Charles University

Faculty of Science

Study programme: Molecular Biology and Biochemistry of Organisms

Jakub Lipka

Advancing Therapeutic Strategies for Prader-Willi Syndrome

Pokročilé přístupy v léčbě Prader-Williho syndromu

Bachelor´s thesis

Supervisor: Mgr. Jan Procházka, Ph.D.

Prague, 2025

Acknowledgements

I would like to thank my supervisor, Mgr. Jan Procházka, Ph.D., for his support and encouragement throughout the process of my bachelor's thesis. My heartfelt thanks also go to David Pachuelo Reguera, Ph.D., for his helpful advice and kind support during my research. I would like to thank everyone in the laboratory for creating a friendly environment and for their help during my experiments.

Declaration / Prohlášení

I declare that I wrote this thesis by myself and listed all the resources I used and the bibliography. Neither this thesis nor its important part was previously used to gain any academic degree.

Prohlašuji, že jsem tuto práci vypracoval samostatně a uvedl jsem všechny použité informační zdroje a literaturu. Tato práce ani její podstatná část nebyla použita k získání žádného akademického titulu.

V Praze, 2025

Signature / Podpis

Abstract

Prader-Willi syndrome is a complex neurodevelopmental disorder caused by genetic abnormalities in the paternally inherited 15q11-q13 chromosomal region. Its hallmark symptom is hyperphagia, which, if not carefully managed, often leads to severe obesity. Additional symptoms include muscle hypotonia, delayed development, emotional regulation difficulties, hypogonadism, and hormonal imbalances, such as growth hormone deficiency. Currently, there is no curative treatment, and existing therapies primarily focus on symptom management, including growth hormone administration to improve body composition and growth. Emerging therapies, such as oxytocin supplementation and medications targeting uncontrolled appetite, show promise as complementary approaches. Recent scientific advancements open the possibility of treatments targeting the genetic root of the disorder. This bachelor's thesis provides a comprehensive review and evaluation of innovative therapeutic strategies, including epigenetic reprogramming of specific genomic regions using methyltransferase inhibitors, CRISPR/dCas9, and zinc-finger motifs, which offer the potential to reactivate the silenced maternal allele. Additionally, it explores the feasibility of gene augmentation therapy. Despite these promising developments, significant challenges remain, such as effective gene therapy targeting, safety concerns, and ethical considerations.

Aims

1) Describe new technologies for possible gene replacement strategies in PWS

2) Describe precise epigenetic reprogramming technologies for PWS

3) Evaluate the role of non-coding RNAs in disease manifestations and possible therapeutic targets

4) Investigate hormonal intervention as a possible mitigation of phenotype

Keywords

Prader-Willi syndrome, rare diseases, Snord116, AAV, gene therapy

Abstrakt

Prader-Willi syndrom je komplikované neurodevelopmentální onemocnění způsobené genetickými abnormalitami v oblasti chromozomu 15q11-q13 zděděného od otce. Mezi charakteristické projevy patří hyperfagie, která při nedostatečné kontrole často vede k těžké obezitě. K dalším symptomům patří svalová hypotonie, opožděný vývoj, hypogonadismus, poruchy zvládání emocí a hormonální nerovnováha, například deficit růstového hormonu. V současnosti neexistuje žádná kauzální léčba a terapie se zaměřuje především na zmírnění symptomů, například podáváním růstového hormonu ke zlepšení tělesné kompozice a růstu. Jako doplněk by k ní mohla sloužit nově se vyvíjející léčba oxytocinem nebo léky zmírňujícími chuť k jídlu. Nejnovější vědecké poznatky však otevírají možnosti léčby zaměřené na samotnou genetickou podstatu onemocnění. Tato bakalářská práce se věnuje podrobnému přehledu a zhodnocení inovativních terapeutických strategií jako jsou metody epigenetického přeprogramování konkrétních úseků v genomu pomocí technologií založených na inhibitorech metyltransferáz, CRISPR/dCas9 a zinc-finger motivech, které nabízejí možnost reaktivace umlčené mateřské alely. Dále se věnuje posouzení možností navrácení funkčních kopií genů zpět do organismu. Navzdory těmto pokrokům přetrvávají zásadní výzvy, jako je efektivní zacílení genové terapie, bezpečnostní rizika a etické otázky.

