER* in the SCN through other molecular interactions, while *SNORD116* has been shown to affect the expression of *UBE3A* and several circadian pacemaker genes [78]. An overexpression of *UBE3A* would be expected to accelerate the oscillatory pattern of BMAL1, while *MAGEL2* may mediate a programmed delay in the feedback loop of the circadian rhythm [75,77]. Thus, a lack of expression for *MAGEL2* and *SNORD116* may lead to a shortened circadian rhythm and dysregulation of diurnally regulated gene expression as well as a further dysregulation of sleep and activity [68,75,78]. As also shown in Figure 4, these interactions produce opposite alterations to the circadian period in both syndromes due to the variable dosages of paternally and maternally expressed genes. In AS, both the expression of *MAGEL2* and lack of expression for *UBE3A* may therefore contribute to a lengthened circadian period, while in PWS both lack of expression for *MAGEL2* and dysregulation in the expression of *UBE3A* due to loss of expression for *SNORD116* are expected to contribute toward a shortened circadian rhythm and dysregulation of diurnally regulated gene expression.

Angelman syndrome

Lengthened circadian period



Prader-Willi syndrome

Loss of programmed delay in diurnally regulated gene expression

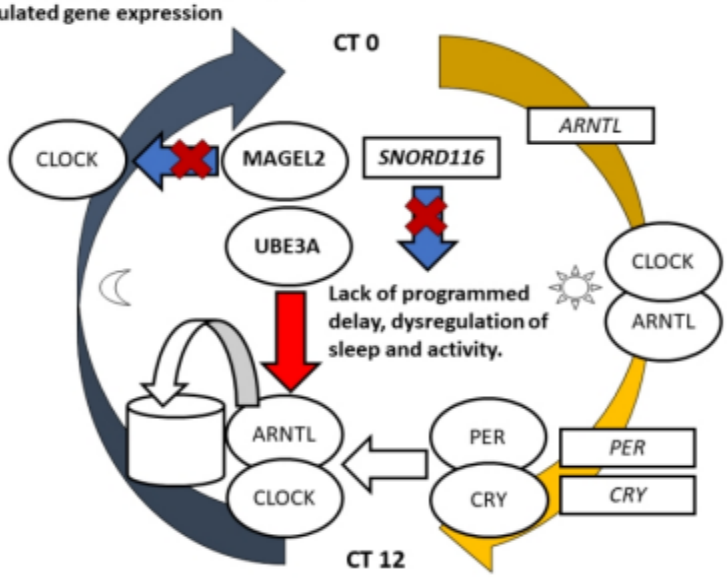


Figure 4. Theoretical gene regulation model proposing partly opposite interactions for AS and PWS between the imprinted genes involved and the non-imprinted genes regulating the circadian rhythm. The gene expression cascade regulating the circadian clock mechanism and its proposed interactions with three imprinted genes are shown for both PWS and AS. The Clock and Arntl (Bmal1) proteins, (marked as round shapes) accumulate in the nucleus after mid-day which activates the transcription of the Per and Cry genes (marked as rectangles) early in the evening. Per and Cry proteins accumulate in the nucleus by nighttime and repress the transcription of Clock and Arntl, simultaneously preventing their own transcription. Paternally and maternally expressed imprinted genes may also affect the genetic regulation of circadian rhythms in partly opposite ways: UBE3A regulates the turnover of BMAL1 via ubiquitination (shown as a simplified diagram of ubiquitination and protein recycling) while MAGEL2 has been hypothesized to mediate the cytoplasmic accumulation of Clock prior to nuclear accumulation of both Clock and Arntl (see above review section for detailed discussion and references).

These opposite alterations of the circadian rhythm may further explain opposite alterations to the timing of sleep onset in both syndromes, as is also shown in Figure 4. AS involves a phenotype of increased sleep latency along with reduced levels of melatonin secretion and increased bedtime resistance. As secretion of melatonin from the pineal gland is directly dependant of input from the SCN, a misaligned rhythm could explain the reduced levels of melatonin secretion in AS. However, the imprinting of Ube3a may be uniquely relaxed in the SCN and so it has been further argued that the increased sleep onset latency in AS may alternatively be due to reduced accumulation of sleep pressure [71]. In contrast to AS, our proposed model suggests that PWS may involve a dysregulation of diurnal gene expression and a relatively shorter subjective day due to the lack of programmed delay in the circadian period, which may further regulate neural and physiological regulation of sleep and wakefulness, which would similarly help explain the reduced sleep onset latency and EDS in the phenotype of PWS.

The evolutionary significance of regulatory mechanisms for sleep and wakefulness can be further understood in the context of human life histories by considering bedtime interactions, which involve numerous soothing routines and can be viewed to represent an important time for maternal bonding. The timing of sleep onset and difficulties with falling asleep may reflect the importance these interactions. Thus, maternal bonding and the regulation of sleep and wakefulness may be subject to an evolutionary tug-of-war between paternally and maternally imprinted genes. Paternally expressed genes may have been selected to favor an innate tendency for increased sleep onset latency and more frequent waking to solicit more maternal resources, while the opposite may be true for maternally expressed genes. Evidently, both AS and PWS

involve extreme phenotypes in the regulation of sleep and wakefulness as well as opposite imbalances in dosages of paternally and maternally imprinted genes.

Eating phenotypes in PWS and AS

Feeding behavior and the development of hyperphagia

For our purposes, feeding behavior can be understood in two overlapping contexts: (1) the evolutionary bases of feeding behavior and life history in human childhood and (2) neural and endocrine mechanisms for the regulation of appetite. Human life histories feature two major transitions of feeding behavior: first, weaning from maternally provided breast milk involves the gradual introduction of complementary foods approximately from the age of 6 months and onwards; the second major transition involves a further nutritional shift from specially prepared complementary foods toward more diverse family foods, coinciding with the development of adequate dentition between ages of 6 and 8 years [13,80]. The age period of complementary feeding coincides with a phase characterized by consistent refusal of new foods (food neophobia) [81], and the tendency for refusal of new foods has also been shown to be highly heritable among humans [82]. Modern practices of complementary feeding can be interpreted to involve specially prepared “baby foods” such as porridge, purees and other foods with constant, soft and smooth textures. Ethnographical records of existing hunter–gatherer societies also indicate that ancestral complementary foods may have consisted mainly of a diverse selection of premasticated foods [83].

The second, gradual transition toward an adult diet can also be viewed to involve a reduced burden of maternal investment. In ancestral human societies, children would begin to contribute to their own nutrition at the ages of about 5–7 years by collecting edibles such as fruit or berries (foraging), which coincides with the transition toward more diverse family foods in modern societies [84]. The relatively early weaning, as compared to ancestors and other great apes, typical of human childhood, and the introduction of complementary foods can be further interpreted as unique evolutionary adaptations for shorter interbirth intervals in humans [13].

The regulation of feeding behaviors in the context of neural and genetic mechanisms, which regulate food intake, is largely based on mouse model studies. The

neurocircuits that regulate feeding behavior are thought to be disrupted in both hyperphagia and hypophagia. The neural circuits that regulate feeding can be divided into homeostatic feeding mechanisms, which maintain the energy balance of the body, and hedonic feeding mechanisms, which are driven by neural signals of reward [85]. The regulation of homeostatic feeding is maintained by peripheral short-term signals of satiety and hunger, as well as long-term signals of energy balance, which are produced in the body and processed by the hypothalamus. Short-term signals of satiety and hunger are produced in the gut and include ghrelin, which stimulates hunger, and cholecystokinin (CCK), glucagon-like peptide-1 and peptide YY, which signal satiety [86]. Long-term signals of energy balance, such as insulin and leptin, are produced in proportion to the levels of adipose tissue in the body and enter the brain through blood circulation [87]. The peripheral signals of satiety, hunger and energy balance converge in the hypothalamus, which regulates food intake and energy expenditure through two opposite neural mechanisms in the ARC. Neuropeptide Y (NPY)-expressing neurons and agouti-related peptide (AgRP)-expressing neurons thus promote food intake, while neurons expressing peptides derived from pro-opiomelanocortin (Pomc) limit food intake[86,87].

The regulation of hedonic eating is based on the rewarding aspect of feeding, as both the consumption and sensory representations of food induce responses in the neural reward circuitry. The neural reward circuitry involves reciprocal connections between monoaminergic, intermediate and ventricular nuclei. Monoaminergic systems are driven by neurotransmitters including serotonin and dopamine, which mediate the motivation for rewarding behaviors such as feeding or mating. In the regulation of feeding, monoaminergic nuclei further project to intermediate nuclei in the lateral hypothalamic area and other brain regions similarly connected to the ARC, which in turn governs feeding via the hypothalamus. The reciprocal connections of the reward circuitry have been shown to promote feeding and to play a particular role in the development of food preferences and increased consumption of palatable foods [85]. Genetic mouse models of PWS and AS show opposite alterations in both dopamine and serotonin levels in the brain [88,89]. Furthermore, dysfunction of the hypothalamus, which regulates both sleeping and feeding, is central to several physiological phenotypes in PWS [90]. Thus, it can be hypothesized that the specific eating behaviors related to these syndromes may involve opposite dysfunctions in the regulation of hedonic feeding mechanisms.

Eating behavior phenotypes in AS and PWS

In this section, we review the evidence from empirical human studies and genetic mouse models on the phenotypes of eating behaviors of AS and PWS, and evaluate if certain traits could be defined as opposites of each other between the two syndromes. In particular, we will focus on the following traits:

1. *Hyperphagia*, that is, significantly increased consumption of food as compared to healthy individuals, regardless of the underlying etiology or associated behaviors.

2. A comparison of *selective* and *unselective* eating, and related behaviors such as food refusal or marked interests for certain types of foods.

3. *Food-seeking behavior*, that is, independent behaviors driven by the condition of hyperphagia including stealing, storing or taking food without approval.

A comparison of hyperphagia in PWS and AS

PWS involves a gradually developing condition of hyperphagia, manifested by low birth weight and an early restriction of growth, followed by rapid weight gain after weaning, and the development of hyperphagic behavior and obesity, consistent across all genotypes [11,12,91]. The rapid weight gain after weaning has been traditionally associated with overeating. However, Miller et al. [11] showed that the changes in weight gain precede the changes in appetite, implying that the development of hyperphagic behavior is preceded by metabolic changes. The authors reviewed complete growth and nutritional records of 58 PWS subjects involved in a longitudinal study to characterize the development of hyperphagia in PWS. The condition was found to follow a gradual progression through several nutritional phases, distinguishable by significant changes in weight gain and dietary intake, as compared to each of the previous phases. The progression and phenotypical changes involved with the nutritional phases can be summarized as follows.

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gain precede the changes in appetite, implying that the development of hyperphagic behavior is preceded by metabolic changes. The authors reviewed complete growth and nutritional records of 58 PWS subjects involved in a longitudinal study to characterize the development of hyperphagia in PWS. The condition was found to follow a gradual progression through several nutritional phases, distinguishable by significant changes in weight gain and dietary intake, as compared to each of the previous phases. The progression and phenotypical changes involved with the nutritional phases can be summarized as follows.

First, infancy is characterized by low birth weight and significant growth restrictions and accompanied by feeding difficulties and overall failure to thrive. Similarly, the infants who did not receive tube feeding also showed metabolic rates indicative of underfeeding. However, the first changes in appetite and weight become apparent approximately at the age of 9 months. At this point, the infant is taking adequate nutrition and the weight gain follows a growth curve similar to typical development. It is notable that the first changes in appetite occur around the age when complementary foods are first introduced to an infant's diet. Furthermore, the infants who began to receive growth hormone treatment at early age to compensate for their low growth hormone levels due to hypothalamic dysfunction also showed a significantly faster development of appetite in infancy, compared to individuals who began to receive growth hormone treatment later, implying a role for endocrine changes in the development of appetite. Second, the rapid weight gain that precedes the gradual rise of appetite and the development of hyperphagia becomes apparent at approximately 2 years of age. This increase in weight gain is also associated with a significant increase in the serum levels of insulin-like growth factor (IGF-1), implying a role for endocrine changes in the development of hyperphagia. As PWS involves a hypothalamic dysfunction, which has been further associated with the growth hormone deficiency typical to the syndrome [90], it can be further postulated that the metabolic changes involved in the development of hyperphagia in PWS may be caused by the hypothalamic dysfunction. Third, the gradual rise of interest toward food and further changes in appetite can be recognized at approximately 4–5 years of age, while the development of independent food-seeking behaviors and visible hyperphagia become apparent at 8–9 years of age.

The concept of distinct nutritional phases has been criticized by Kotler et al. [12] who performed a retrospective review of clinical records for 55 individuals with PWS and

found that routinely collected clinical records contained inadequate information for assigning an individual to one of the nutritional phases defined earlier by Miller et al. [11]. Furthermore, the identification of the later nutritional phases relies on changes in appetite and behavior, but as pointed out by Kotler et al. the analyses applied by Miller and colleagues did not control for changes in appetite with age, effects of psychiatric medication or any restrictions in the availability of food. Kotler et al. note that PWS involves incomplete pubertal development, but the progression of early stages of puberty is accelerated, as compared to typical development [92]. Thus, it can be postulated that the imprinted genes involved in the development of PWS may affect the comparably earlier onset of adrenarche in PWS, and that the onset of extreme hyperphagic behavior approximately at the age of 8–9 years coincides with the beginning of this juvenile phase [11,13].

In comparison to PWS, few studies have characterized the development of hyperphagic behavior in AS. Berry et al. [93] note that behaviors indicative of hyperphagia were reported in AS individuals in significantly greater proportions compared to a control group of children with intellectual disabilities. Approximately one third of the AS individuals in the study were reported to steal or gorge on foods regularly, indicating a tendency for hyperphagic behavior. However, no significant differences in reported behaviors were found between different genotypes. A comparative questionnaire study on food-related behaviors among five genetic neurodevelopmental syndromes similarly found that their group of AS subjects displayed a significantly higher degree of behaviors indicating impaired satiety, compared to subjects with Cornelia de Lange syndrome but also a significantly lower degree of impaired satiety compared to PWS subjects [94].

In contrast to the results of Berry et al. [93] and the development of hyperphagia in PWS, the phenotype and development of which has not been found to differ between PWS genotypes [11], hyperphagic behavior in AS have been strongly associated with imprinting defects and paternal disomy, which involve increased dosage for the paternally expressed genes in the 15q11-q13 locus [95,96]. In particular, Mertz et al. [95] found that AS individuals with patUPD had significantly increased birth weights and also showed a significant increase in BMI at approximately 3 years of age and afterward, as compared to AS subjects with the deletion genotype or UBE3A mutations [95]. Similarly, a study on early childhood development in AS also found that AS individuals with

imprinting defects or the patUPD genotypes developed a disproportionally high BMI within the first 4 years of age, in comparison to individuals with UBE3A mutations [96]. In addition, Mertz et al. [95] found that AS individuals with the patUPD genotype showed significantly higher degrees of hyperphagic behavior, drive and severity, compared to AS individuals with the deletion genotype [95]. However, the study design of the authors did not enable precise assessments on the age of onset for the hyperphagic behaviors. Thus, it is not possible to estimate if the hyperphagic behavior in AS develops at an early age, as suggested by the early change in BMI, or if the condition involves a more gradual development of appetite, similar to that of PWS.

In conclusion, PWS involves a gradually developing condition of hyperphagia consistent across all genotypes. In comparison, hyperphagic behaviors are reported with approximately one third of AS individuals, while rapid weight gain at an early age and significantly increased degrees of hyperphagic behavior are further associated with genotypes showing relatively more paternal imprinted gene biases. Furthermore, while patUPD and imprinting defects each account for approximately 2%–3% of all AS cases [9], we note that relatively more paternal genotypes were disproportionally represented (18% UPD, 6% imprinting defect, 5% abnormal methylation) in the study of Berry et al [93]. However, it is currently unclear if hyperphagic behaviors are exclusively associated with relatively more paternal genotypes in AS.

Selective and unselective eating in PWS and AS

Due to the central role of hyperphagia in the behavioral phenotype of PWS, food preferences have been studied extensively in this syndrome [97]. Individuals with PWS have been noted to show a consistent preference for sweet foods over other tastes [97–102]. Kotler et al. [12] also note that about one third of their participants (17 out of 55) were described as “picky eaters” during their clinical visits. An avoidance of meat and chunky or non-pureed foods was shown at an age of 1–3 years, while preferences for starchy foods and avoidance of meat were common throughout all age groups. However, as food neophobia is a consistent feature of typical childhood development at early ages, it is difficult to estimate if these food preferences are consistently narrower or broader compared to typically developing individuals [12]. While tendencies for particular food preferences are present in PWS, two behavioral studies further suggest that the amount of food available is consistently more important compared to taste. Glover et al.

[99] showed that PWS subjects would consistently choose a larger amount of a less preferred food over a smaller amount of their favorite food, while obese control subjects instead showed a tendency toward choosing their preferred foods. Similarly, Joseph et al. [103] showed that adult PWS subjects consistently chose larger amounts of food regardless of any preference in taste or any delay in presentation. In addition, behavioral studies also suggest that PWS subjects are more likely to accept contaminated or inappropriately placed foods. Dykens [104] used photos of food items to assess acceptance of different foods and found that PWS subjects were significantly more likely to endorse contaminated or highly unusual foods compared to both typically developing controls and intelligent quotient (IQ)-matched controls with varied intellectual disabilities. Similarly, Young et al. [105] found that both PWS subjects and children with varied intellectual disabilities were significantly more likely to express acceptance of inappropriately placed foods, such as on food the floor or in a trash can compared to typically developing individuals, indicating a consistent tendency for unselective eating. Furthermore, a group of three individuals with a mean age of approximately 12 years was found to actively seek and consume inappropriately placed food in an experimental setting, while older individuals (mean age of approximately 20 years) did not show a similar tendency.