Cíle

1) Popsat nové technologie použitelné pro náhradu inaktivovaných genů podílejících se na PWS.

2) Popsat metody epigenetického přeprogramování konkrétního úseku genomu PWS pacientů.

3) Zhodnotit roli nekódujících RNA v projevech onemocnění a posoudit vhodné terapeutické cíle.

4) Prozkoumat využití hormonální léčby pro možné zmírnění fenotypu.

Klíčová slova

Prader-Williho syndrom, vzácná onemocnění, Snord116, AAV, genová terapie

List of Abbreviations

5mC	5-methylcytosine
AAV	adeno-associated virus
AgRP	agouti-related protein
AI	artificial intelligence
AS	Angelman syndrome
ASE	antisense element
ATF	artificial transcription factor
ATP10C	ATPase phospholipid transporting 10C
ATS	antisense
CpG	5'-cytosine-phosphate-guanine-3'
CRISPR	clustered regularly interspaced short palindromic repeats
CRISPRa	CRISPR activation
CRISPRi	CRISPR interference
crRNA	CRISPR RNA
dCas9	deactivated CRISPR-associated protein 9
DCCR	diazoxide choline controlled-release tablets
DMR	differentially methylated region
DNA	deoxyribonucleic acid
FDA	U.S. Food and Drug Administration
GABA	gamma-aminobutyric acid
GH	growth hormone
GLP-1 RA	glucagon-like peptide-1 receptor agonist

gRNA	guide ribonucleic acid
H3K4	histone 3 lysine 4
H3K9	histone 3 lysine 9
H3K9me2	dimethylation of lysine 9 on histone 3
HIV	human immunodeficiency viruses
IC	imprinting centre
IGF	insulin-like growth factor
iPSC	induced pluripotent stem cells
KRAB	Krüppel-associated box
lncRNA	long non-coding RNA
MAGEL 2	melanoma antigen L2
MKRN3	makorin ring finger protein 3
NAG neurons	neuropeptide Y, agouti-related protein, and gamma-aminobutyric acid neurons
NPAP1	nuclear pore-associated protein 1
NPC	neural progenitor cells
NPY	neuropeptide Y
PAM	protospacer adjacent motif
PC1	prohormone convertase
pre-mRNA	precursor messenger RNA
PWS	Prader-Willi syndrome
PWSCR	PWS critical region
RNP	ribonucleoprotein particle
rRNA	ribosomal RNA

sgRNA	single RNA
SNHG14	small nucleolar host gene 14
snoRNA	small nucleolar RNA
snRNA	small nuclear RNA
SNRPN	small nuclear ribonucleoprotein polypeptide N
SNURF	SNRPN upstream reading frame
SRO	shortest region of deletion overlap
T2D	type 2 diabetes
^{Tet1}dCas9	Tet1-fused dCas9
^{Tet1v4d}Cas9	Tet1-fused dCas9 with extended linker
tracrRNA	trans-activating RNA
TSS	transcriptional start site
UBE3A	ubiquitin-protein ligase E3A
^{VP64}dCas9^{VP64}	VP64-fused dCas9
WT	wild-type
ZFP	zinc finger protein

Table of Contents

1 INTRODUCTION

Genomic imprinting is a characteristic attribute of mammals and flowering plants. While most genes are expressed from both paternal and maternal chromosomes, a smaller subset (approximately 1 % in mammals) is expressed from only one allele. This phenomenon occurs in two primary forms: paternal imprinting, where the maternal copy of a gene is transcriptional active while the expression from the paternal copy is repressed, and maternal imprinting, where, in contrast, gene expression relies solely on the paternal copy. Genomic imprinting is associated with several genetic pathological conditions. (McGrath & Solter, 1984; Moore & Haid, 1991; Tucci et al., 2019)

One example is Prader-Willi syndrome (PWS), a rare neurodevelopmental disorder resulting from the loss of expression of paternally inherited genes in the 15q11-q13 chromosomal region (Butler & Palmer, 1983). Since the maternal copy of these genes is silenced, it cannot compensate for this loss. This critical region contains five protein-coding genes and a group of small nucleolar RNA (snoRNA) genes (reviewed by Cassidy et al., 2012). PWS is considered to be the most common genetic cause of human-related obesity, with around 400,000 individuals suffering worldwide (Butler & Thompson, 2000).