In comparison to PWS, both narrow food preferences and marked interests for certain types of foods have been reported in studies of AS. Clarke and Marston conducted a caretaker questionnaire on problematic behaviors, comparing a group of AS subjects aged 5–33 years to previously studied groups with varied intellectual disabilities [106]. The authors noted that a range of varied food-related problem behaviors, including overeating or a narrow range of food preferences, were reported in 64% of the participants. Similarly, according to Berry et al. [93], narrow food preferences are reported with a prevalence between 33% and 100% in the relevant literature concerning AS. The authors also found that behaviors concerning narrow food preferences were also reported in significantly greater proportions among AS subjects (aged between 1 and 40 years, with a mean age of 13.6 years), compared to a control group of children with intellectual disabilities, with a prevalence of approximately 70% among the AS individuals. Finally, AS has been noted to involve specific interests toward certain foods. In particular, “marked preference for certain foods, particularly those that do not require much chewing such as bread, pasta or banana” has been noted [107]. Hence, while the

evidence is limited in nature, studies concerning AS support the notion of a consistent tendency for relatively selective eating in AS, along with an exaggerated interest in foods resembling specially prepared complementary foods in texture.

Although relevant studies characterizing narrow or limited food preferences are few in AS, behavioral tendencies for narrow food preferences are well documented in subjects with ASDs (reviewed in Marí-Bauset et al. [108]). Given the relatively high degree of comorbidity between autism and AS [2–4], a certain resemblance of feeding behavior patterns and food preferences between the two conditions may be assumed. Children with ASDs display a consistent tendency for significantly increased selectivity toward food, as compared to typically developing children: Raiten and Massaro [109] compared food preferences among children with ASDs and typically developing children with a 7-day food diary and a parental questionnaire and found that children with ASDs showed a significantly higher degree of food selectivity compared to typically developing children. Similar results have been further shown in several behavioral studies [110–116]. While the association of narrow food preferences and ASDs is consistently reported across studies, it is less clear if the selectivity is based on taste, difficulty in consumption or other aspects such as visual representation. For example, Schreck et al. [110] found that children with ASDs were significantly more likely to require specific utensils or particular presentation of food items, compared to typically developing children, and that children with ASDs were also more likely to accept foods with constant texture, such as purees or mashed potatoes. Similarly, Hubbard et al. [117] noted that children with ASDs were significantly more likely to refuse foods based on their texture, smell and taste compared to typically developing children.

In conclusion, while the evidence is limited, AS may involve a tendency for a narrow range of preferred foods, which resemble complementary foods in texture. In comparison, PWS subjects tend to choose larger amounts of food over preferred foods and may endorse both contaminated and inappropriately placed foods. These behaviors suggest a tendency for unselective eating, which develops gradually along with the gradual rise in appetite at the age of 4–5 years.

Food-seeking behaviors in PWS and AS

The hyperphagic condition of PWS has been characterized to involve an exaggerated preoccupation with food and the development of independent food-seeking behaviors, including stealing or taking food without approval as well as bargaining for food and snacks with their caretakers [91,118]. As food-seeking behaviors have also been reported in studies of AS [93], it should be assessed whether any quantifiable differences in hyperphagic behavior can be found between AS and PWS. Two large-scale questionnaire studies have documented a wide range of hyperphagic behaviors in PWS: Russell and Oliver designed a questionnaire for food-related behaviors based on structured interviews with parents and caretakers, and derived subscales for behavioral traits to further characterize preoccupation with food, impaired satiety and negative food-related behaviors, which include taking or stealing food, eating inappropriate items (pica) and reacting inappropriately when food is taken away [118]. The PWS subjects scored consistently higher in all subscales, compared to a control group of children with intellectual disabilities living in a similar community setting, indicating a consistent tendency for food-seeking behaviors and an exaggerated preoccupation with food [118]. Dykens et al. assessed hyperphagic behaviors in PWS with a specifically designed questionnaire [91]. Hyperphagic behaviors were categorized into different factors and a principal components analysis was performed to further characterize which of the factors best explained the variance in the results. While the drive for food (hyperphagic drive) was found to be consistent across all age groups, extremely obese individuals with PWS displayed significantly greater drive for food, compared to other over- or normal-weight PWS subjects. In addition, PWS subjects above the age of 10 were found to show a significantly greater variety of hyperphagic behaviors compared to younger children with PWS. Hyperphagic severity, indicating the individual's preoccupation with food, was found to be similar in all other age groups, while the oldest age group (30 years and above) showed significantly lower scores. In other words, hyperphagic problem behaviors only become evident in late childhood, while the preoccupation with food diminishes as the individual matures [91]. In contrast to the results of Mertz et al. [95] concerning AS subjects with PatUPD, no significant correlations were found between genotypes and any degree of hyperphagic behavior, drive or severity among PWS individuals, further indicating that the condition of hyperphagia is consistent across all the genotypes in PWS [91].

Food-seeking behaviors have been described in three studies concerning food-related behaviors in AS. As noted earlier, stealing food or overeating has been reported in a significantly larger proportion of AS subjects (approximately one third) compared to control subjects with intellectual disabilities [93]. Furthermore, in a direct comparison of food-related problem behaviors between five genetic neurodevelopmental syndromes, both AS and PWS individuals were reported to show greater degrees of food-seeking behaviors, compared to the groups of CdLs and 1p36 deletion syndrome individuals. However, individuals with PWS also showed significantly greater preoccupation with food compared to individuals with AS [94]. Similarly, as noted earlier, the patUPD genotype has been characterized to display a significantly greater degree of food-seeking behaviors and a greater degree of hyperphagic drive and severity, compared to AS subjects with the deletion genotype [95].

In summary, both AS and PWS show a significant tendency for food-seeking behaviors, but while food-seeking behaviors are consistent across all genotypes in PWS, in AS the patUPD genotype shows a higher degree of food-seeking behaviors. In addition, behaviors indicating a preoccupation with food are reported to a greater degree in PWS.

Developing models of eating behavior for PWS and AS

To further characterize the behavioral phenotypes of feeding behavior in PWS and AS, we review current research in PWS (1) and relevant mouse model studies (2) on the mechanisms of hyperphagia and food preferences and use this information to develop models of feeding behavior for both PWS and AS:

Human studies on the regulation of hunger and satiety in PWS

Several behavioral and physiological human studies have characterized the hyperphagic condition of PWS as involving an impairment of satiety. Firstly, Holland et al. [119] showed that PWS subjects consumed significantly higher amounts of calories during a meal session, compared to typical control subjects. As expected, the blood levels of CCK, associated with regulation of satiety, were also significantly higher in the PWS subjects compared to controls. The increase in blood CCK levels during the meal

session thus indicates that the hyperphagic behavior is not associated with a failure in the release of peripheral satiety signals. Similarly, physiological studies have indicated that two other peripheral signals of satiety, leptin and peptide YY, are also significantly elevated in PWS subjects [120]. While significantly elevated levels of circulating ghrelin, a peptide involved in the peripheral signaling of hunger, have been noted in several studies [121,122], it is unlikely that hyperghrelinemia would be causal to the hyperphagia in PWS, as the levels of fasting ghrelin are also elevated in infants and children in early nutritional phases before the onset of hyperphagic behavior [123]. As the elevated levels of peripheral signals of satiety would be expected to regulate meal size, these results suggest that the apparent dysregulation of food intake may instead be connected to the hypothalamic dysfunction central to PWS.

Neuroimaging studies have provided evidence that impaired satiety in PWS is connected to a hypothalamic dysfunction and a failure in neural recognition of peripheral satiety signals. Hinton et al. [98] measured neural activation in response to an overnight fast followed by a high-energy breakfast and found that the PWS subjects showed a comparable lack of neural activation in brain regions previously associated with satiety after meal consumption, indicating a dysfunction in neural regulation of food intake.

Recently, a post-mortem transcriptional analysis of brain tissues in PWS subjects has confirmed that PWS subjects display an imbalance in the expression of hypothalamic neurotransmitters involved in the regulation of hunger and satiety [124]. Comparing gene expression, some ~3600 genes were found to be differentially expressed in the hypothalamic tissues of PWS subjects, as compared to controls with comparable BMI. Furthermore, the expression of AgRp and other genes predominantly expressed in AgRp-expressing neurons was found to be significantly upregulated in PWS subjects, while the expression of Pomc and other genes predominantly expressed in Pomc-expressing neurons were found to be significantly downregulated. Comparisons with the gene expression profiles of fasted animals indicated that the genes upregulated in PWS subjects also represented genes commonly upregulated in response to hunger. In addition, a dysregulation of genes involved in the regulation of energy homeostasis and adipocyte tissues is also implied. Finally, using a targeted deletion of SNORD116 in a human cell model and analysis of predicted splicing targets for the SNORD116 SnoRNA, the researchers showed that lack of expression for SNORD116 may lead to marked neurodegeneration and reduced neuronal differentiation through dysregulation

of alternative splicing of several genes previously implied in neuron development and synaptic plasticity [124]. Taken together, these results indicate that the pleiotropic effects of neuronal loss and reduced neuronal differentiation may also lead to a dysregulation of hunger and satiety in the hypothalamus in PWS.

The complex nature of the genetic mechanism for hyperphagia and obesity in PWS is further highlighted in studies of Schaaf–Yang syndrome. Schaaf–Yang syndrome is caused by truncating mutations or deletions of the paternally derived copy of the MAGEL2 gene, and so the genotype of Schaaf–Yang syndrome has partial overlap with the genotypes of PWS. While both Schaaf–Yang syndrome and PWS involve intellectual disability, hypotonia and feeding problems during infancy, hyperphagia is described in only 35% of subjects with Schaaf–Yang syndrome, whereas excessive weight gain has been reported in 47% of the subjects [125]. In a partially overlapping study, McCarthy et al. [126] reported that all nine of the studied subjects with Schaaf–Yang syndrome showed elevated levels of fasting ghrelin, while hyperphagia had not been reported in any of the patients involved in the study. The consistent features between the phenotypes of PWS and Schaaf–Yang syndrome indicate that the lack of expression for MAGEL2 may also play a role in the development of early feeding restrictions and the later development of obesity. However, the partial penetrance of hyperphagia in Schaaf–Yang syndrome indicates that other paternally expressed genes of the 15q11-q13 locus further affect the phenotype of hyperphagia in PWS.

Genetic mouse model studies of the regulation of hunger and satiety in PWS

Several mouse model studies have investigated the genetic mechanisms for the central features in the phenotype of PWS: an early growth restriction followed by the subsequent development of hyperphagia and obesity. Bischof et al [127] showed that mice with two inactivated copies of Magel2 exhibited an early growth deficit from the first week after birth until weaning, which was followed by rapid weight gain and obesity after weaning. However, the mutant mice showed a ~10% reduction in food intake and were also less active compared to controls. These results resemble the phenotype of PWS closely, which may not only indicate a central role for Magel2 in the development of both early growth restrictions and later changes in weight gain but also indicate that losses of expression for other paternally expressed may be responsible for the development of

hyperphagia in PWS. The role of MAGEL2 in the development of obesity has been shown to involve interactions with leptin in the hypothalamus: Mercer et al. [128] showed that model mice with deletions of the Magel2 gene lack the anorexigenic response to leptin, which induced restrictions in food intake in the control mice. Based on the observations of genetic markers for neural activation, it was shown that leptin fails to activate the Pomc-expressing neurons in the cells of MAGEL2-null mice, indicating a neural dysregulation of food intake. As also shown in Figure 5, leptin regulates food intake via the hypothalamus. Thus, the authors hypothesized that Magel2 may have a role in regulating intracellular leptin responses in hypothalamic neurons. Recently, another mouse model study confirmed that Magel2 interacts with necdin and three ubiquitin pathway proteins (Rnf41, Usp8 and Stam1) to regulate the lysosomal degradation of the leptin receptor [129]. As Magel2 was found to regulate the stability of Rnf41 and Usp8, the authors postulated that Magel2 may regulate the abundance of leptin receptors in the hypothalamus indirectly through ubiquitination pathways. Together, these results indicate that MAGEL2 may regulate long-term energy homeostasis via its interactions with leptin, and lack of expression for MAGEL2 may be the central mechanism for the obesity characteristic to the phenotype of PWS.

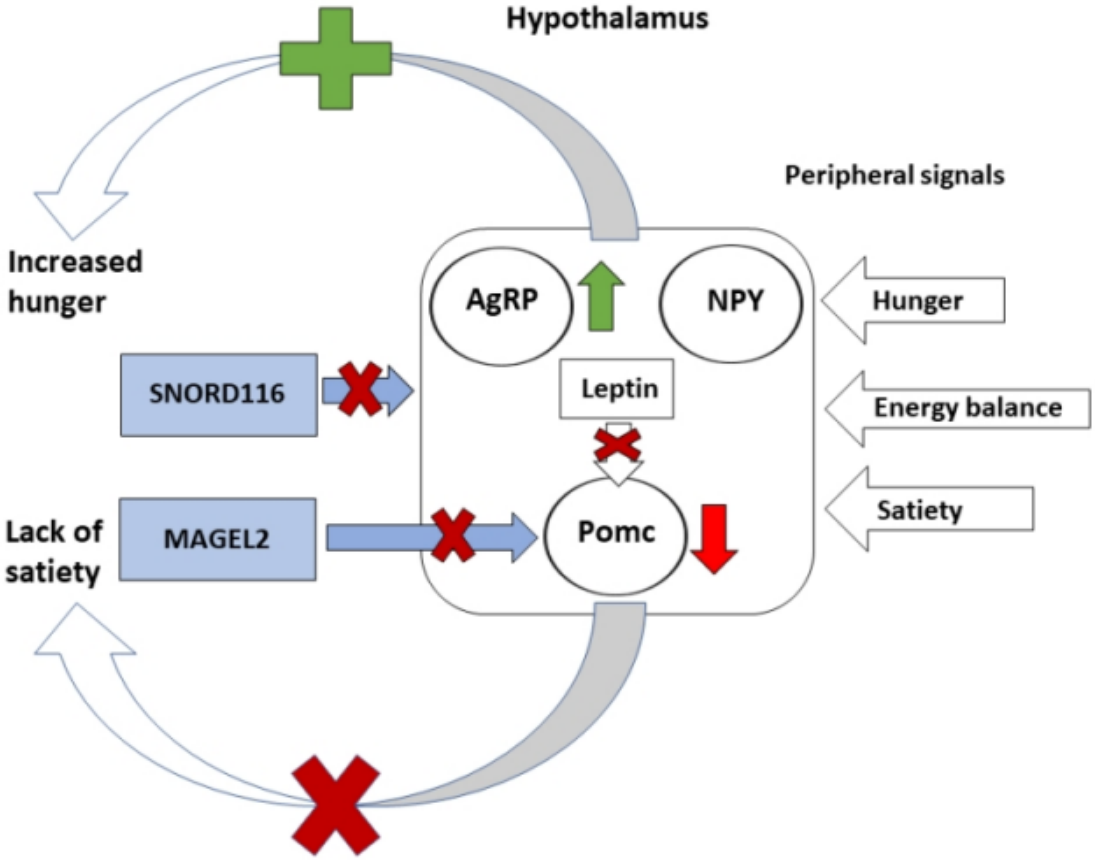


Figure 5. Theoretical gene regulation model on how the PWS genotype may affect the hypothalamic regulation of hunger and satiety. Mouse models indicate that lack of expression for both Magel2 and SNORD116 may in part contribute to lack of satiety with the PWS phenotype.

Several mouse model studies have suggested that the mechanism for the early growth restrictions and late development of hyperphagia in PWS may also be partially dependent on the lack of expression for SNORD116 snoRNA. Ding et al. [130] showed that mice with paternally inherited deletions of Snord116 develop normally in prenatal stages but show a growth reduction at early ages, followed by the development of hyperphagia at a later age. However, the model mice did not replicate the phenotype of PWS in full; despite their hyperphagic condition, the model mice would stay lean and showed altered metabolism, with higher rates of oxygen consumption compared to control mice. Other mouse model studies have addressed the role of SNORD116 in the

development of hyperphagia in PWS with partly contradicting results, but none of the mouse models have replicated the full phenotype of hyperphagia and obesity in PWS. Qi et al. [131] showed that lack of expression for Snord116 may alter the regulation of food intake via the NPY-expressing neurons in the hypothalamus. Model mice with a selective deletion of Snord116 in the NPY-expressing neurons replicated the phenotype of early growth restriction and late development of hyperphagia closely. Thus, Snord116 may be critical for regulating the expression of Pomc and NPY, as the model mice also showed a significant upregulation of both NPY and Pomc mRNA in the hypothalamus. In particular, the anorexigenic response of Pomc-expressing neurons was hypothesized to play a role in the growth reductions at an early age, while the drive for increased food intake induced by the NPY-expressing neurons would take hold later in life [131].

However, another recent mouse model study by Polex-Wolf et al. [132] partly contradicted the results of previous studies [130,131] as the authors found that a mouse model with a paternal deletion of Snord116 did not develop hyperphagia at a later age. However, mice with a selective deletion of Snord116 in the mediobasal hypothalamus induced at adult age showed the development of hyperphagia 10 weeks after the procedure. The model mice also showed a significantly greater weight gain in comparison to controls, though only a small subset (5 out of 21) of the mice would develop significant obesity and increased fat mass. The results seemed to contradict previous findings on the neural mechanisms that lack of expression for SNORD116 was affecting, as the expression of Pomc, NPY and leptin receptor mRNAs during a fast was not shown to be significantly different from controls. Similarly, the expression of prohormone convertase 1 (Pcsk1) and its upstream regulator did not differ significantly from controls, indicating that the increase in food intake could not be explained by an imbalance in the homeostatic regulation of feeding and that an alternative explanation for the mechanism of hyperphagia would be required.

Although mouse model studies have implied that the lack of expression for both SNORD116 and MAGEL2 may play roles in the development of hyperphagia and obesity in PWS, the analysis of these studies is complicated by a recent study which notes the paradoxical leanness of model mice with a deletion of the PWS imprinting center. Showing a close resemblance to the PWS phenotype, the model mice showed an early reduction of growth and failure to thrive in infancy, with later development of food hoarding behaviors. However, significantly increased food intake as compared to

controls would only develop with model mice on high-fat diets [133]. As also shown in the results of Ding et al [130] and Qi et al. [131], the model mice also showed a significant reduction in body weight and fat mass. In further investigation, the model mice did not show an elevation of white fat mass in a thermoneutral environment, indicating that increased energy usage in maintaining body temperature is unlikely to cause the leanness of PWS model mice. As the model mice similarly failed to gain weight on a high-fat diet, the authors postulated that the leanness of the model mice may result from a failure of lipid accumulation in white adipocytes, which may further indicate that the model mice failed to model the full phenotype of PWS due to metabolic differences between humans and mice [133].

In summary, mouse model studies have shown that the lack of expression for MAGEL2 may be connected to the development of obesity due to dysregulation of leptin-induced anorexigenic responses in the hypothalamus. Furthermore, while all of the currently reviewed mouse model and human studies indicate that the development of hyperphagia in PWS is associated with lack of expression for SNORD116 in the hypothalamus, further research is necessary for understanding the precise mechanisms of how SNORD116 and alternative splicing mechanisms might alter the regulation of both neuronal development [124] and homeostatic feeding as regulated by the hypothalamus [131].

A model for hypothalamic dysregulation of homeostatic feeding in PWS

Currently available studies on both humans and mouse models suggest that the hyperphagia typical to PWS results from a dysregulation of homeostatic feeding in the hypothalamus. Based on these findings, we have developed a model for the dysregulation of homeostatic feeding mechanisms in PWS. By this model, peripheral signals of satiety and long-term energy balance are produced at elevated levels, but the processing of these signals in the hypothalamus is disrupted due to an imbalance in hypothalamic neurotransmitters, ultimately due to the lack of expression for the paternally expressed genes MAGEL2 and SNORD116:

First, we note that the regulation of long-term energy balance in the hypothalamus is dependent on the anorexigenic response induced by leptin. However, lack of expression for Magel2 leads to dysregulation in the ubiquitination of leptin receptors, [128] so leptin fails to induce the fasting response in the hypothalamus due to

an accelerated turnover of leptin receptors at Pomc-expressing neurons, contributing to both increase in food intake and the development of obesity. Second, SNORD116 has been shown to be involved in the regulation of food intake via the hypothalamus [130,131], and transcriptional analysis of hypothalamic tissues of PWS subjects suggests that the lack of expression for SNORD116 may alter the regulation of neuronal development [124] leading to an imbalance in the regulation of the hypothalamic feeding mechanism. The expression of AGRP, previously implied in the regulation of hunger, is significantly upregulated in the hypothalamus, while the expression of POMC, previously implied in the regulation of satiety, is significantly downregulated. Thus, the impaired satiety central to phenotype of eating behaviors in PWS may be induced by continuous signaling of hunger and diminished signaling of satiety in the hypothalamus.

As PWS involves a gradual transition from poor feeding in infancy to the development of hyperphagia in late childhood, further research is necessary to understand how a lack of expression for paternally expressed genes would gradually alter the regulation of food intake from infancy to early childhood and further from childhood to the juvenile phase. As the development of hyperphagia coincides with early adrenarche, it has been suggested that the changes in appetite may reflect changes in the expression of adrenal androgens during childhood development [12]. A model of the central hypothalamic mechanisms in the regulation of food intake and their currently known interactions with the paternally expressed genes, MAGEL2 and SNORD116, is shown in Figure 5.

Mouse models on the role of palatability in food intake

Changes in appetite may also be driven by the reward circuitry of the brain, in addition to the homeostatic mechanisms regulating hunger and satiety. While mouse model studies of PWS have focused primarily on the development of hyperphagia and the mechanisms in regulation of homeostatic feeding, the role of palatability in the regulation of feeding behavior has also been highlighted in a number of mouse model studies. Exploring the role of hedonic eating in PWS, Davies et al. [134] characterized the feeding behavior in a mouse model with a paternally inherited deletion corresponding to the imprinting center of the PWS–AS locus. The model mice showed significantly increased food consumption after an overnight fast as compared to wild-type controls, with both regular chow and high-sugar content food. In order to dissociate the impact of

nutrition and taste, the researchers studied licking behavior. The mice were accustomed and given limited access to a lick-measuring device which would dispense sugared water or alternatively a solution with saccharin, an artificial sweetener with no nutritional value. With sucrose, no significant differences between the model mice and wild-type controls were found in comparisons of repeated licking behavior. However, with saccharin, the model mice showed a significant reduction in the number of total licks as compared to wild-type controls. In addition, when trained with a treat-dispensing device, the model mice again matched the behavior of the wild-type controls with a sugar-based treat but showed a significant reduction in the number of responses when rewarded with a saccharin-based treat instead. The authors concluded that the PWS model mice appeared to be particularly sensitive to the calorific content of palatable food, rather than taste, thus resembling the lack of satiety and the tendency for unselective eating among PWS subjects.

The role of dopamine in the development of food preferences has been shown in a multitude of studies. For example, Cooper and Al-Naser [135] found that dopamine may influence the development of preference for palatable foods. Food intake of fasted rats was measured in experimental settings, with comparisons based on the palatability of the food offered. While highly palatable food was consistently consumed in greater amounts compared to regular food pellets, treatment with a selective D1 dopamine receptor agonist significantly increased the consumption of the palatable food as compared to the control, while treatment with a selective D2/D3 dopamine receptor agonist significantly decreased the consumption of the highly palatable food and increased the consumption of regular chow. Hence, preference for highly palatable foods is at least partly driven by the dopamine reward system.

The consumption of palatable foods thus shows a definite connection to the dopamine reward system and opposite alterations to both dopamine and serotonin neurochemistry have been shown in mouse models of both AS and PWS. Farook et al. [88] studied mice models with either duplications or deletions of Ube3a. Mice with a maternally inherited deletion of Ube3a resembling the genotype of AS had elevated levels of dopamine in the striatum and frontal cortex and elevated levels of serotonin in the striatum and cortex, as compared to controls. Conversely, mice with a maternal duplication of Ube3a, resembling the matUPD genotype of PWS, had elevated levels of dopamine in the midbrain and the striatum. Mercer et al. [89] also showed that Magel2-

null mice had significantly decreased levels of serotonin in the cortex, prefrontal cortex and hypothalamus as well as significantly decreased levels of dopamine in the hypothalamus. Thus, studies of mice models indicate that AS may involve highly elevated levels of dopamine in brain regions critical to feeding. In contrast, PWS may involve decreased levels of dopamine and serotonin in multiple brain regions critical to feeding.

A model for the development of hyperphagia and food preferences in AS and PWS

Considering the existing evidence from both empirical studies in AS and PWS and findings from relevant mouse model studies, we have developed a model to explain the development of hyperphagia and food preferences in both AS and PWS. First, we assume that the hyperphagic condition of PWS is caused by impaired satiety due to a disruption of the homeostatic feeding mechanism, as shown in Figure 5. Furthermore, we predict that PWS confers a consistent tendency for unselective eating, as suggested by the mouse model study of Davies et al. [134] as well as the observation that PWS patients consistently choose larger amounts of food regardless of preference in taste or delay in presentation [98,103].

Second, both increased selectivity toward food and marked preferences for certain foods, in particular foods with consistent and soft texture, are prominent in AS. Thus, we have hypothesized that the hyperphagic phenotype of AS is connected to an increased interest toward complementary foods. In contrast to the unselective eating in PWS, this behavior may be driven by an influence of paternally expressed genes, as the hyperphagic phenotype of AS is strongly associated to the patUPD genotype, which confers a higher dosage of the paternally expressed genes in the 15q11-q13 chromosome region [95]. It can be predicted that complementary foods, which differ from adult diets and could be more difficult to obtain and prepare, may involve increased maternal costs. Paternally expressed genes may thus have been selected to favor an increased preference for complementary foods and prolonged parental care in juvenile stages, so a disruption of the genetic conflict could further result in extreme behaviors such as overeating and limited preferences for specific foods, as seen with the phenotypes of both AS and autism.

To illustrate our hypothesis, we have developed a framework around hedonic feeding mechanisms and the known alterations of dopamine neurochemistry in AS and

PWS, as shown in Figure 6. First, food selectivity similar to ASD may contribute to increased selectivity toward food across all genotypes. Second, both a lack of expression for UBE3A and an increased dosage of paternally expressed genes such as MAGEL2 are expected to contribute toward elevated levels of dopamine in the brain, so the more paternal patUPD and imprinting defect genotypes of AS are expected to show both elevated hedonic value of food and tendency for the development of hyperphagia with selective eating. In contrast, all genotypes of PWS lack expression of MAGEL2, so asymmetrical alterations to hedonic feeding can be expected between AS and PWS, as shown in Figure 6. However, the exact mechanism of hyperphagia in AS does not need to depend on hedonic feeding mechanisms to fulfill our initial expectations on the influence of paternally expressed genes in the development of hyperphagia and selective eating.

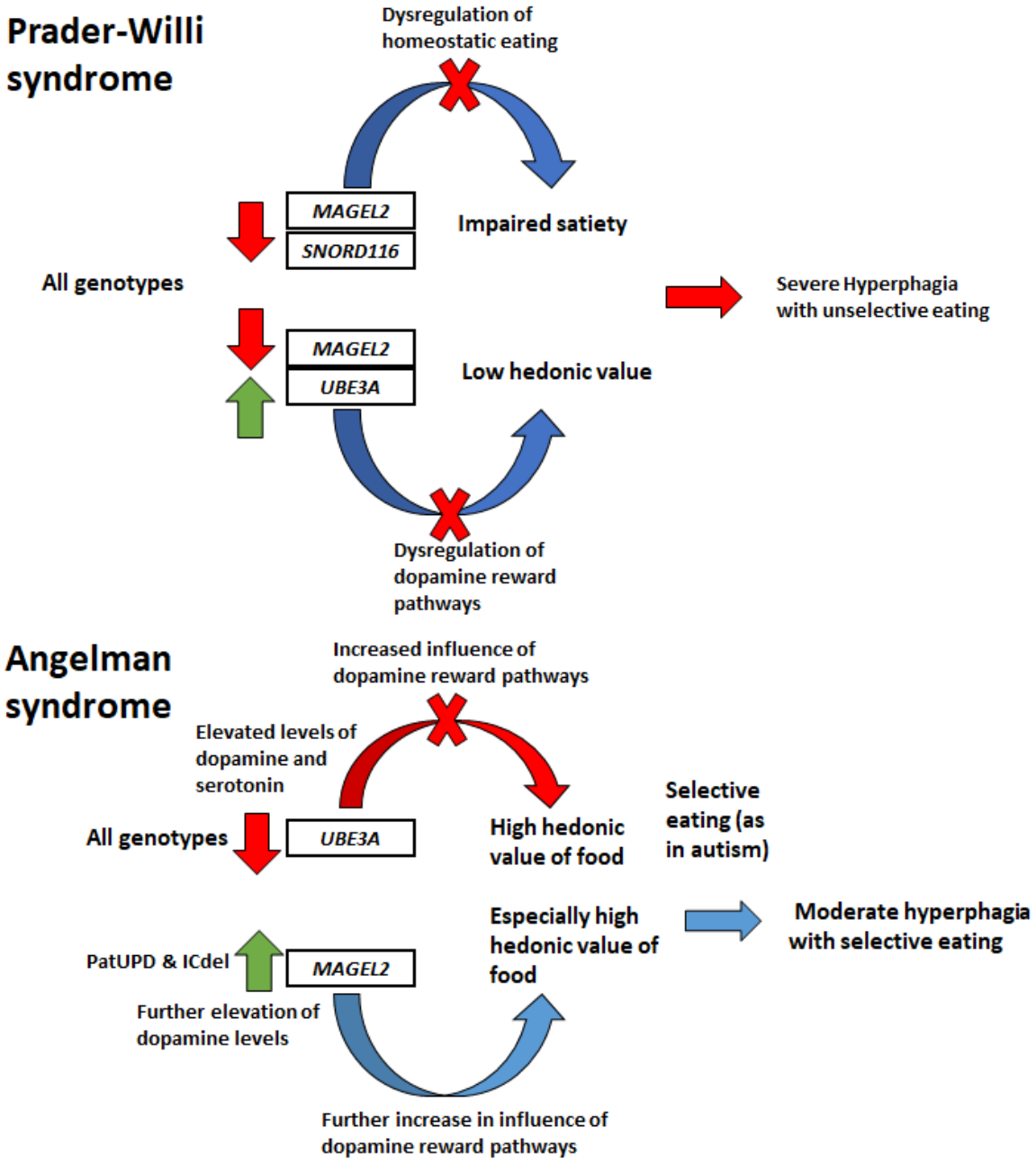
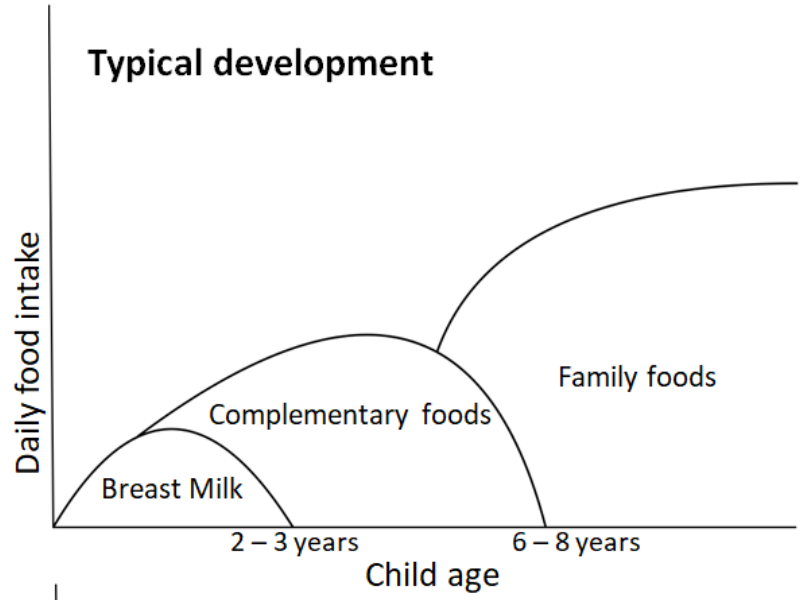
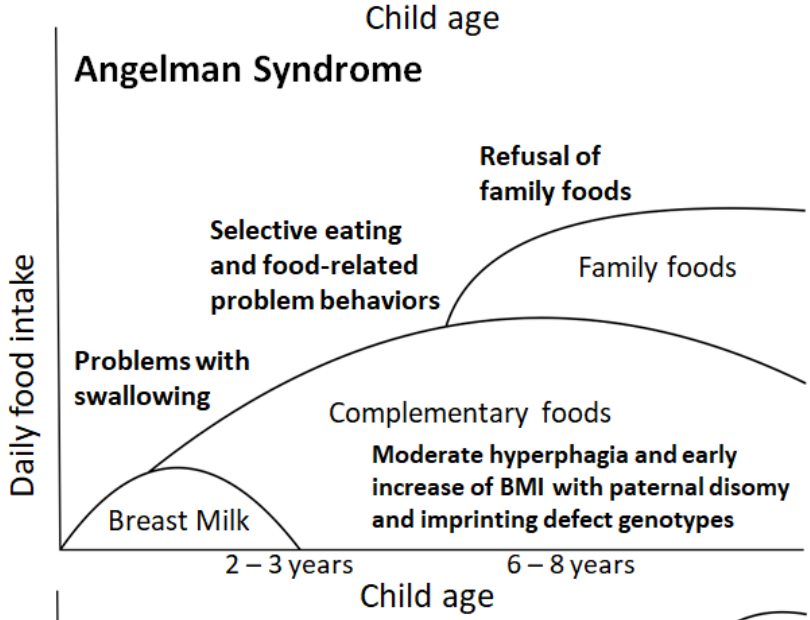


Figure 6. A hypothetical model involving dysfunctions of hedonic and homeostatic feeding mechanisms and the development of different hyperphagia phenotypes in AS and PWS. PWS and AS may involve opposite dysfunctions of dopaminergic pathways due to losses and gains in dosages of imprinted genes. The effect is more pronounced with uniparental disomies and imprinting defects due to an increased dosage of paternally (or maternally) expressed genes. The increased dosage of paternally expressed genes and the expected increases in dopamine levels may explain the tendency for selective eating and the early development of hyperphagia in AS associated with patUPD and imprinting defects.

Currently available evidence on the timing of the development of hyperphagic behavior in PWS indicates that the development of hyperphagia coincides approximately with the timing of early adrenarche, as well as the development of adult dentition and simultaneous transition toward an adult diet of diverse family foods [12,13]. While exact information on the timing of the development of hyperphagia in AS is currently lacking, the rapid increase of BMI between 2 and 5 years of age [95,96] suggests that the development of hyperphagic behavior in AS may coincide with the usual period of dependence on complementary foods, which are the primary source of nutrition for the child after weaning until the development of mature dentition at the age of 6–8 years [80]. Thus, we have further complemented our model with predictions on the timing of the development of hyperphagia and food preferences in both AS and PWS. As shown in Figure 7, our model predicts increased interest toward complementary foods and early development of hyperphagia in AS and conversely a gradual rise of interest toward food and late development of hyperphagia and indiscriminate “foraging” of diverse foods in PWS in accordance to earlier work [13].