In PWS cases, the underlying genetic cause varies. Most (50–70 %) of the cases are due to a *de novo* deletion (5–6 Mbp) in the PSW locus, approximately 30–40 % result from maternal disomy, where both copies of chromosome 15 are inherited from the mother instead of one from each parent, the remaining cases arise from various imprinting defects, which disrupt regular gene expression in this region (Bohonowych et al., 2019; Lionti et al., 2015). These genetic disruptions all lead to the characteristic features of PWS: infantile hypotonia (low muscle tone), which leads to poor sucking ability in newborns, resulting in failure to thrive, mental deficiency, hypogonadism/hypogenitalism, hyperphagia resulting in obesity, short stature, and many hormone deficiencies (reviewed by Cassidy et al., 2012).

There is no adequate remedy for PWS nowadays. The only options are growth hormone (GH) replacement therapy, food intake control and regular physical activity (National Institutes of Health, 2021). Individuals treated with GH show significant improvement in growth and body proportions (Eiholzer & l'Allemand, 2000), body composition and developmental motor skills (Carrel et al., 2004).

Interestingly, a similar genetic disruption in the same region leads to Angelman syndrome (AS). Unlike PWS, which arises from the loss of paternal gene expression, AS is caused by the loss or dysfunction of the maternally inherited UBE3A gene within this region. (Kishino et al., 1997)

Innovations in gene therapy, including adeno-associated virus (AAV) and lentiviral delivery systems, CRISPR/dCas9-based epigenetic modulators, transcription activators, and methyltransferase inhibitors, present promising opportunities for addressing the fundamental genetic defects in PWS. These approaches can potentially improve the quality of life for affected individuals by targeting the root causes of the disorder rather than only mitigating symptoms. This bachelor's thesis aims to explore and summarise novel therapeutic strategies for managing PWS.

2 PWS/AS LOCUS

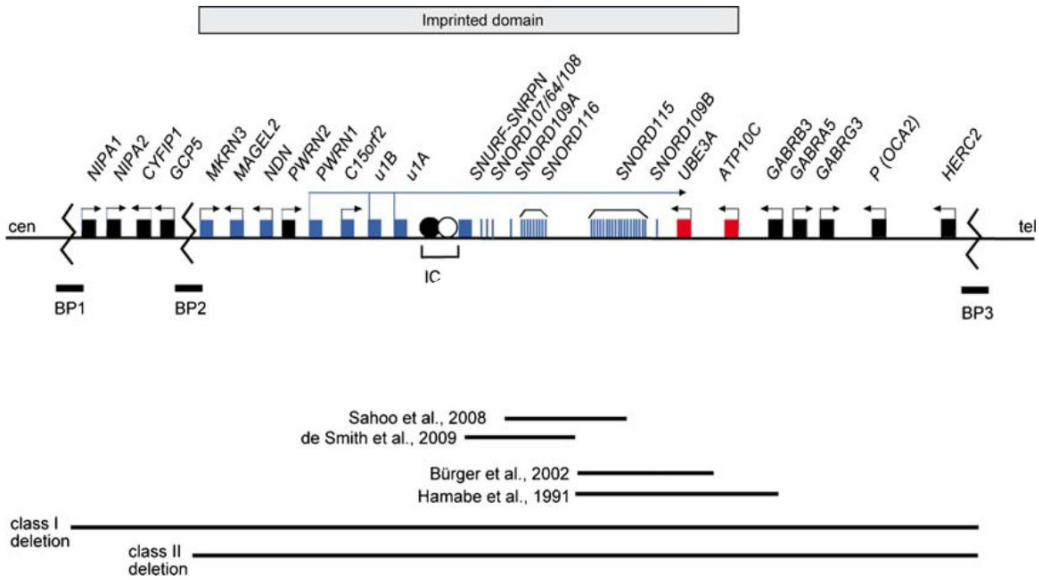


Figure 1: A schematic overview of human chromosomal region 15q11q13. Genes expressed from the maternal chromosome only are drawn as red boxes, genes expressed from the paternal chromosome only are drawn as blue boxes, snoRNAs are drawn as vertical lines, and genes expressed from both parental alleles are drawn as black boxes. The orientation of transcription is indicated by horizontal arrows. IC (imprinting centre), BP (breakpoint) (adapted from Buiting (2010); modified).

The maternally imprinted PWS region contains MKRN3, MAGEL2, NDN, NPAP1 (C15orf2), imprinting centre (IC), SNURF-SNRPN, and SNHG14. (Boccaccio et al., 1999; Färber et al., 2000; Jay et al., 1997; Jong et al., 1999; Leff et al., 1992). Downstream of this region lie the two paternally imprinted genes, UBE3A and ATP10C (Kishino et al., 1997; Meguro et al., 2001). One of the major causes of PWS, deletions in the 15q11-q13 region, arises due to

recombination events between homologous repetitive sequences at the ends of the locus. (Amos-Landgraf et al., 1999). The most common PWS-associated deletions extend from breakpoint 1 (BP1) to breakpoint 3 (BP3) (class I deletion) or from BP2 to BP3 (class II deletion) (Christian et al., 1998) (see Figure 1). Extended deletion variants occur less frequently, involving breakpoints downstream of BP3 (Butler et al., 2008; Sahoo et al., 2007). Although maternally imprinted genes are widely considered the primary contributors to the core clinical manifestations of PWS, the loss of specific non-imprinted genes within the deleted region may also modulate the phenotype (reviewed by Buiting, 2010). Disruptions in the IC can lead to either PWS or AS (Johnstone et al., 2006).

If we disregard deletions in the IC, defining the minimal deletion resulting in the PWS phenotype is more complex than it may initially seem. Generally, the key region responsible for PWS is considered to be SNORD116 (De Smith et al., 2009; Sahoo et al., 2008). However, some recorded cases exhibit deletions that do not include SNORD116, which still result in PWS-like features. Such deletions may disrupt long non-coding RNAs (lncRNAs) and influence the efficiency of splicing for critical small nucleolar RNAs (snoRNAs) (Lei et al., 2019). Various atypical deletions have been described by (Grootjen, Juriaans, et al., 2022), further highlighting the complexity of the molecular mechanisms underlying PWS.

2.1 Imprinting centre

Genomic imprinting is regulated through epigenetic marks on DNA (5mC within a CpG dinucleotide) and histones. Imprinted genes are typically organised in clusters, each controlled by a single IC in *cis* that carries parent-specific methylation. This methylation pattern is established during gametogenesis and maintained throughout an individual's lifetime. (reviewed by Li & Sasaki, 2011)

Imprint establishment, in general, is carried out by the DNA methyltransferases DNMT3A and DNMT3B, which require the stimulatory factor DNMT3L for proper functionality (Gowher et al., 2005; Okano et al., 1999). During the cell cycle, it is essential to restore epigenetic marks, as half of them are lost with each round of DNA replication. This process is mediated by DNA methyltransferase DNMT1 and transcriptional repressor ZFP57 that contains a KRAB domain, which binds to KAP1, serving as a scaffold protein for heterochromatin protein HP1, histone methyltransferases, and deacetylases (Bestor et al., 1988; Kurihara et al., 2008; Li et al., 2008; Sripathy et al., 2006).