Typical development
Daily food intake
Family foods
Complementary foods
Breast Milk
2 – 3 years
6 – 8 years
Child age


Angelman Syndrome
Daily food intake
Refusal of family foods
Selective eating and food-related problem behaviors
Family foods
Problems with swallowing
Complementary foods
Moderate hyperphagia and early increase of BMI with paternal disomy and imprinting defect genotypes
Breast Milk
2 – 3 years
6 – 8 years
Child age

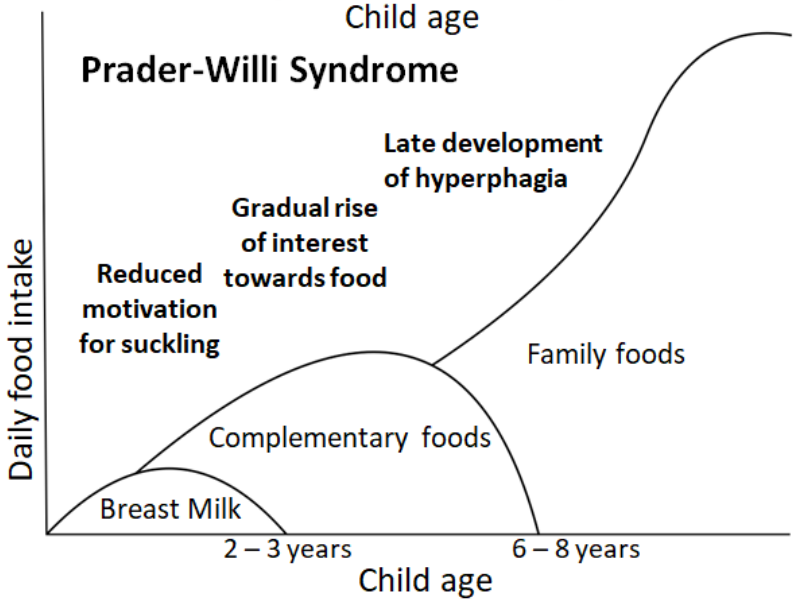

Prader-Willi Syndrome
Daily food intake
Late development of hyperphagia
Gradual rise of interest towards food
Reduced motivation for suckling
Family foods
Complementary foods
Breast Milk
2 – 3 years
6 – 8 years
Child age

Figure 7. Theoretical model for how AS and PWS may affect the nutritional transitions in childhood development. Development of partly opposite food preferences and hyperphagia in AS and PWS, as compared to typical childhood development. Maternally provided breast milk is the primary source of nutrition for the infant until the age of weaning at the age of 2–3 years, while specially prepared complementary foods are gradually introduced from the age of 6 months and onwards. Diverse family foods resembling an adult diet typically replace complementary foods by the age of 6–8 years. Poor feeding during infancy is prominent in both AS and PWS, although for different reasons. The behavioral phenotype of PWS involves a gradual rise of interest toward food and late development of hyperphagia, whereas AS may involve comparably earlier development of hyperphagia and a specific interest for complementary foods and prolonged refusal of family foods.

3.5. Conclusions

In this article, we have compared sleeping and eating behavior phenotypes of PWS and AS and evaluated, for the phenotypes with sufficient data, which phenotypes are opposite to one another and which are not. Furthermore, we have assessed these behavioral phenotypes in the context of relevant mouse model studies and developed genetic and physiological models for sleeping and eating behavior to help explain how the different genetic alterations of these syndromes could produce opposite phenotypes, especially from alterations to dosages of different imprinted genes. Finally, we have evaluated our findings in the context of human childhood development and the kinship theory of imprinting. Our main findings are as follows:

First, relevant articles and our meta-analysis showed evidence of opposite phenotypes for sleep onset latency between AS and PWS, and partially opposite phenotypes for sleep duration, while other traits of interest showed relatively similar phenotypes in both syndromes. As relevant mouse model studies have indicated that both paternally and maternally expressed genes may regulate circadian rhythms and sleep, we suggested a model (Figure 4) to explain how variable dosages of paternally and maternally expressed genes inherent to each syndrome could produce opposite phenotypes of sleep onset latency in AS and PWS. Thus, in PWS, both the increased expression of UBE3A due to lack of expression for SNORD116 and a lack of expression for MAGEL2 may contribute to a lack of programmed delay and acceleration of the circadian period. In contrast, both a lack of expression for UBE3A and the expression of MAGEL2 are expected to contribute to a deceleration of the circadian period in AS. These opposite alterations to the circadian rhythm and diurnally regulated gene expression patterns may lead to opposite alterations to the timing of sleep onset as also shown in the results of the relevant studies reviewed here. In AS, a lengthened circadian

period may lead to a phase delay in the 24-h circadian rhythm and a longer subjective day and increased sleep onset latency. In PWS, the opposite pattern is shown, with a lack of programmed delay leading to a shorter subjective day and reduced sleep latency.

Second, we describe evidence that that AS and PWS show a shared tendency for overeating and food-seeking behaviors [12,95,96] but apparent opposite tendencies for selective and unselective eating preferences [93,98,103]. However, while the hyperphagic phenotype of PWS is consistent across all genotypes [11,12], in AS hyperphagic phenotypes are strongly associated with the patUPD and imprinting defect genotypes [95,96] which are further characterized by increased dosages of paternally expressed genes as also shown in Figure 2. Since relevant human and mouse model studies indicate that PWS may involve both a hypothalamic dysregulation of the homeostatic feeding mechanism and diminished hedonic value of food due to lack of expression for maternally imprinted genes, we have also suggested a model (Figure 6) to explain how selective versus unselective food preferences in AS and PWS, as well as the association of hyperphagia with relatively “more paternal” genotypes in AS, may be explained by opposite alterations to dopamine reward circuitry and hedonic feeding mechanisms.

As human life history can be interpreted to involve a unique evolutionary adaptation to shortened birth intervals with early weaning and the introduction of complementary feeding with specially prepared “baby foods” [13,80], we hypothesize that the development of hyperphagia and food selectivity in both AS and PWS may reflect nutritional shifts in human childhood as shown in Figure 7. In AS, increased selectivity toward foods may result from a prolonged interest in mother-provided complementary foods, while hyperphagic behavior is driven by a further exaggeration of this interest. Conversely, as described previously, PWS involves a gradual rise in interest toward food, leading to the development of hyperphagia around the age of adrenarche [12], which coincides with a transition from the mother-provided complementary foods to diverse family foods [13]. This hypothesis is readily testable, as it further predicts that the timing and development of both selective food preferences and hyperphagia in AS should coincide with the period of complementary feeding in early childhood.

Third, we have assessed how varying dosages of the paternally expressed MAGEL2 and SNORD116 as well as the maternally expressed UBE3A may affect phenotypes of both sleep and eating behavior. Due to the wide-reaching roles of MAGEL2 and UBE3A in regulating numerous gene networks through ubiquitination pathways, [136,137] both UBE3A and MAGEL2 may affect several behavioral phenotypes including both the regulation of circadian rhythms and the regulation of long-term energy balance and feeding through a variety of different molecular mechanisms. Furthermore, UBE3A and MAGEL2 as well as SNORD116 are expressed in the same brain region, the hypothalamus. Numerous studies have indeed shown that the hypothalamus plays a dual role in both sleep–wake regulation and the regulation of feeding. For example, lesions of the NPY-expressing neurons in the mediobasal hypothalamus have been shown to cause hyperphagia and a lack of circadian variation for the distribution of non-REM sleep in mouse model studies [138], and orexin neuropeptides expressed by neurons in the lateral hypothalamic area play a role in the regulation of both sleep and eating [139]. Lack of expression for SNORD116 in the mediobasal hypothalamus is also involved in the development of hyperphagia in PWS [130–132], while a lack of expression for Magel2 has been connected to reduced levels of orexin and orexin-expressing neurons in the lateral hypothalamus, along with fragmentation in circadian regulation of activity and rest [75] as well as reductions in growth followed by later development of obesity [127,128].

The role of the hypothalamus in the development of AS is currently understudied. As alterations to the expression levels of MAGEL2, SNORD116 and UBE3A may affect the phenotypes of both eating and sleeping via the hypothalamus, our hypothesis predicts that other human neurogenetic syndromes that may also resemble AS or PWS phenotypically [140,141] or result from alterations to partially overlapping genes [136,142] may also exhibit joint effects on sleeping, eating and other hypothalamus-mediated phenotypes. The opposite alterations to sleep onset latency described here can be interpreted to follow from extreme manifestations of paternally and maternally expressed genes, as also previously discussed by Kotler and Haig [13]. Since settling to sleep often involves separation of the mother and the infant, bedtime can be seen as an important time for maternal bonding [143]. Furthermore, as the behavioral phenotype of AS has been argued to reflect an extreme development of phenotypes related to affect signaling, [16,17] paternally expressed genes may have been selected to favor

increased bedtime resistance and more frequent waking in solicitation of both nutrition and social interaction from the mother, which may also lead to increased sleep onset latency and difficulties in falling asleep later in life. In contrast, maternally expressed genes may have been selected to favor reduced bedtime resistance and less frequent waking to reduce maternal stress during early infancy, which may further lead to a consistent tendency for reduced sleep onset latency and excessive sleepiness in later life.

Declaration of conflicting interests

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Ethical approval

We confirm that our work is solely a scientific review, and thus ethics approval was not applicable.

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Chapter 4. Does SNORD116 mediate aspects of psychosis in Prader-Willi syndrome? Evidence from a non-clinical population.

4.1. Abstract

The paternally expressed gene SNORD116 encodes a set of short nucleolar RNAs that affect the expression of hundreds of other genes via epigenetic interactions. Lack of expression for SNORD116 has been implicated in major phenotypes of Prader-Willi Syndrome (PWS). Rates of psychosis and autism spectrum disorders are greatly increased in PWS, but the genetic and epigenetic causes of these increases remain unknown. We genotyped a large population of typical individuals for five SNPs within SNORD116 and phenotyped them for variation in schizotypal and autism spectrum traits. SNORD116 SNP and haplotype variation mediated variation exclusively in the Schizotypal Personality Questionnaire - Ideas of Reference subscale, which reflects variation in aspects of paranoia. The effect was restricted to females. SNORD116 represents, in addition to UBE3A and NDN-MAGEL2, a third, independent locus in the 15q11-q13 imprinted region that preferentially or exclusively affects levels of paranoia. This convergent pattern may reflect a common neural pathway affected by multiple genes, or an effect of interactions between the imprinted loci.

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4.2. Introduction

Psychotic-affective disorders, which include schizophrenia, bipolar disorder, and depression, are chronic mental disorders of overlapping symptomology and genetic components [1]. Psychotic-affective disorders grade in their diagnostic phenotypes into typical individuals, in non-clinical, personality-associated form [2]. While over 100 small-effect SNP loci have been associated with increased susceptibility to psychotic-affective disorders [1,3], such disorders also show highly increased rates of prevalence in so-called 'neurogenetic syndromes', which result from large-scale alterations to a single gene or a set of genes and involve well-defined behavioral phenotypes [4–6]. Although neurogenetic syndromes such as Prader-Willi syndrome (PWS) typically involve large-scale dysfunctions in neural and endocrine systems, associations between neurogenetic syndromes and psychological disorders may also be mediated by partly overlapping mechanisms, and these syndromes thus present valuable models for understanding the genetic and neurological basis of such disorders.

PWS is a disorder of genomic imprinting, resulting from large-scale alterations (usually paternally inherited deletions or maternal uniparental disomy, mUPD) that cause a lack of expression for a set of ~ 20 – 30 paternally-expressed imprinted genes within the 15q11-q13 locus [7]. Under genomic imprinting, only one copy of a gene (paternally or maternally inherited, depending on the locus and transcript) is expressed, while the other copy is silenced by epigenetic mechanisms [8]. PWS affects between ~ 1:10 000 and 1:30 000 births, and is characterized by hypotonia, developmental delay and feeding difficulties in infancy, followed by the development of hyperphagia and obesity in late childhood (Cassidy et al., 2012). PWS also involves relatively mild intellectual disability, and a distinct behavioral phenotype with temper tantrums, stubbornness, and both manipulative and compulsive behaviors, typically over food and daily routines [7,9]. PWS also predisposes the brain to the development of several psychiatric conditions. Firstly, the prevalence of autism spectrum disorders within PWS is estimated (by systematic review of the literature) to be about 27% [10], much higher than rates among typical populations, ranging between about 0.5% to 5.4 [11]. Secondly, the prevalence of psychotic conditions is also greatly increased in PWS. Two independent studies of large cohorts [5,6,12], show rates between 17 – 28% for the deletion genotype and notably higher rates (~ 64% in both studies) for the mUPD genotype. High rates of psychotic

conditions in PWS and especially among the mUPD genotype have similarly been reported in earlier studies [13–15], (see [16] for review). The phenotypes of the psychotic conditions in PWS prominently involve second-person hallucinations, persecutory delusions and paranoia [6]. It has been postulated that the course of psychiatric illness and psychosis in particular may be more severe among individuals with the mUPD genotype of PWS because this subtype involves both a lack of expression for the paternally expressed genes in the 15q11-q13 chromosome region and doubled dosage of the maternally expressed gene UBE3A [12,13].

No naturally occurring single gene alterations have been identified in humans with PWS. However, six cases of PWS individuals with small atypical deletions encompassing SNORD116 and varying numbers of adjacent non-coding RNA genes, including IPW and SNORD109A, appear to recapitulate the major phenotypes of hypotonia, developmental delay and hyperphagia as well as the obsessive behaviors typical to this syndrome [17–22]. Due to the small number of known PWS individuals with small atypical deletions, as well as the varying size of the deletions and numbers of non-coding RNA genes included in these deletions, current evidence does not lend itself to accurately assessing the role of SNORD116 in the PWS phenotype. In addition, only two of the individuals in these studies were over 20 years of age at the time of assessment [17,22] and no study to date has specifically addressed psychological phenotypes in PWS individuals with small atypical deletions, inversions or translocations. Thus, the potential role of SNORD116 in the psychiatric phenotypes of PWS remains unclear.

The paternally expressed SNORD116 (short C/D box non-coding RNA) gene encodes a group of short regulatory RNAs (reviewed in [23]). The majority of SNORDs act as guides in sequence-specific 2′-O- methylation of ribosomal RNAs and facilitate RNA processing in protein synthesis, but SNORD116 has no known complementary sequences with ribosomal RNAs [23]. Instead, the short SNORD116, processed from the paternally expressed UBE3A -antisense transcript (UBE3A-ATS) have been shown to aggregate in cloud-like patterns in the nucleus, and to affect the expression of other genes [24,25].

Several mouse model studies of PWS indicate that SNORD116 may regulate the expression of other genes in neural pathways via its effects in the hypothalamus. Firstly,

model mice with a paternally derived deletion overlapping Snord116 and IPW show phenotypes of hypotonia and feeding difficulties, closely resembling these characteristics of PWS in infancy [26]. Secondly, mouse models with selective deletions of Snord116 further implicate the role of the hypothalamus with phenotypes relevant to PWS; a selective deletion of Snord116 induced only in NPY-expressing neurons largely recapitulates the phenotypes of the mouse model with the global deletion of Snord116 [27]. Similarly, a mouse model with an adult-onset deletion of Snord116 induced in the mediobasal hypothalamus was shown to develop a phenotype of increased food consumption over time [28]. Thirdly, Coulson et al. [25] showed that Snord116 is involved in the regulation of hundreds to thousands of genes through indirect epigenetic mechanisms and downstream effects in neurological pathways; overall, ~ 23 000 differentially methylated DNA sequences (CpG sites) distributed over ~ 4300 genomic regions were shown to display a pattern of diurnally cycling methylation, which was almost entirely disrupted in the Snord116+/− model mice. Thus, mouse models and molecular studies of Snord116 indicate that disruption in mechanisms of gene regulation in the hypothalamus, due to lack of expression for SNORD116, may underlie major phenotypes of PWS (also reviewed in [29,30]).

Given that a lack of expression for SNORD116 may underlie major phenotypes of PWS, including aspects of behavior, it follows that more limited genetic alterations such as single nucleotide polymorphisms segregating in typical populations, may also exert comparably smaller effects on the same underlying neural, behavioral and developmental mechanisms. Thus, genetic variation within SNORD116 may also be expected to affect variation of psychological traits in range of typical development. In this study, we genotyped a population of typical individuals for genetic variation in the SNORD116 locus and characterized them for variation in both autism spectrum and schizotypal traits. Given that rates of both ASDs and psychotic disorders are greatly increased in PWS, we hypothesized that genetic variation in SNORD116 would be associated with individual variation in autism spectrum traits, schizotypy or both, in typical populations. In addition, based on previous studies of the NDN, MAGEL2, and UBE3A, located within the imprinted 15q11-q13 chromosome region [31,32], and the prominent psychosis phenotypes in PWS, we also hypothesize that genetic variation of SNORD116 may specifically affect phenotypes related to paranoia.

4.3. Methods

The study was approved by the ethics boards at both the University of Alberta (Pro00015728) and Simon Fraser University (2010s0554), with all participants providing prior written informed consent. Subjects were students enrolled in introductory psychology courses and received partial course credit for participation. Subjects completed the questionnaire package in pencil on paper form, in groups of approximately ten to twenty, sitting in a room with adequate spacing such that their responses could not be seen by either experimenters or other subjects. At the start of each experimental session, subjects were given a 50 ml centrifuge tube containing 30 ml of mouthwash. The mouthwash was swished for 30 s before being expelled back into the tube, which was then collected, and put on ice until the end of the session when it was frozen. Students of Caucasian descent (self-identifying) were chosen for genetic analyses, resulting in a study population of 546 undergraduate students (315 females and 231, mean age ~19 years). As only partial questionnaire data was available for a portion of individuals, we chose to exclude individuals with partial answers, resulting in a final dataset of 480 individuals with full genotype questionnaire data available. However, questionnaire data for individuals with partial answers and genotype data (540 individuals) have been included in supplementary data available with the publication.

Typical variation and forms of schizotypal traits were quantified with the Schizotypal Personality Questionnaire-Brief Revised (SPQ-BR) [33]. The questionnaire consists of 32 items using a 5-point Likert scale ranging from ‘strongly disagree’ to ‘strongly agree’. The questions are further divided across 7 subscales of personality traits and social behavior include 1. Ideas of Reference, 2. Constricted Affect, 3. Eccentric Behavior, 4. Social Anxiety, 5. Magical Thinking, 6. Odd Speech and 7. Unusual Perceptions. The subscales 2 and 4 factor into the aggregate scale of Interpersonal aspects while 1, 5, and 7 form the scale of Cognitive-Perceptual aspects and subscales 3 and 6 form the scale of Cognitive-behavioral Disorganization which further sum to total schizotypy.