The PWS imprinting centre (PWS-IC) overlaps the SNURF-SNRPN gene. This region contains two differentially methylated regions (DMRs), separated by a biallelically methylated segment. The first DMR is located at the 5' end of the SNURF-SNRPN gene and is methylated exclusively on the maternal allele. The second DMR is positioned at the 3' end and is methylated solely on the active paternal allele. (Shemer et al., 1997)

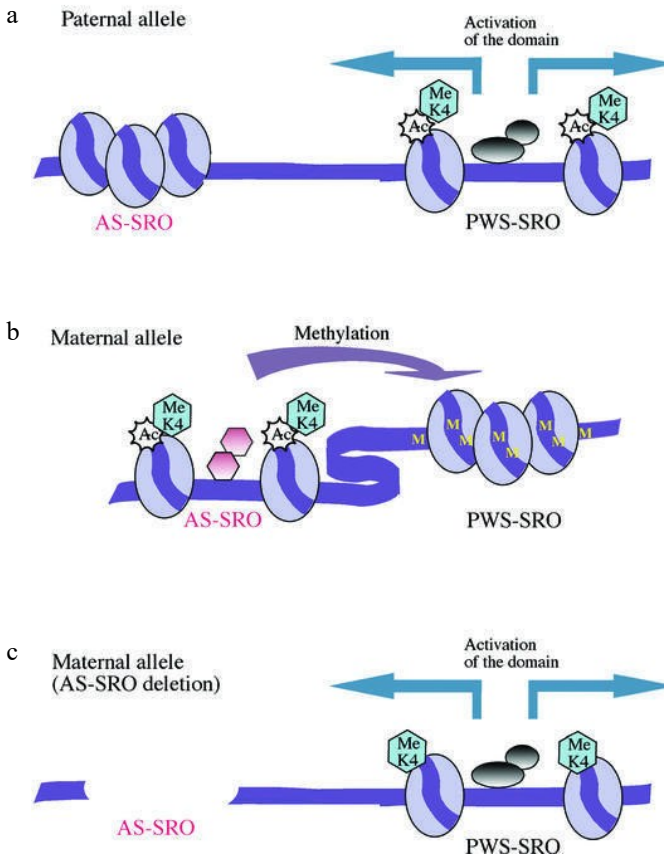


Figure 2: Model of the imprinting mechanism at the PWS/AS regional control centre. (**a**) On the paternal allele is AS-SRO in an inactive conformation and does not interact with PWS-SRO. The function of the active paternal PWS-SRO is to activate genes in the PWS/AS domain. (**b**) The maternal AS-SRO is differentially packaged with acetylated histones. The function of this active AS-SRO is to methylate the adjacent PWS-SRO and put it in an inactive chromatin structure. (**c**) This epigenetic state fails to form on the PWS-SRO if the AS-SRO is deleted or positioned too far from the PWS-SRO.(adapted from Perk et al. (2002); modified)

Horsthemke & Buiting (2008) analysed various imprinting centre (IC) deletion case reports and identified two distinct regions where deletions lead to either PWS or AS. These regions are known as the shortest region of deletion overlap (SRO). The PWS-SRO, measuring 4.1 kb, is located at the same site as the first DMR, whereas the AS-SRO, 880 bp long, lies 35 kb upstream. Nevertheless, the majority of IC defects result from epimutations rather than known DNA sequence changes (Buiting et al., 2003).

The AS-SRO plays a crucial role in regulating the methylation of PWS-SRO, ultimately leading to the repression of PWS genes expression (see Figure 2). On the maternal allele, AS-SRO adopts active conformation and interacts with PWS-SRO, triggering its methylation. This methylation silences PWS genes. In contrast, on the paternal allele, AS-SRO remains in an inactive conformation, preventing interaction with PWS-SRO. As a result, PWS-SRO remains unmethylated, allowing transcriptional activation of the entire locus. (Perk et al., 2002)

2.2 Functional roles of PWS genes

The maternally imprinted region encodes several proteins with diverse functions. MKRN3 (makorin ring finger protein 3) is believed to function as a probable E3 ubiquitin ligase and serves as a major regulatory element in puberty onset; mutations in this gene lead to central precocious puberty (Känsäkoski et al., 2015; Macedo et al., 2018). The NPAP1 (nuclear pore-associated protein 1) is expressed exclusively from the paternal chromosome in the brain during the foetal period. As development progresses, its expression becomes biallelic. It is thought to be a component of the nuclear pore complex. (Buiting et al., 2007; Neumann et al., 2012).