The Autism Spectrum Quotient (AQ) [34] was used to quantify variation in personality traits and behaviors associated with the autism spectrum. The questionnaire includes 50 statements with responses ranging from ‘definitely agree’ to ‘definitely disagree’ (with items are scored as one or zero) which reflect the domains of 1. Social

skills, 2. Attention Switching, 3. Attention to Detail, 4. Communication and 5. Imagination and sum into the total AQ score.

To characterize genetic variation in the SNORD116 locus and to what extent this variation may be associated with AQ or SPQ scores, each individual was genotyped for 5 common single nucleotide polymorphisms (SNPs) which were chosen to reflect overall genetic variation across the locus. The CEU (Utah residents of Caucasian descent, n = 99) population in the 1000 genomes project was selected to characterize the haplotype structure of the locus (defined as chr15: 15:25048477–25110000, hg38). The haplotype structure of the locus was visualized with Haploview [35] and defined with methods described in Gabriel et al. [36]. Based on the initial characterization of the haplotype structure we chose 4 SNPs that would allow us to distinguish between the most common and the second most common haplotype in each major haplotype block within the locus. A 5th SNP, rs17115143, was later chosen, since molecular and bioinformatic analyses ([37]; The ENCODE Project Consortium, 2012) accessed through the regulomeDB web site indicated that this polymorphism may affect a binding site for ZNF274, a zinc-finger-protein which regulates the silencing of the maternally inherited copy of SNORD116 via histone modifications [38].

Mouthwash samples were stored −20 °C and genomic DNA was extracted from each sample. The mouthwash samples were first centrifuged, and the resulting pellet of buccal cells and debris was subsequently lysed with proteinase K. DNA was purified using an adapted version of the phenol-chloroform extraction method [39]. Phenol-Chloroform-isoamyl-alcohol was used to separate lysed proteins and nucleic acids into distinct phases, followed by precipitation in ethanol. The resulting pellet of nucleic acids was subsequently purified with 70% ethanol, dried in a vacufuge, and resuspended in water. DNA was stored in −20 °C for long-term and later diluted to 10–20 ng/μl for genotyping.

The SNPs rs1812905, rs17115143, rs11637737, rs8031260 and rs11161166 were genotyped using sequence-specific pairs of dye-labelled primers, each specific to one of the common polymorphisms of each SNP (Taqman human SNP genotyping assay, Thermofisher), and analyzed with a Roche Light-Cycler 96 Real-Time PCR machine. Fluorescence signal data were analyzed under Endpoint genotyping with the

LightCycler 96 Software v. 1.1.0.1320 (2011) and genotyping success for individuals with full questionnaire data available was 99.6%.

Each of the markers in the final dataset was in Hardy-Weinberg equilibrium (χ-squared test, rs1812905: $p = 0.747$, rs17115143: $p = 0.328$, rs11637737: $p = 0.375$, rs8031260: $p = 0.995$, rs11161166). ANOVA tests for differences in means between genotype groups, specified as homozygote for common polymorphism, heterozygote, and homozygote for rare polymorphism, were conducted for each SNP marker, for both sexes combined as well as for males and females separately. Our decision to conduct tests for females and males separately stemmed from results of previous studies showing sex differences on AQ and SPQ phenotypes in typical populations [40–42], and sex differences in some PWS behavioral phenotypes [43,44]. We also conducted two-way ANOVA tests for sex differences and interactions between sex and genotype for all AQ and SPQ phenotypes, on all 5 SNORD116 SNPs.

Dominant-recessive models (GT1+GT2 vs GT3, and GT1 vs GT2+GT3) and post-hoc analysis (Tukey's Honest Significance Difference, (Tukey, 1949)) for evaluating the effect of each genotype were also conducted for all ANOVA tests. The post-hoc analysis also included a comparison of the two homozygote genotypes in relation to one another (GT1 vs GT3), given that this test avoids the ambiguity involved in allele expression patterns in heterozygotes under imprinted gene expression. All ANOVA and post-hoc tests were conducted with R version 3.5.1. (R Core Team, 2018). In addition, to analyze the data at the level of multilocus haplotypes, combined haplotypes of the 5 SNORD116 SNPs for each individual were estimated with PHASE 2.1 [45,46] and haplotype-based analyses were conducted with PLINK 1.07 [47].

4.4. Results

Genetic variation of SNORD116

All individuals within the study population were genotyped for 5 SNPs within SNORD116 (rs1812905 G/T, rs17115143 G/A, rs11637737 G/A, rs8031260 G/A and rs11161166 T/A) and each SNP was analyzed in respect to the three genotypes; the common homozygote of the polymorphism, the heterozygote and the rare homozygote (referred as GT1, GT2 and GT3 respectively). The five genotyped SNPs of SNORD116

showed high to moderate degrees of linkage disequilibrium with each other (D' between 1.0 and 0.8, r2 between 0.7 to 0.4; see Supplementary Table 1); their genotypes are thus non-independent, although the r2 values well under 1.0 indicate that they cannot substitute for one another.

Analysis of variance for AQ and SPQ phenotypes

Across all five SNPs, genotype variation was nominally, significantly, and exclusively associated with variation on the SPQ-BR Ideas of Reference subscale. These effects were found among females (ANOVA; GT1 vs GT2 vs GT3, unadjusted p values from 0.0016 to 0.0369, Table 3 and Supplementary Table 3, available with the publication), but not among males (ANOVA, GT1 vs GT2 vs GT3, unadjusted p values 0.33 to 0.78, Table 3). These results were non-significant after Benjamini-Hochberg adjustments for multiple testing. Analyses with two-way ANOVA tests indicated significant interactions between genotype and sex for the Ideas of Reference subscale, for 3 of the 5 SNPs (Supplementary Table 5, available with publication).

Table 4. AQ and SPQ phenotype data for each rs11161166 genotype and dominant-recessive genotype models with ANOVA tests between genetic variance of the SNORD116 SNP and AQ and SPQ phenotypes. A Benjamini-Hochberg false discovery rate (FDR) was applied for 12 independent comparisons. Results for post-hoc comparisons (Tukey) between the two homozygote genotypes and dominant-recessive genotype models are also shown.

Phenotype (Females)	**GT1 (74) Mean/S.D.**	**GT2 (138) Mean/S.D.**	**GT3 (62) Mean/S.D.**	***p* (GT1 vs GT2 vs GT3)**	***p* FDR**	***p* GT1 (74) vs GT3 (62)**	***p* GT1+2 (212) vs GT3 (62)**	***p* GT1 (74) vs GT2+3 (200)**
Social (AQ)	2.5/2.2	2.4/2.2	2.3/2.0	0.826	0.993	0.810	0.612	0.616
Switch (AQ)	5.0/1.9	4.9/2.1	4.8/1.9	0.767	0.993	0.782	0.688	0.480
Detail (AQ)	5.5/2.1	5.2/2.2	5.3/2.2	0.579	0.993	0.865	0.947	0.335
Comm (AQ)	2.3/1.7	2.3/1.9	2.3/1.7	0.993	0.993	0.996	0.908	0.991
Imag (AQ)	2.1/1.6	1.9/1.5	2.2/1.4	0.401	0.993	0.782	0.230	0.848
Autism (AQ)	17.4/6.0	16.6/5.9	16.9/5.3	0.664	-	0.891	0.967	0.401
Ideas (SPQ)	**17.7/4.5**	**16.6/4.3**	**15.8/3.4**	**0.034**	0.408	**0.029**	0.066	**0.019**
Constrict (SPQ)	14.5/5.0	14.6/5.2	14.7/4.8	0.975	0.993	0.972	0.848	0.859
Eccentric (SPQ)	12.7/3.8	11.6/4.0	11.8/3.8	0.148	0.828	0.338	0.665	0.052
Anxiety (SPQ)	11.7/4.2	11.5/4.3	11.7/3.9	0.925	0.993	0.999	0.856	0.786
Magic (SPQ)	8.9/4.1	8.5/3.7	8.4/3.1	0.675	0.993	0.723	0.687	0.379
Speech (SPQ)	13.6/2.9	13.4/2.9	13.1/3.0	0.690	0.993	0.666	0.455	0.514
Perceptions (SPQ)	10.3/2.9	10.5/3.1	9.7/2.7	0.207	0.828	0.436	0.084	0.855
Interpersonal (SPQ)	26.2/7.8	26.1/8.3	26.4/7.7	0.972	-	0.992	0.830	0.976
Cog.percep (SPQ)	36.9/8.8	35.5/8.6	33.9/6.2	0.112	-	0.091	0.080	0.097
Disorganized (SPQ)	26.3/5.6	25.0/5.9	24.9/5.8	0.257	-	0.349	0.501	0.100
Schizotypy (SPQ)	89.4/18.1	86.7/17.1	85.2/15.1	0.331	--	0.325	0.328	0.167

Phenotype (Males)	GT1 (67) Mean/S.D.	GT2 (107) Mean/S.D.	GT3 (32) Mean/S.D.	*p* (GT1 vs GT2 vs GT3)	*p* FDR	*p* GT1 (67) vs GT3 (32)	*p* GT1+2 (174) vs GT3 (32)	*p* GT1 (67) vs GT2+3 (139)
Social (AQ)	1.9/1.9	1.8/1.8	2.4/2.2	0.257	0.909	0.361	0.102	0.788
Switch (AQ)	4.6/1.8	4.5/1.7	4.9/2.0	0.462	0.909	0.745	0.270	0.825
Detail (AQ)	5.4/2.2	5.4/2.0	5.3/2.0	0.926	0.991	0.938	0.696	0.922
Comm (AQ)	2.0/1.7	2.0/1.7	2.0/1.7	0.991	0.991	0.992	0.929	0.903
Imag (AQ)	2.4/1.7	2.5/1.5	2.6/1.8	0.929	0.991	0.925	0.711	0.837
Autism (AQ)	16.3/5.4	16.2/4.8	17.2/5.2	0.602	-	0.711	0.324	0.929
Ideas (SPQ)	15.9/4.1	16.6/3.8	16.5/4.0	0.571	0.909	0.765	0.789	0.290
Constrict (SPQ)	14.4/4.1	15.3/4.0	15.4/5.9	0.397	0.909	0.544	0.577	0.177
Eccentric (SPQ)	12.0/3.9	12.1/3.9	12.7/3.3	0.667	0.909	0.665	0.375	0.683
Anxiety (SPQ)	10.4/3.5	11.1/3.7	11.3/3.3	0.413	0.909	0.506	0.504	0.194
Magic (SPQ)	7.3/3.6	6.9/3.2	6.8/2.8	0.682	0.909	0.731	0.634	0.399
Speech (SPQ)	12.4/2.9	12.7/2.7	13.3/3.0	0.312	0.909	0.278	0.166	0.303
Perceptions (SPQ)	9.9/2.3	9.9/2.7	11.0/2.6	0.09	0.909	0.107	0.028	0.437
Interpersonal (SPQ)	24.8/6.6	26.3/6.5	26.7/8.1	0.288	-	0.414	0.476	0.120
Cog.percep (SPQ)	33.1/6.6	33.4/7.2	34.3/7.2	0.735	-	0.715	0.463	0.627
Disorganized (SPQ)	24.4/5.6	24.7/5.4	26.0/5.2	0.376	-	0.349	0.184	0.415
Schizotypy (SPQ)	82.3/14.1	84.5/14.1	87.0/13.0	0.283	-	0.268	0.217	0.187

Dominant-recessive models among females (GT1 vs GT2+3) showed that individuals homozygous for the common alleles of each SNP also had nominally significantly higher mean scores for the Ideas of reference -subscale as compared to the two other genotypes (Table 3 and Supplementary Table 3.) We also compared the two homozygote groups to one another using a post-hoc comparison (Tukey's HSD, GT1 vs GT3). Given that the SNORD116 locus shows imprinted expression and parental genotypes are not available in these data; as such, the expressed allele of heterozygotes is unknown. For each SNP, these analyses indicated that female individuals homozygous for the common allele showed nominally significant higher mean scores as compared to the other homozygote genotype, expressing the variant allele (Table 3 and Supplementary Table 3.) Finally, we identified nominally significant effects for the subscales of Social Skills (on the AQ) and Disorganization (on the SPQ) among

males in a dominant-recessive (GT1+2 vs GT3) model between rs1812905 genotypes (Supplementary Table 3.), but these effects were not found with any of other SNPs and were non-significant after Benjamini-Hochberg adjustments for multiple testing.

Haplotype analysis

Considering the high degree of linkage disquilibrium between the 5 SNPs, we also combined the information provided by the genetic markers in a haplotype analysis using the expectation-maximization algorithm in PLINK [47]. The three most common haplotypes represented ~ 80% of the genetic variation present in the study population (Supplementary Table 2, available with the publication) The haplotype analysis, comparing a model assuming a unique effect for each haplotype to a null model where all haplotypes have a similar effect on the phenotype showed a significant result among females, for the SPQ-BR Ideas of Reference subscale, indicating that the two most common haplotypes, associated with the common and variant alleles of each genotyped SNPs respectively, show opposite phenotypic effects (Table 4). The haplotype model was not significantly more probable as compared to the null model among males (overall F-statistic comparison, $p = 0.536$, all tests with $p > 0.30$).

Table 5.Haplotype model analyses based on the 3 most common haplotypes of all 5 SNORD116 SNPs, and variation of the Ideas of reference -phenotype among females and males. The results of the haplotype model are based on F-test comparing null and alternate models, with the alternate model assuming that each haplotype has a unique effect on the phenotype, while the null model assumes all haplotypes to have a similar effect (Overall f-statistic p = 0.0414 among females, p = 0.536 among males).

Haplotype and Gender	Freq	Ideas weighted Mean/SD	Haplotype p
Females			
GGGAA	0.379	16.19/3.98	0.0247
GGGGA	0.104	16.5/4.47	0.688
TAAGT	0.517	17.23/4.42	0.0126
Males			
GGGAA	0.342	16.27/3.63	0.712
GGGGA	0.097	16.92/4.01	0.346
TAAGT	0.561	16.32/3.7	0.352

4.4. Discussion

Our primary results showed a nominally significant association between the SNORD116 SNP genotypes and the SPQ-BR Ideas of Reference subscale, a trait that can be broadly characterized as paranoid ideation. The effect was significant among females, but not among males or with both sexes. The association was apparent with all five of the SNPs, and the effect among females was similarly shown in analyses comparing homozygote groups, which are relevant considering the imprinted expression of this locus. Whereas the SNPs analyzed individually showed only nominal, unadjusted associations, the haplotype analyses indicate that genetic variation of SNORD116, as characterized by the two most common haplotypes (TAAGT and GGGAA) (Table 4) was significantly associated with variation in SPQ-BR Ideas of Reference subscale among females.

These findings are particularly relevant in the context of two previous studies (Table 5) which also characterized effects of genetic variation within the imprinted 15q11-q13 chromosome region in populations of typical individuals. Firstly, genetic variation within the UBE3A locus showed an association with SPQ Total Schizotypy [32]. Individuals homozygous for the rare T-allele of rs732739 variant allele showed a significantly lower mean schizotypy score as compared to other genotypes, and the strongest effect among all subscales was shown for Ideas of Reference. This effect was significant among females and both sexes combined, but not among males [32].

Table 6. Summary of the main results and genotypes of the current and previous studies characterizing variation of AQ and SPQ phenotypes associated with genetic variation of PWS-AS loci. Haplotype sizes are based on estimations of linkage disequilibrium between markers, with the CEU (Caucasian individuals from Utah) 1000 genomes population.

MAGEL2 & NDN (31. Crespi et al., 2018)	SNORD116 (this article)	UBE3A (32. Salminen et al., 2019)
Ideas of Reference *p* = 0.00098 (GT1 vs GT2 vs GT3)	**Ideas of Reference *p* = 0.0369 to 0.0016 (5 SNPs)** (GT1 vs GT2 vs GT3)	**Ideas of Reference *p* = 0.00564** Constricted Affect *p* = 0.0176 Eccentric Behavior *p* = 0.0194 Interpersonal scale *p* = 0.0163 Total Schizotypy *p* = 0.0184
Significant among males and both sexes, but not females	Significant only among females	Significant among females and both sexes, but not males
CC(300) vs CT(397) vs TT(134) (rs850807)(both sexes) (estimated haplotype size ~ 20 kb)	TAAGT vs GGGAA (SNPs within ~ 50 kb of each other) GGGAA/TAAGT (89 females) TAAGT/TAAGT (59 females) GGGAA/GGGAA (35 females) GGGGA/TAAGT (28 females) GGGGA/GGGAA (16 females)	CC(382) vs CT(111) vs TT(8) (rs732739) (females) (estimated haplotype size ~ 0.1mb)

Secondly, genetic variation for the MAGEL2-NDN locus, as characterized by rs850807, also showed a significant association exclusively with the SPQ-BR Ideas of Reference subscale [31]. The effect was significant among males and both sexes, while a similar, nominally significant effect was shown among females under the dominant-recessive model ((CC vs CT+TT), analysis based on data published with the article [31]). The SNORD116 SNPs reported in this study are not fully independent of SNPs within of UBE3A, but show low degrees of linkage disequilibrium with them (D' = ~ 0.2, r2 = 0.01, CEU 1000 genomes population), as characterized by rs732739 [32] and are in linkage equilibrium with rs850807 [31] (D = 0.097, r2 = 0.007 in CEU) (Table 3.) Given the low degrees of linkage disequilibrium between the three loci, our results imply that these three loci may either mediate Ideas of Reference phenotypes through a common

mechanism or neural pathway independently of one another, or that the loci interact in regulation of gene expression in some interdependent manner.

These results fit with evidence from previous work on how different dosages of both paternally and maternally expressed genes may explain the heightened incidence of psychoses and bipolar disorders in PWS individuals with the mUPD genotype, as shown in both case studies and large cohorts [5,6,12], (see supplementary material in [31] for a comprehensive list of studies). The mUPD genotype of PWS involves both doubled dosage of the maternally expressed UBE3A, as well as a lack of expression for the ~ 20–25 paternally expressed genes, shared across all genotypes of PWS. Hence, it has been previously suggested that the combination of both increased dosage of UBE3A and the lack of expression for the paternally expressed genes of the PWS locus may predispose the brain for development of psychosis in PWS [12]. However, it is not clear if the greatly increased risk for development of psychosis with the PWS mUPD genotype is due to an epistatic effect between one paternally expressed locus and the maternally expressed UBE3A or due to additive effects resulting from lack of expression for several paternally expressed genes. Our results imply that lack of expression for more than one paternally expressed locus may be involved with the psychosis phenotype of PWS and that genetic variation segregating among typically developing individuals may exert similar but much weaker effects, particularly with behavioral phenotypes relevant to Ideas of Reference.

Relevant mouse and cell models of PWS and Angelman syndrome (AS) indicate three separate mechanisms for how expression of imprinted genes in the 15q11-q13 chromosome region may interact with each other. Firstly, the short SNORD116 RNAs are typically expressed from the paternally inherited chromosome as a part of the long UBE3A-ATS and processed into their shorter forms, which are known to aggregate in cell nuclei, and may be involved in regulating gene expression and methylation of other loci [23]. Secondly, the UBE3A-ATS overlaps with SNURF-SNRPN, SNORD115 and UBE3A, (but not the MAGEL2-NDN loci) and it has been hypothesized to be involved in the epigenetic silencing of the paternally inherited copy of UBE3A in neurons. Specifically, transcription of UBE3A and UBE3A-ATS is thought to occur simultaneously, but in opposite direction, in the paternally inherited chromosome, and thus, the transcription of UBE3A-ATS would physically interfere with the transcription of the UBE3A mRNA (reviewed in [48]). GC-rich sequences within SNORD116 may also pair

with open single-stranded DNA during transcription of the UBE3A-ATS, forming RNA:DNA loops, which may further regulate levels of UBE3A-ATS expression in typical neuron development [49]. Sequence level variation within the SNORD116 locus may thus affect the expression of UBE3A and other genes within the UBE3A-ATS indirectly. Thirdly, the maternally inherited copy of SNORD116 is typically silenced by an epigenetic mechanism separate from the PWS imprinting center (reviewed in [50]). The maternal copy of SNORD116 locus thus acts as a recognition site for the ZNF274 zinc-finger protein that regulates the silencing of several paternally expressed loci via histone modifications [50]. Inactivating the SETDB1 gene which interacts with the ZNF274 zinc-finger protein at the maternal chromosome also activates the expression of other paternally expressed loci (excluding Necdin) in a PWS mouse model without significantly affecting the expression of Ube3a [38]. While disruptions in silencing mechanisms of imprinted genes may not be directly relevant to genetic variation segregating in non-clinical populations, mechanisms affecting the regulation of gene expression for several imprinted loci in tandem may imply that typical expression of imprinted loci could be similarly co-regulated, which would in part explain how genetic variation of different loci might affect phenotypic variation in an interdependent manner.

The effects shown here for the 5 SNORD116 SNPs (Table 3. and Supplementary Table 3.) are significant only among one gender group, which may implicate sex-specific differences in the mechanisms or common neural pathways that the locus affects. We note that statistical analyses on whether psychosis phenotypes differ between the sexes have not been reported in any large-scale studies or meta-analyses concerning PWS [5,12–15,51,52]. However, a behavioral study of PWS individuals profiling each participant with one of four psychiatric phenotypes (‘basic’, characterized by immaturity, ‘impulsive’, ‘compulsive’ or ‘psychotic’) found a significant positive association between male sex and the impulsive behavior profile, characterized by low tolerance to frustration and open aggression [44]. Significant sex differences in food-related problem behaviors (as measured by Food Related Problems Questionnaire, FRPQ) have also been shown among PWS individuals [43]. Specifically, males with a deletion genotype had a higher score on average as compared to females with the same genotype, while males with mUPD genotype showed lower scores as compared to females of the same genotype. Finally, a later onset of hyperphagia for the mUPD genotype as opposed to the deletion genotype was shown among females but not among males [53].

Sex-specific differences in methylation levels have also been identified for both imprinted and non-imprinted loci [54,55] and in addition, a number of loci appear to show an imprinted pattern of expression in one sex, but not the other [56]. Sex may also affect the regulation of imprinted genes in 15q11-q13 locus, as typical females were found more likely (compared to males) to show monoallelic expression of the imprinted gene ATP10A while most individuals showed a biallelic expression pattern [57]. These studies imply that behavioral phenotypes of PWS may interact with the PWS genotypes either through epigenetic or developmental mechanisms. Thus, analyzing the sexes separately or including sex as a factor in the analysis would be important in either case.

Several limitations apply to this study. First, our study population consisted solely of Caucasian undergraduate students (mean age ~ 19 years) and thus, any possible interactions between age, genotype and phenotype or socio-economic factors cannot be ruled out. Replication in independent populations, and ones that differ in ethnicity, is recommended. A previous study has indicated that younger subjects show comparably higher Ideas of Reference scores on average [58], which may suggest that age, in addition to sex and genotype may affect the phenotype for Ideas of Reference, but our results do not lend themselves for the evaluation of any such effect. Second, we have analyzed only a subset of PWS phenotypes related to autism spectrum traits and schizotypy; future studies can usefully also include phenotypes related to hyperphagia, obsessive behavior, and other PWS-associated phenotypes.

In conclusion, we have identified an independent effect of genetic variation in SNORD116 on psychological phenotypes related to paranoid ideation that fits with previous evidence on greatly increased incidences of psychotic conditions in PWS, especially involving the genotype of maternal disomy [5,6,12,14,15]. Beyond psychotic conditions, tendencies for paranoid ideation may also be especially relevant in the context of social behaviors with PWS, as also shown in a recent study indicating that individuals with PWS are particularly prone to misjudge the intentions of others in a negative light [59]. Our results indicate that genetic variation of PWS loci segregating among non-clinical populations may also mediate psychological tendencies to assign negative intentions to others, the harmful extreme of which may be perceived as paranoid ideation. The results also support a model of psychosis liability that is mediated by interactions among multiple loci, which can be tested directly for this locus in future work.

CRediT authorship contribution statement

Iiro Salminen: Investigation, Data curation, Formal analysis, Software, Visualization, Writing - original draft, Writing - review & editing. Silven Read: Investigation, Data curation, Methodology, Software. Pete Hurd: Conceptualization, Funding acquisition, Investigation, Data curation, Methodology, Supervision, Project administration, Resources, Writing - review & editing. Bernard Crespi: Conceptualization, Funding acquisition, Supervision, Project administration, Validation, Resources, Methodology, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Chapter 5. Do the diverse phenotypes of Prader-Willi syndrome reflect extremes of covariation in typical populations?

5.1. Abstract

The phenotypes of human imprinted neurogenetic disorders can be hypothesized as extreme alterations of typical human phenotypes. The imprinted neurogenetic disorder Prader-Willi syndrome (PWS) features covarying phenotypes that centrally involve altered social behaviors, attachment, mood, circadian rhythms and eating habits, that can be traced to altered functioning of the hypothalamus. Here, we conducted analyses to investigate the extent to which the behavioral variation shown in typical human populations for a set of PWAS-associated traits including autism spectrum cognition, schizotypal cognition, mood, eating, and sleeping phenotypes shows covariability that recapitulates the covariation observed in individuals with PWS. To this end, we collected data from 296 typical individuals for this set of phenotypes, and showed, using principal components analysis, evidence of a major axis reflecting key covarying PWS traits. We also reviewed the literature regarding neurogenetic syndromes that overlap in their affected traits with PWS, to determine their prevalence and properties. These findings demonstrate that a notable suite of syndromes shows phenotypic overlap with PWS, implicating a large set of imprinted and non-imprinted genes, some of which interact, in the phenotypes of this disorder. Considered together, these findings link variation in and among neurogenetic disorders with variation in typical populations, especially with regard to pleiotropic effects mediated by the hypothalamus. This work also implicates effects of imprinted gene variation on cognition and behavior in typical human populations.

Keywords: Prader-Willi Syndrome, Hypothalamus, Genomic Imprinting, Schizophrenia, Attachment, Sleep, Feeding.

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5.2. Introduction

The hypothalamus is a highly conserved region in vertebrate brains, that has been found to regulate physiological homeostasis via several neural mechanisms and specific signaling cascades, including biorhythmicity, sleep-wake control and the regulation of satiety and hunger states (reviewed in [1,2]). The primary functions of the hypothalamus have been highlighted by syndromes involving hypothalamic dysfunction, which demonstrate phenotypes involving disrupted metabolic control and altered regulation of sleep [3,4]. Furthermore, mice model studies that involve knockouts of genes active in the hypothalamus tend to show altered phenotypes of food intake, sleep or both [4–8]. Recent gene knockout studies also appear to show that hypothalamic neuron populations primarily expressing a specific neural transmitter may simultaneously exert pleiotropic effects on several behaviorally distinct aspects of energy homeostasis. For example, model mice with a specific lack of expression for the melatonin-concentrating hormone (MCH), typically expressed exclusively in the hypothalamus, show a pleiotropic phenotype with increased wakefulness and an increase in activity during fasting periods, which may indicate that MCH neurons typically function to promote sleep and suppress food-seeking behaviors for conservation of energy balance [9]. In contrast, mouse knockout studies with orexin-expressing neurons have shown that orexin may simultaneously promote feeding, energy expenditure and wakefulness [1,10,11]. While pleiotropy in homeostatic neural circuits may merely represent an ancestral state conserved across vertebrate lineages, it may also be theorized that hypothalamic neural circuits have been selected to promote a multitude of co-adapted behavioral and physiological responses to facilitate comprehensive behavioral strategies. The pleiotropy of hypothalamic neural circuits appears to extend beyond homeostatic mechanisms, as animal models have also shown that maternal care, which is intricately linked to both feeding and sleep-wake cycles in infancy, is in part mediated by hypothalamic neural circuits in both the mother and the offspring [12,13]. Finally, such pleiotropic effects in hypothalamic control may sometimes be subject to maladaptive effects in humans, as eating disorders, mood and anxiety and schizophrenia may all be associated with dysfunctions of hypothalamic neural circuits [14,15]

Syndromic conditions of hypothalamic dysfunction, particularly neurogenetic conditions involving altered gene dosage, may be hypothesized as extreme

manifestations of genetic regulatory mechanisms involved in hypothalamic function. Prader-Willi syndrome, a neurogenetic disorder involving hypothalamic dysfunction [3] highlights alterations in genomic imprinting, an epigenetic regulatory mechanism in which a specific gene is expressed mainly or entirely from one parental allele. Imprinted genes are typically found in evolutionarily conserved clusters where several epigenetic mechanisms including DNA methylation, non-coding RNAs, histone modifications and sequence-specific regulatory proteins function in tandem to regulate imprinted gene expression [4,16–18]. In PWS, affected individuals carry a genomic alteration, typically due to a meiotic error, which leads to a complete lack of expression for a co-regulated set of ~ 20 imprinted genes which are typically expressed solely from paternally inherited alleles in healthy individuals [19]. The neurobehavioral phenotype of PWS shows evidence for disruption of homeostatic mechanisms regulated in the hypothalamus: PWS involves failure to thrive and comparably increased somnolence particularly in infancy, followed by gradual development of hyperphagia, with lack of satiety and obsessive food-seeking behaviors later in childhood [20,21]. The behavioral phenotypes of PWS also extend beyond altered homeostatic mechanisms, as individuals with PWS typically show mood disturbances, anxiety and frustration towards changes in routine [22].

Individuals with PWS also show a high prevalence of psychotic symptoms, particularly within the genotype of maternal uniparental disomy (matUPD) which involves both a lack of expression for the affected paternally expressed imprinted genes and increased dosage for the maternally expressed imprinted gene UBE3A. Several independent cohort studies have shown that ~ 60 % of PWS individuals with the matUPD genotype develop psychotic symptoms in adulthood, and thus, it has been theorized that an imbalance in genomic imprinting via increased dosage of UBE3A may predispose PWS matUPD individuals for development of psychosis [23–26]. The hypothesis may thus imply that the matUPD genotype of PWS represents an extreme imbalance towards psychological phenotypes mediated by maternally expressed imprinted genes [21,27]. Interestingly, it has also been shown that dysfunctions of the hypothalamus are also associated with psychotic disorders [14,28], which might further imply that the extreme psychological phenotype shown with the matUPD genotype of PWS is causal due to hypothalamic dysfunction.

Both within and beyond the scope of PWS, mice models of genomic imprinting further show that paternally expressed genes in particular may take part in regulation of

hypothalamic function and simultaneously modify both homeostatic and social behaviors [4]. In a genome-wide characterization of imprinting in the mouse brain, a significantly larger number of paternally expressed imprinted genes were expressed in the adult hypothalamus, as compared to the number of maternally expressed imprinted genes, while the embryonic mouse brain showed a comparably larger number of active maternally expressed genes [29]. A dysfunction of a developmental transition in genomic imprinting and hypothalamic control of feeding and somnolence may in part explain the early failure to thrive and the gradual development of hyperphagia in the PWS phenotype [30,31].

Mouse models of PWS also show evidence of pleiotropic phenotypes mediated by altered hypothalamic function, from several lines of evidence. Firstly, SNORD116-deficient model mice show phenotypes of altered food intake [8,32] and sleep-wake cycles [33] and also show a reduced number of orexin-expressing neurons in their lateral hypothalamus as compared to controls [7]. As orexin-expressing neurons promote wakefulness and food consumption [1], these results show an interesting parallel to the PWS phenotype. Lack of expression for MAGEL2 has also been shown to produce a reduction in orexin-expressing neurons, and thus SNORD116 and MAGEL2 may act in a dose-dependent manner in PWS ([6,7]).

Secondly, the MAGEL2 and NDN genes have also been shown to interact with circadian clock proteins in the suprachiasmatic nucleus, affecting phenotypes of circadian rhythmicity [6,34]. Circadian rhythmicity affects energy homeostasis and the regulation of sleep-wake cycles via diurnally regulated epigenetic cascades affecting gene expression patterns of several thousands of genes downstream [35]. It has also been shown that lack of expression for SNORD116 alters the diurnal rhythm of gene methylation in model mice, potentially affecting energy homeostasis and epigenetic regulation of several thousands of differentially methylated sites [36]

Thirdly, the social behavioral changes found in PWS may also be altered by a hypothalamic imbalance of oxytocin and oxytocin receptors [37]. Individuals with PWS show reduced numbers of oxytocin-expressing neurons [38,39] and reduced gene expression for the oxytocin receptor gene, OXTR [40], as compared to controls. However, individuals with PWS also appear to show elevated levels of oxytocin in both blood and cerebrospinal fluids, as compared to controls [41,42], which may further

indicate a disruption of feedback in oxytocin signaling. Mouse models of PWS, with deletions of MAGEL2 further show that lack of expression for MAGEL2 leads to deficits in social behaviors, but the phenotype may be rescued with post-natal oxytocin treatment [43,44]. Thus, evidence from both PWS and other neurogenetic disorders involving hypothalamic dysfunction and animal models of genomic imprinting show that hypothalamic mechanisms may simultaneously affect phenotypes of sleeping, eating, social behaviors and affection, perhaps via multiple co-adapted and overlapping neural pathways.

The prevalence for the traits analyzed here within the diagnostic criteria for PWS range from ~90 % for hyperphagic behaviors to 30 - 50 % for minor criteria such as sleep disturbances [45]. While individual variation in behavior is known within PWS, the shared genetic origin and further evidence from animal models of hypothalamic function implies that intellectual disability alone cannot account for the observed suite of behaviors in PWS [46], thus further implying that phenotypes of social behaviors and affection may also be affected by hypothalamic mechanisms.

Amongst typical human populations, co-adapted pleiotropic phenotypes of hypothalamic function might be manifested as overlapping effects across multiple co-associated phenotypes. As we might suppose that small-effect genetic and epigenetic variation for both imprinted and non-imprinted genes involved in regulation of hypothalamic function may also be circulating among typical human populations, might we also expect to detect non-clinical degrees of co-occurring behavioral variation across a corresponding set of behavioral phenotypes among typically developing individuals? To evaluate this hypothesis, we phenotyped a population of typical individuals for psychological and behavioral traits central to PWS, including autism spectrum cognition, schizotypal cognition, mood, eating, and sleeping, and we estimated the pattern and degree of covariation shown for these traits via principal component analysis. While this analysis is limited to behavioral variation and cannot directly implicate specific underlying genetic variation, the co-occurrence of the behavioral variation shown across the relevant phenotypes resembles the set of phenotypes also altered in PWS, thus linking a neurodevelopmental axis highlighted by phenotypical variation in neurodevelopmental disorders to behavioral variation among populations of typical individuals.

5.3. Materials and methods

Questionnaire data was collected between 2019 and 2020 from 589 psychology students (416 females, 170 males, 3 other) at Simon Fraser University and the University of Alberta. To ensure quality of the collected data, the analysis was limited to individuals who had answered every question in the set of questionnaires. After these procedures, a population of 296 individuals (106 males, 190 females) remained. Ethical approval was obtained from the Simon Fraser University and University of Alberta Departments of Research Ethics. The study was advertised in a university questionnaire study portal for psychology students and course credits were offered for participation. The students all provided written informed consent.

A set of demographic variables including biological sex, age and self-identified ethnicity were collected from all participants. The Autism spectrum quotient (AQ) [47] was used to characterize the extent of variation for autism spectrum cognitive traits and social behaviors, in ranges relevant to typical human populations while typical variation in schizotypy was assessed with the Schizotypal Personality Questionnaire-Brief Revised (SPQ-BR) [48] questionnaire. Variation in styles of attachment across relationships with the individual's mother or mother-like figure, father or father-like figure, close friend and romantic partner were characterized with the Experiences in Close Relationships – Relationship Structures Questionnaire (ECR-RS) [49]. The Oxford Happiness Questionnaire [50] was used in assessing the mood and psychological well-being of the subjects and answers for depression items of the NEO Personality Inventory (NEO-PI) [51] were also collected to assess the phenotypes of mood. A modified version of the Pittsburgh Sleep Quality Index [52] without the questions filled out by the partner or roommate was collected to assess the variation in the sleeping habits of the students. The Reduced Morningness-Eveningness Questionnaire (RMEQ) [53] was used to assess the ranges of variation in biological rhythmicity. The Dutch Eating Behavior Questionnaire (DEBQ) [54] was used to characterize variation in eating habits and preferences. The questionnaire data was typically collected in early to late afternoon although the timing of the collection was not specifically regulated.