2.2.1 MAGE family genes

The MAGEL2 (melanoma antigen L2) gene is responsible for Schaaf-Yang syndrome, a disorder with clinical features similar to PWS. Various loss-of-function mutations in MAGEL2 have also been associated with autism spectrum disorder and a broad spectrum of clinical manifestations, including neonatal hypotonia, developmental delay, intellectual disability, excessive weight gain, eye abnormalities, and other PWS-like phenotypes. Additionally, MAGEL2 is implicated in the proper functionality of the hypothalamus, particularly in the regulation of neuropeptide secretion, such as oxytocin and GH, which are commonly dysregulated in PWS patients. MAGE proteins function as regulators of E3 RING ubiquitin ligases, enzymes responsible for attaching ubiquitin molecules to proteins or DNA. This ubiquitin tagging can influence various cellular processes, including proteasomal degradation, protein trafficking, endocytosis, cellular signalling, DNA repair, and histone modifications. Specifically, MAGEL2 is implicated in regulating retrograde vesicular trafficking, a process essential for intracellular transport and signalling. (Hao et al., 2013; McCarthy et al., 2018; Meziane et al., 2015; Schaaf et al., 2013; ubiquitinoylation reviewed by Yau & Rape, 2016)

The NDN (necdin) gene, which shares structural similarity with MAGEL2, interacts with the tumour suppressor gene p53 in postmitotic neurons, where it is involved in neuronal differentiation and survival. High expression levels of NDN were observed in the hypothalamus. Disruptions in NDN and MAGEL2 contribute to impaired mental development, growth, and sensitivity to hormonal signals. Notably, defective leptin signalling, a hormone responsible for sensing satiety, has been recorded. This impairment is likely mediated through a ubiquitin-dependent mechanism. (Chen et al., 2020; Taniura et al., 1999; Uetsuki et al., 1996).

2.2.2 SNURF-SNRPN

The SNRPN (small nuclear ribonucleoprotein polypeptide N) is a part of a ribonucleoprotein particle (RNP) and is involved primarily in neuron-specific pre-mRNA splicing and hypothalamus development (McAllister et al., 1989; Özçelik et al., 1992). The exact function of the SNURF (SNRPN upstream reading frame) gene remains unclear.

Deletions in bicistronic SNURF-SNRPN lead to PWS-like phenotype, neonatal hypotonia, intellectual disability, and obesity. The question is whether the loss of this gene's expression is indeed the cause or if it results from disruptions in the nearby imprinting centre (IC) and downstream snoRNAs. (Cao et al., 2017; Kuslich et al., 1999)

2.2.3 SNHG14

The SNHG14 (small nucleolar host gene 14) is a long non-coding RNA (lncRNA) with multiple alternative promoters and terminators, which contains several small nucleolar RNAs (snoRNAs; SNORD64, SNORD107, SNORD108, SNORD109A/B, SNORD115, and SNORD116), and the UBE3A antisense (ATS) transcript (see Figure 1) (Cavaillé et al., 2000; Runte et al., 2001). Among snoRNAs, the loss of the active SNORD116 cluster is believed to be the most significant contributor to PWS pathogenesis (De Smith et al., 2009; Sahoo et al., 2008).

The SNHG14 serves as a host gene for snoRNAs, which are processed from introns during transcript splicing. The primary SNORD116 transcript undergoes neuron-specific splicing mediated by the splicing factor RBFOX3/NeuN. During this process, introns give rise to individual snoRNAs, while exons are spliced into the 116HG transcript (see Figure 3). Both

products play crucial roles in PWS pathophysiology. (Coulson et al., 2018; Powell et al., 2013; Runte et al., 2001)