The principal components analysis was conducted in R (stats 3.6.3, 2020) with the princomp function, which calculates eigenvalues and eigenvectors from either a covariance or a correlation matrix of multiple variables to express the covariability within

a given data set in principal components. As the set of questionnaires varied highly across their scales, we chose to restrict our analysis to a correlation matrix, which standardizes the effects for each of the variables.

The analysis was further limited to variables considered relevant for hypothalamic function, in respect of the behavioral phenotype of PWS, including total score autism spectrum cognition (AQ), the dimension of cognitive perception in schizotypy (SPQ-BR), attachment-related anxiety in relationship with the individual's mother (ECR-RS), total score for the Pittsburgh Sleep Quality Index and the scale of Emotional eating (DEBQ). Two different measures for phenotypes of depression and foul mood were also included: the total score for the Oxford Happiness Questionnaire was reversed by subtracting the individual's score from the highest score in the data set, creating a scale from 0 to 138 to represent one's unhappiness in themselves and their circumstances. The scored values for the depression-endorsing items (8 in total) of the NEO personality inventory were also summed up to create an alternative measure of low mood.

A literature review (supplementary Table 1.) was also conducted on known syndromes and case studies of disorders displaying 'Prader-Willi –like' phenotypes. Articles were searched using the Web of Science search engine using 'Prader-Willi' and the known, hypothalamic phenotypes as search terms. The review was further supplemented by manual searches using references of previously published reviews [55,56].

5.4. Results

The analyzed data consisted of a set of 296 individuals for whom complete data was available (190 females and 106 males) with a mean age of 19.6 years (standard deviation of 3.4). The majority of the participants reported their ethnicity as either Caucasian or Asian descent, 46 % and 27 % respectively. Within the combined population, the reversed score for the OHQ, representing unhappiness, correlated strongly and positively with all of the other traits hypothesized to be relevant for the PWS phenotype of hypothalamic dysfunction while the other traits were also strongly and positively correlated with one another (Table 6). The alternative measure for low mood (NEO-PI depression items) also correlated strongly and positively with traits relevant to

the PWS phenotype, indicating a high degree of robustness for the interaction between low mood and PWS-related phenotypes. The correlations, calculated via Pearson’s product-moment correlation, remained significant after correction for false discoveries with the Benjamini-Hochberg method. Finally, we find that the phenotypes of Restrained eating and External eating, which are not characteristic of PWS and can thus serve as forms of 'control' variables, did not show significant correlations with the reversed OHQ score or the total PSQ score but did correlate strongly with Emotional eating (results not shown).

Table 7. Pearson moment-product correlations across a of set psychological and behavioral traits deemed to reflect typical degrees of variation in phenotypes relevant to the phenotype of Prader-Willi syndrome. Correlations remained significant after Benjamini-Hochberg correction for false discoveries, as shown in the p adjusted -column.

Phenotype x	Phenotype y	Pearson's correlation	t	df	p	P adjusted
AQ total score	Cognitive-Perceptual scale (SPQ-BR)	0.174	3.028	294	0.003	0.004
	Neo-pi depression items	0.298	5.360	294	1.69^{-7}	5.07^{-7}
	OHQ-reversed	0.320	5.7940	294	1.77^{-8}	7.43^{-8}
	Anxiety in mother relationship (ECR-RS)	0.172	2.997	294	0.003	0.004
	PSQ total score	0.165	2.8612	294	0.005	0.005
	Emotional eating (DEBQ)	0.189	3.3009	294	0.001	0.002
Cognitive-Perceptual scale (SPQ-BR)	OHQ-reversed	0.241	4.2522	294	2.85^{-5}	6.65^{-5}
	Neo-pi depression items	0.373	6.8855	294	3.49^{-11}	1.83^{-10}
	Anxiety in mother relationship (ECR-RS)	0.171	2.9843	294	0.003	0.004
	PSQ total score	0.184	3.2156	294	0.001	0.002
	Emotional eating (DEBQ)	0.222	3.9031	294	0.0001	2.25^{-4}
OHQ-reversed	Anxiety in mother relationship (ECR-RS)	0.243	4.3031	294	2.30^{-5}	6.04^{-5}
	Neo-pi depression items	0.675	15.693	294	2.20^{-16}	2.31^{-15}
	PSQ total score	0.458	8.8298	294	2.20^{-16}	2.31^{-15}
	Emotional eating (DEBQ)	0.238	4.2057	294	3.46^{-5}	7.27^{-5}
Neo-pi depression items	Anxiety in mother relationship (ECR-RS)	0.212	3.7263	294	2.33^{-4}	4.08^{-4}
	PSQ total score	0.447	8.569	294	6.00^{-16}	4.20^{-15}
	Emotional eating (DEBQ)	0.311	5.6199	294	4.44^{-8}	1.55^{-7}
Anxiety in mother relationship (ECR-RS)	PSQ total score	0.145	2.5041	294	0.013	0.0135
	Emotional eating (DEBQ)	0.119	2.0463	294	0.042	0.0416

The first principal component (PC1) accounted for ~ 35 % of the total variance within the population and included positive loadings for all of the PWS-related traits. (see table 7.) PC2 accounted for ~ 15 % of the variance and included negative loadings for the reversed OHQ scores, and the total PSQI score, while showing positive loadings for the other traits (See Tables 7 and 8). The three largest principal components accounted for 65 % of the total variance within the combined population. Alternative analyses using the NEO-PI depression items instead of the reversed OHQ score performed in a similar manner, but PC2 also included a negative loading for Emotional eating (DEBQ) (data omitted, see additional tables available in the publication). Analyses divided by sex showed minor differences to the multivariate structures shown for the combined population. The proportion of variance covered by the largest principal component was somewhat larger (~ 42 % males, 39 % for females %) compared to combined population. The largest principal component was similarly characterized by positive loadings for all six variables with both sexes. However, females uniquely show a negative loading for anxiety in mother relationships for PC2, while the trait loaded positively on PC2 with males (see Table 9, additional data available with the publication.)

Table 8. Principal components analysis of the variation for the reversed OHQ score and phenotypes in ranges of typical variation for schizotypy, autism spectrum cognition, attachment-related anxiety, sleep and emotional eating. The principal components were calculated from eigenvalues on the correlation matrix, only the three largest principal components are shown for each analysis.

Rev-OHQ	**PCA**	**Variance proportion**	**Cumulative proportion**	**S.D.**
Both (N = 296)	PC1	0.352	0.352	1.452
	PC2	0.151	0.502	0.951
	PC3	0.149	0.651	0.945
Females				
(N = 190)	PC1	0.330	0.330	1.406
	PC2	0.190	0.520	1.068
	PC3	0.143	0.662	0.925
Males				
(N = 106)	PC1	0.378	0.378	1.507
	PC2	0.171	0.549	1.013
	PC3	0.161	0.710	0.983

Table 9. Loadings for each variable across the three largest principal components in the analysis using the reversed OHQ score with the combined population.

Rev-OHQ	Variable	PC1	PC2	PC3
Both n = 296	Total AQ score	0.38837	0.13650	0.16058
	Cognitive-perceptual	0.37179	0.48263	-0.23554
	Unhappiness (OHQ)	0.52514	-0.35915	0.05906
	Anxiety in Mother relationship (ECR-RS)	0.32930	0.32214	0.75914
	Total PSQI score	0.44100	-0.62766	-0.10832
	Emotional eating (DEBQ)	0.36366	0.34888	-0.57202

Table 10. Loadings for each variable across the three largest principal components in the subdivided analyses limited to Females and Males respectively.

Females Rev-OHQ		PC1	PC2	PC3
	Total AQ score	0.39672	0.11636	0.38052
	Cognitive-perceptual	0.36298	0.47936	0.01189
	Unhappiness (OHQ)	0.54545	-0.23769	-0.24759
	Anxiety in Mother relationship (ECR-RS)	0.36903	-0.26135	0.70616
	Total PSQI score	0.46599	-0.35988	-0.52000
	Emotional eating (DEBQ)	0.24498	0.70877	-0.15718
Males Rev-OHQ		PC1	PC2	PC3
	Total AQ score	0.42474	0.07000	0.11739
	Cognitive-perceptual	0.30949	0.45395	0.66039
	Unhappiness (OHQ)	0.50714	-0.16530	0.16649
	Anxiety in Mother relationship (ECR-RS)	0.31433	0.65815	-0.44884
	Total PSQI score	0.40077	-0.55053	0.13253
	Emotional eating (DEBQ)	0.45519	-0.15958	-0.55078

The covariability shown for the diverse set psychological and physiological traits analyzed may indicate that typical variation for phenotypes of schizotypy, attachment, mood, sleep and eating habits are partly mediated by a hypothalamic axis of neural regulation. The variation for each of the traits correlated positively with one another, showing for example that high degrees of schizotypy were positively correlated with less secure attachment, comparably higher tendencies for emotional eating and comparably

more severe sleep problems. In addition, the reversed OHQ, which corresponds to one's unhappiness and lack for sense of fulfilment, showed a strongly positive and significant correlation with the other traits as also shown in Table 7, while the NEO-PI depression items performed similarly, indicating robustness for the effect observed.

To further explore if hypothalamic dysfunctions may simultaneously alter covarying sets of behavioral phenotypes, we reviewed a large body of literature, focusing on conditions where individuals have been reported to show behavioral phenotypes partly overlapping with the set of behavioral phenotypes also altered in PWS. As also shown in Supplementary table 1 (available with the publication), a set of clinical conditions and differential diagnoses or 'Prader-Willi –like' syndromes can be recognized, whereby individuals present with a set of phenotypes resembling some of those of PWS yet lack the methylation alteration, maternal uniparental disomy, or large deletion diagnostic of this syndrome.

The main findings from this review were threefold: Firstly, a number of imprinted and non-imprinted genes that are implicated in 'Prader-Willi –like' syndromes, may also interact directly with the imprinted loci involved in PWS. The IPW non-coding RNA gene, located within the 15q11-q13 genomic region has been found to also interact with transcription of the imprinted genes within DLK1-DIO3 imprinted locus, which in turn is affected in the Kagami-Ogata and Temple syndromes. It has been proposed that such crosstalk between imprinted loci may reflect common evolutionary origins and co-adaptation between the two or perhaps several imprinted loci [57]. Secondly, while phenotypical presentations showed variation on the individual level due to varying genomic alterations across cases, rather than resembling PWS with one specific phenotype, PWS-like conditions tend to show pleiotropic sets of behavioral phenotypes, partly resembling the full set of phenotypes similarly also altered in PWS. Thirdly, mouse and cell model studies focusing on potential neural mechanisms of the candidate genes involved in PWS-like conditions further highlight both hypothalamic mechanisms and mechanisms related to the excitation-inhibition balance of the brain in particular. This review thus provides evidence that a diverse set of covarying phenotypes are altered in both PWS and the 'Prader-Willi –like' syndromes and dysfunctions of hypothalamic mechanisms may be further implicated in these conditions via alterations of both imprinted and non-imprinted genes, further implying a hypothalamic axis of neural and genetic effects on human behavior that are not wholly separate from one another, such

that alterations on a singular regulatory mechanism may have several pleiotropic phenotypic effects downstream.

5.5. Discussion

In this study, we have collected and analyzed questionnaire data from a population of healthy individuals to evaluate if a set of PWS-associated psychological and physiological traits relevant to hypothalamic function, would also show a corresponding pattern of covariation in the ranges of typical variation found among healthy individuals. The questionnaire data showed that phenotypes of sleeping and emotional eating, governed in part by the hypothalamus, appear to co-vary with phenotypes of schizotypy, mood and anxiety-related aspects of attachment. The overlap of such a diverse set of physiological and behavioral traits in part mediated by the hypothalamus may imply an evolutionary history whereby neural circuits responsible for regulation of hunger, satiety and biorhythmicity were co-opted to jointly regulate affection and social behaviors in the context of maternal care.

The genomic conflict theory of imprinting [20,21] which states that genomic imprinting has evolved due to a conflict in resource allocation between maternally and paternally inherited alleles, provides a useful explanation for why hypothalamic traits might be mediated by genomic imprinting to regulate phenotypes of sleep, feeding and attachment, as the phenotypes of these traits among the offspring may be expected to jointly affect the maternal investment of care into their offspring. Thus, alleles of paternally expressed imprinted genes, which may not share their genotype with other members of the offspring due to the statistical probability for mixed parenthood, may have been selected to favor a hypothalamic phenotype comparably more demanding towards the mother and might thus be under selection for effects that involve spending a larger proportion of the subjective day active while also demanding more affection and food from their mother. By contrast, PWS phenotypes, which are due to lack of expression for such paternally expressed imprinted genes and thus involve a bias in gene dosage towards expression of maternally inherited imprinted genes, would be expected to reflect an extreme manifestation of mechanisms that select for a comparably more equal distribution of maternal investment across offspring, and less individually demanding phenotypes with increased somnolence, reduced affection and reduced food intake in infancy, when the child is most dependent on their mother for sustenance

([21,30]. As healthy individuals may also show less extreme, non-clinical variation for gene expression of imprinted and non-imprinted genes involved in hypothalamic function, co-variation across a corresponding set of behavioral phenotypes may also be shown, due to neural and genetic regulatory mechanisms that underlie typical function of these phenotypes.

In addition to the altered phenotypes of PWS, several lines of evidence also show that hypothalamic mechanisms may also modify phenotypes of social behavior. Firstly, evidence for structural abnormalities and functional deficits in hypothalamic circuits has also been shown in schizophrenia [14,28]. Secondly, oxytocin pathways have been consistently associated with bonding between the mother and offspring [58] but maladaptive effects of oxytocin pathways in social behavior may be highlighted in studies showing that variation in oxytocin function may be associated with schizotypy, autism spectrum disorders and depression [59]. In PWS, variation of sleep phenotypes has also been significantly associated with psychosis-risk symptoms, linking a correlate of hypothalamic dysfunction to psychotic symptomatology in PWS [60]. A small number of subjects both with and without PWS have also been reported to develop manic symptoms or panic attacks due to hypothalamic stimulation with electrodes, which might similarly suggest an association between hypothalamic dysfunction and psychotic symptoms [61,62].

We have also reviewed a large body of literature (supplementary Table 1.) on disorders displaying phenotypes implying of hypothalamic dysfunction via altered phenotypes of food intake, sleep or both. Our approach was primarily guided by the phenotype of PWS, which involves a lack of expression for multiple paternally expressed imprinted genes and shows a highly pleiotropic phenotype of hypothalamic dysfunction with altered sleep, feeding, affection, mood and social cognition ([3,26,30]. While the set of disorders resembling PWS is varied and involves dysfunctions of both imprinted and non-imprinted genes [56], we also find that candidate genes that are associated with hypothalamic phenotypes also tend to be associated with altered behavior among these disorders. Of particular note are phenotypes shown in Kleefstra syndrome, which involves hypotonia, food seeking behaviors and obesity with a sleep phenotype of repeated night awakenings and hyperactivity, while a limited number of cases also involve phenotypes of frustration and temper tantrums in childhood and psychosis, bipolar disorders and apathy among adult cases [63–66]. Thus, the syndrome features

behavioral phenotypes that resemble phenotypes present in PWS and physiological phenotypes mediated by the hypothalamus which both resemble PWS in part while also showing opposite tendencies for altered sleep phenotypes. We also find, as noted in previous work (reviewed in Ivanova and Kelsey, 2011) that paternally expressed imprinted genes appear to be particularly active in the hypothalamus as is also shown by the genome-wide pattern genomic imprinting in the mouse brain [29]. The mouse hypothalamus showed a larger number of active paternally expressed imprinted genes as compared to maternally expressed imprinted genes, a pattern opposite to that of the embryonic mouse brain, which may thus highlight the hypothalamus as a hotspot of paternal-origin allelic expression [4,29]. Paternally expressed imprinted genes expressed in the hypothalamus also show phenotypes relevant to allocation of maternal resources in model mice: In particular, a large of paternally expressed imprinted genes were active in hypothalamic neural circuits and were also associated with altered phenotypes relevant to maternal care including sleep, feeding, parenting behaviors, anxiety or social cognition (supplementary table 2. for references), reflecting the affected phenotypes also shown PWS.

Our findings may also be evaluated in the context of previous behavioral studies which have also indicated correlations between pairs of behavioral traits shown in our questionnaire data. Firstly, as PWS has been found to show varying incidence rates for ASDs in prior studies (Dykens et al., 2011), a measure of autism spectrum cognition was also included in our analysis. While our data showed degree of covariation between typical variation of autism spectrum cognition and variation of other phenotypes deemed to be relevant for the PWS phenotype, earlier questionnaire studies have also shown that both clinical and non-clinical variation in behavioral traits that extend towards the spectrums of autism and schizotypy tend to co-occur or correlate positively with one another to some degree [67–69]. Studies among individuals with clinical forms of psychosis also appear to show elevated rates of ASDs as compared to the typical human populations and autism –like behavioral traits are similarly precent in high percentages, varying between 9 – 61 % of individuals diagnosed with psychotic symptoms [68]. It has been traditionally theorized that the co-occurrence of schizotypy and autism spectrum traits may be partly due to differential diagnosis and overlapping behavioral phenotypes, particularly between negative psychotic symptoms and social deficits in ASDs (reviewed in (Kincaid et al., 2017)). However, studies concerning non-

clinical variation in schizotypy and autism spectrum traits have shown correlations between both negative and positive schizotypy and autism spectrum traits [67,68].

In the data analyzed here, the cognitive-perceptual dimension of schizotypy, as measured via the SPQ-BR questionnaire may be interpreted to represent healthy variation within a spectrum of behaviors that extends towards the positive symptoms of schizophrenia, which include ideas of reference (“I often feel that others have it in for me”), magical thinking (“Have you ever felt that you are communicating with another person telepathically?”) and unusual perceptions (“Are your thoughts sometimes so strong that you can almost hear them?”). Both the cognitive-perceptual scale and SPQ-BR total scores were significantly and positively correlated with AQ total scores in our data. Hence, in the context of our current study and earlier studies, it is not entirely clear whether genetic variation and neural mechanisms prevalent in ASDs may (1) function as risk factors for development of psychotic symptoms later in life, or (2) if the two disorders overlap in phenotype only but are mediated by separate genetic and neural mechanisms. Considering the psychological phenotypes of hypothalamic dysfunction shown in both PWS and the altered phenotypes of attachment and social behavior shown in mouse models with deletions of paternally expressed imprinted genes (supplementary table 2), it may be further theorized that genetic variation for imprinted genes and genes related hypothalamic function might simultaneously predispose individuals to covarying effects for altered phenotypes of social behaviors and insecure attachment, which may contribute towards variation involving overlap of social deficits between schizotypy and autism spectrum cognition. Relevant to this hypothesis and in similarity to covariation shown in our results, variation in the continuum of paranoia has been shown to be positively correlated with insecure and anxious attachment styles [70,71], which has been further theorized to indicate that dysfunctional theory-of-mind cognition may predispose individuals to insecure attachment via overly negative models of how others might perceive oneself [72].

Correlations between sleep problems, typically measured by the PSQI questionnaire have also been observed to correlate positively and significantly with eating disorders and emotional eating in prior studies [73–75]. It has also been previously theorized that such correlations might indicate neural mechanisms in the hypothalamic-pituitary axis jointly regulating sleep and feeding might underlie the correlations observed between sleep problems and disordered eating [74].

Our analyses are affected by three main limitations that must be considered in relation to the results shown. Firstly, our analyses are limited to self-evaluated behavioral variation and cannot directly implicate specific underlying genetic variation within the study population. Secondly, the set of behavioral phenotypes studied is intended to represent a set of evolutionarily relevant correlates in ranges of typical function rather than traits directly and fully comparable to PWS phenotypes. As PWS typically involves severe hyperphagia with lack of satiety and food-related obsessive behaviors [76], the observed behaviors may also lack a clear correlate within ranges of typical eating behaviors. For the purposes of our study, we have primarily considered the phenotype of Emotional eating, which involves overeating due to emotional responses and may thus partially reflect hypothalamic function rather than external eating, which involves overeating due to how appetizing the food seems or restrained eating, which may involve a conscious decision to restrict eating [54]. Thirdly, individual neural mechanisms of hypothalamic function may only be considered from a theoretical viewpoint, as we lack experimental evidence to assign any specific mechanism of neural function to the behavioral phenotypes shown in the study population.

Apart from the aforementioned limitations, it may also be questioned whether genetic polymorphisms affecting hypothalamic function are circulating among typically developed human populations. We note that as shown within a population of healthy human donors from Miami Brain Bank, published under the Genotype-Tissue Expression Portal project (GTEx Portal genome browser, accessed on 7th Oct 2022), the long non-coding RNA, PWRN1 shows a high number of expression quantitative trait loci SNPs for gene expression differences in hypothalamic tissues, located both within and in the promoter regions of the gene. PWRN1 may also be expressed in the brain as a part of the SNURF-SNRPN (SNHG14) RNA transcript [77]. Furthermore, the SNORD116 snoRNA, which is the only single locus implicated in the main behavioral phenotypes of PWS (reviewed in [78]) is also processed from the introns of the SNURF-SNRPN transcript [79], and hence, polymorphisms affecting expression of PWRN1 might also indirectly affect the expression of SNORD116. While differences in gene expression within hypothalamic tissues are not in in themselves indicative of behavioral variation, these polymorphisms nevertheless showcase a theoretical mechanism for non-clinical variation in regulation of hypothalamic function.

In conclusion, the co-occurring set of physiological and psychological phenotypes shown in PWS and other 'Prader-Willi –like' neurogenetic syndromes implies that of hypothalamic neural circuits and their genetic regulatory mechanisms may have become co-adapted to jointly regulate phenotypes of sleep, food intake and social behaviors in the context of maternal investment. In particular, we highlight the sets of physiological and behavioral phenotypes associated with (1) oxytocin- and (2) orexin-expressing neurons in animal model studies and neurogenetic disorders. Alterations in function of oxytocin neurons have been independently associated with alterations in maternal care [12], feeding [80] and psychological functioning [14] while alterations in orexin-expressing neurons have similarly been associated with alterations in sleep and feeding [7] as well as alterations of social behaviors [81], pair-bonding and parenting behaviors [12,82]. Here, we show that behavioral variation present among healthy individuals shows evidence for patterns of co-variation for a set of behavioral and physiological phenotypes which may represent a non-clinical extension of neural functions in part mediated by hypothalamic neural circuits. Thus, alterations in regulation of hypothalamic circuits might predispose individuals to a diverse set of co-occurring behavioral and psychological alterations, which should be considered in both research of psychological and physiological disorders and counseling among healthy individuals.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Iiro Salminen took part in the collection of the questionnaire data, conducted a literature review on neurogenetic disorders, analysed questionnaire data and took part in writing the article. Silven Read organized and quality-checked the questionnaire data. Bernard Crespi contributed to the design of the study and took part in writing the article.

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Chapter 6. Conclusions

Genomic imprinting appears to have evolved due to an evolutionary conflict between maternally and paternally inherited alleles and may thus affect the evolution of genes involved in regulation of behaviors that may influence the allocation of maternal resources across offspring. In the context of human behavior, we might expect maternally and paternally expressed imprinted genes to alter the phenotypes of social behaviors, mood, sleep and eating preferences in opposite ways as is also highlighted by the extreme and maladaptive phenotypes shown across neurogenetic syndromes of genomic imprinting. In this thesis, we have explored hypotheses derived from these considerations to characterize how imprinted genes may affect regulation of human behaviors and to further highlight regulatory mechanisms of behavior across neural pathways. Our work offers three main conclusions:

Firstly, the imprinted genes within the PWS-AS genomic region may through either pleotropic effects or a tendency towards an overly active theory-of-mind cognition, due to the child focusing one's attention towards the mother, mediate differences in paranoid ideation. These phenotypes made apparent by the high prevalence of psychotic disorders shown in both PWS [1–3] and among individuals with maternally inherited duplications of the PWS-AS genomic region [4,5], while our results further imply that genetic variation within PWS-AS genomic region may also mediate non-clinical variation in social behaviors in typical human populations [6,7]. This result is important because it may highlight genes and neural mechanisms affecting theory-of-mind cognition for further research and which may also be utilized in genetic counseling to map alleles and genomic regions of concern to identify individual vulnerabilities in mental health.

Secondly, as shown in chapter 3, PWS and AS show opposite phenotypes for sleep latency and eating preferences, which may imply that evolutionary conflicts in solicitation of maternal care have also affected the evolution of genes and neural systems that regulate phenotypes of feeding, circadian rhythms, and sleep. This result is important in that the neural and genetic circuits which regulate the phenotypes of sleep and feeding may further affect the expression of several thousands of other genes, as is also shown with mouse models of SNORD116 [8,9]. Thus, genetic variation of imprinted

genes might also subject individuals to indirect epigenetic effects, and which may importantly be predicted from evolutionary theory and the altered phenotypes displayed in syndromes and mouse models of genomic imprinting. Research hypotheses drawing from these predictions may be further applied to research on neural and genetic systems in regulation of behavior and for development of novel treatments.

Finally, in chapter 5 we show that the phenotypes of behavior that are jointly altered in PWS due to hypothalamic dysfunction may also show co-variation with one another among typically developing individuals, which may reflect an underlying neural axis of phenotypes that are jointly altered by hypothalamic mechanisms. This result is important because it implies genomic imprinting may also exert indirect effects on human behavior via regulation of neural pathways and highlights a set of behavioral phenotypes affected these mechanisms. It should also be explored whether imprinted genes beyond the PWS-AS genomic region may also affect behavior via hypothalamic pathways. Taken together, this research highlights genes and neural mechanisms that affect underlying vulnerabilities for depression, sleeping problems, disordered eating, anxiety, psychotic disorders and autism spectrum cognition, which should be of direct interest to both further research and counseling in physical and mental health.

6.1. Evidence from four independent sources of evidence implies that dosage of UBE3A may exert opposite effects on phenotypes of social behaviors

Genomic imprinting may be expected to alter phenotypes of behaviors that may affect the allocation of maternal resources among offspring. However, the complex behavioral phenotypes shown in AS and PWS appear to also imply that genomic imprinting might also exert effects on phenotypes of social behaviors with a stubborn and easily irritable behavioral phenotype shown in PWS [10–12] and a happy demeanor and easily excited bouts of laughter shown in AS [13,14]. If behavioral effects associated with genomic imprinting are exerted via regulatory proteins that affect proteins of neural function, we might further expect that imprinted genes may also show opposite directional effects on phenotypes of social behaviors due to pleiotropic interactions in other brain regions or neural pathways also expressing the regulated neural protein, which thus offers a possible mechanism to the partly opposite phenotypes of social behaviors shown AS and PWS. In chapter 2, we first reviewed studies in several

independent clinical populations showing that different dosages of UBE3A, characterized firstly by lack of expression in AS, and increased dosage in the mUPD genotype of PWS, are associated with an increased prevalence of ASDs in AS [15] and increased risk for psychotic disorders in PWS [1,16]. Secondly, increased dosage of UBE3A was also associated with increased risk for schizophrenia shown among individuals with maternally, but not paternally inherited duplications of the PWS-AS genomic region [4,5]. In our work, we showed that genetic variation of UBE3A associated with differences in schizotypy and paranoid ideation in particular [7], which shows an interesting parallel to the phenotypes of psychotic behaviors shown in PWS, which typically involve hallucinations and delusions related to perceptions and intentions held by other individuals [1], which may be attributable to overly active theory-of-mind cognition. We may also theorize that imprinted genes might also mediate for phenotypes of more or less active theory of mind -cognition and the ability to perceive the intentions of the mother in particular. In contrast, the behavioral phenotype of AS, that shows a tendency towards autism spectrum cognition and continuous smiling and happy demeanor which may be interpreted as a dysfunctional extreme of affect signaling particularly towards the mother, might represent an extreme of behavior phenotypes promoted paternally expressed genes. Hence, PWS and AS might also represent opposite and extreme maladaptive phenotypes of social behaviors. Finally, we may also note that (4) animal model studies have shown opposite effects of social behavior for different dosages of UBE3A; lack of expression has been associated with overly active and inappropriate sociability on both mouse and rat models [17,18] while overexpression of UBE3A was associated with a lack of preference for social interaction, which the researchers likened to phenotypes of social behaviors also observed in ASDs [19]. The main results presented in chapter 2 thus provide an additional line of evidence in how imprinted genes may affect phenotypes of social behaviors and also implies that maladaptive phenotypes of these effects may be manifested as individual vulnerabilities for mental disorders involving deficits in social cognition.

6.2. Maternally and paternally expressed genes may exert opposite effects on phenotypes of feeding and sleeping in AS and PWS.

Imprinted genes have been shown to affect both phenotypes of sleeping and feeding, particularly via hypothalamic mechanisms [9,20,21] and both AS and PWS show altered phenotypes of sleeping and eating in relevant literature [22–25]. Hence, in chapter 3 we describe a comparative review on these phenotypes among AS and PWS, and our primary conclusions are that (1) AS and PWS show evidence for opposite phenotypes of sleep onset latency which is increased as compared to peers in AS and shortened as compared to peers in PWS [25,26] and that (2) AS and PWS show opposite phenotypes for selective food preferences with low selectivity prominent among PWS subjects, and subjects with AS showing highly specific food preferences, particularly for foods which may resemble specially prepared complimentary foods [26]. The first result shows an interesting parallel to the sleep phenotypes shown among mouse and cell models of AS and PWS: Magel2 has been shown to interact with proteins that regulate circadian rhythms [27,28] while UBE3A has also been shown to interact with circadian rhythm proteins, and lack of expression for UBE3A alters circadian rhythmicity and sleep patterns in both mouse and nerve cell models [29–31]. Two recently published studies that reveal novel details on interactions between circadian rhythm proteins and imprinted genes involved in PWS may also fit our hypotheses previously discussed in chapter 3, proposing that circadian rhythms may be altered in opposite ways between PWS and AS respectively. Firstly, MAGEL2 has been found to promote the ubiquitination of CRY1 in a human cell model [32], while NDN was also shown to regulate *Bmal1* stability in mouse and cell models [33], and both of these interactions could be interpreted to function so as to lengthen the period of night in PWS. By this hypothesis, lack of expression for MAGEL2 would mean that CRY1 may suppress the expression of CLOCK and BMAL1 for a comparatively longer period during the dark period, while lack of expression for NDN would mean that BMAL1 protein function may be impaired due to decreased stability, which may also lengthen the dark period, and decrease activity during the day as the enhancer activity of the CLOCK:BMAL1 heterodimer may also be affected. Thus, evidence from both neurogenetic syndromes and mouse models indicates that paternally and maternally expressed genes may alter sleep phenotypes in opposite ways, and non-clinical

variation in expression of imprinted genes might also result in less extreme effects such as individual vulnerabilities towards sleep problems among typically developing individuals. Secondly, our results imply previously unrecognized and opposite tendencies for food preferences between AS and PWS. We note that these results also appear to fit with previous hypotheses on how the timing of transitions from breast milk as primary form of nutrition to the consumption of complementary foods and independent foraging may also be partly regulated by imprinted gene expression in humans [34]. In this hypothesis, paternally expressed genes would be expected to prolong these transitions, perhaps via an increased preference for complementary foods and maternal provision, while maternally expressed genes would in turn promote less sensitive food preferences and earlier transitions between different sources of nutrition which would increase the inclusive fitness of maternally inherited genes via shortened birth intervals. It may be interesting to consider if variation in expression levels of imprinted genes might also contribute to individual vulnerabilities towards disordered eating among typical human populations.

6.3. The paternally expressed genes of the PWS-AS region may also regulate phenotypes of social behavior.

PWS shows a distinct behavioral phenotype with stubbornness, aggression and mood fluctuations and these behavior problems may persist to adulthood while also showing a more severe phenotype with the matUPD genotype [10]. The paternal deletion genotype of PWS may also show a moderate tendency for development of depressive illness, and it has been hypothesized that the development of affective psychosis in PWS may involve a ‘two-hit’ model, where the lack of expression for the paternally expressed genes of PWS-AS region and increased dosage of UBE3A unique to the matUPD genotype, may have synergistic effects [1]. In chapter 4, our results show that genetic variation of SNORD116 may also be mediating differences in schizotypy. This result further implies that paternally expressed genes of the PWS-AS genomic region may also exert an effect over behavioral phenotypes and should be further considered in the context of other relevant studies, including another behavioral study showing that the genetic variation of the MAGEL2 may also mediate differences in schizotypy among typical human populations [35] and studies of Schaaf-Yang Syndrome showing that paternal deletions of the MAGEL2 gene are associated with a distinct behavioral and

developmental phenotype different from that of PWS [36–38]. Mouse models have shown that deletions of SNORD116 are associated with dysregulation of sleep and feeding, deficits in development of orexin- and melatonin concentrating hormones in the lateral hypothalamus and an altered pattern of hypothalamic gene regulation which may involve several thousands of genes [8,9]. The phenotypes of these mouse models thus resemble the phenotype of hypothalamic dysregulation shown with PWS and raise questions over which behavioral phenotypes may be affected by genetic variation in SNORD116 in typical human populations.

6.4. Paternally expressed genes may mediate a jointly altered set of behavioral phenotypes via hypothalamic pathways.

The behavioral phenotypes of PWS and AS imply that genomic imprinting may affect a wide range of behavioral phenotypes and mouse models of PWS further show that imprinted gene expression may regulate complex gene expression cascades via several hypothalamic pathways. While one might expect that complex systems which affect the expression of thousands of other genes indirectly would be fine-tuned according to constraints set by neural development and the maintenance of homeostatic systems, it remains unknown whether hypothalamic pathways are regulated in a rigid manner in typically functioning subjects or if comparably less extreme genetic or epigenetic variations in regulation of hypothalamic pathways may also be circulating in typical human populations. If the former is true, then any variation observed in phenotypes also regulated by hypothalamic pathways would be due to more specific mechanisms and thus, would not be expected to co-vary with one another by chance. In chapter 5, we tested this hypothesis by developing a set of human behavioral phenotypes which may be partly affected by a hypothalamic neural axis on the basis that these phenotypes show extreme alterations in PWS and other syndromic conditions involving hypothalamic dysfunction (see supplementary table 1 in chapter 5) and investigated if the same corresponding phenotypes might also show patterns of co-variation among typical subjects. Importantly, our results showed that co-variation within this diverse of phenotypes was not limited to co-variation of sleep and feeding phenotypes or co-variation of traits affected by social behaviors. Instead, our results showed significant correlations for non-clinical phenotypes of schizotypy, autism

spectrum cognition, mood, social anxiety, sleeping problems and emotional eating. While our results are limited to co-variation found among behavioral phenotypes and as such we can not point towards genetic or neural mechanisms in a direct manner, the results also imply that vulnerabilities to sleep problems and disordered eating may also overlap with risks in mental health which should be considered both in research and counseling.

6.5. Concluding remarks and future directions

This thesis highlights how imprinted genes may alter neural pathways towards maladaptive extreme through the dysregulation of several behavioral phenotypes, as shown in the phenotypes of PWS and AS. Our results further imply that biased misexpression of imprinted genes might subject typically developing individuals to underlying vulnerabilities for conditions affected by impaired neural and genetic mechanisms in regulation of mood, social behaviors and sleep and feeding. In addition, the regulatory effects exerted by imprinted genes may also highlight neural pathways and gene expression cascades that interact with imprinted genes, and both the affected phenotype and the direction of the effect may be readily deduced from the kinship model of genomic imprinting. Thus, discovering the regulatory mechanisms that imprinted genes tap into also represents potential advancements in research of neural development and regulation of growth and metabolism.

6.6. References

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