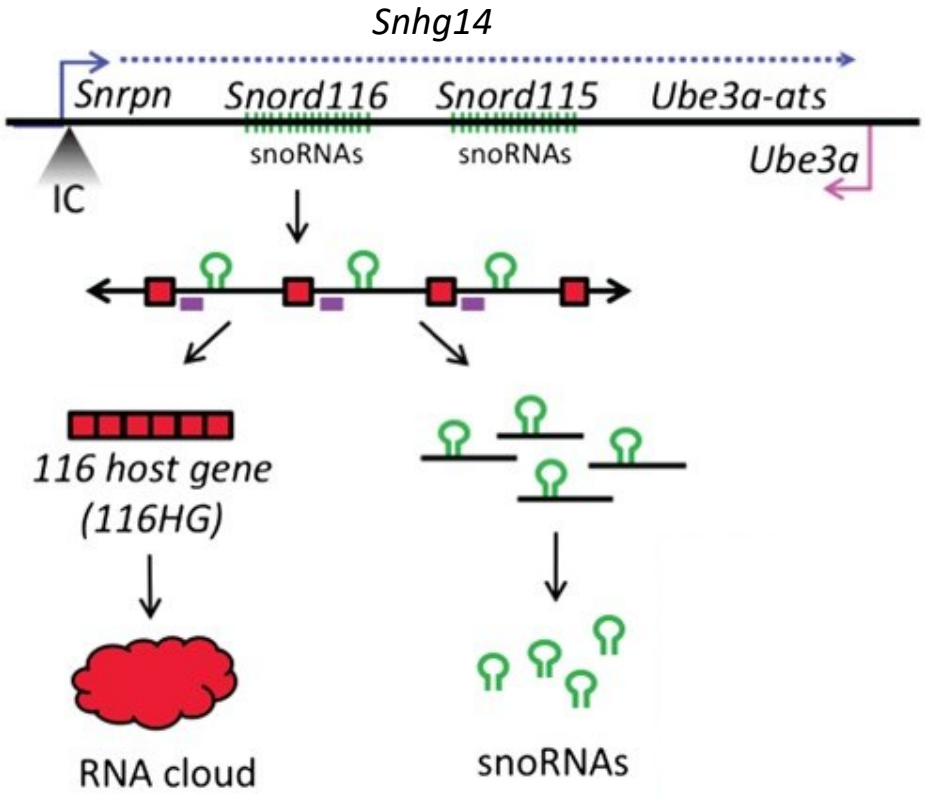


Figure 3: A schematic overview of Snord116 processing in mouse (*mus musculus*). The long paternally expressed transcript Snhg 14 is shown in blue and maternally expressed Ube3a is shown in pink. The primary Snord116 transcript is comprised of a repeating array of exons (red) flanking intron-embedded snoRNAs (green). Processing of this primary transcript produces snoRNAs from the introns of Snord116 and the 116HG from the spliced exons of Snord116. (adapted from Coulson et al. (2018); modified)

2.2.4 SNORD116

The SNORD116 belongs to the group of orphan snoRNAs, which contain conserved C (RUGAUGA), D (CUGA), and less conserved C'/D' box motifs, and variable antisense elements (ASEs) that enable specific target binding. While canonical snoRNAs are involved in rRNA and small nuclear RNA (snRNA) modifications, such as pseudouridylation and 2'-O-ribose methylation, orphan snoRNAs interact with pre-mRNAs, regulate alternative splicing, and even influence gene expression. The specific targets of SNORD116 remain largely unknown, but recent studies in human cells have identified a wide range of potential targets (e.g. PAX6, FGF13, IRX5), including PWS-associated genes themselves, notably MAGEL2. SNORD116 is believed to play an important role in brain development, regulation of neuronal pathways, and gene expression regulation, making it a key factor in the pathology of PWS.

(Falaleeva et al., 2016; Ganot et al., 1997; Gilmore et al., 2024; Kiss-Laszlo et al., 1998; Qi et al., 2016).

In the human genome, SNORD116 is arranged in 30 tandem repeats, which Baldini et al. (2022) have categorised into three distinct classes (I–III). By contrast, the mouse (*Mus musculus*) carries 79 repeats of Snord116. Despite its higher repeat count, the mouse variant exhibits lower variability, consisting only of class I repeats. Additionally, loss of Snord116 does not result in hypotonia or obesity, characteristic features observed in affected humans. Instead, the mouse model maintains a lean body composition despite increased food intake. Energy homeostasis remains unaffected, and Ding et al. (2008) have suggested that the observed phenotype is caused more by behavioural factors (overeating) than metabolic dysfunction. (Baldini et al., 2022; Ding et al., 2008; Qi et al., 2016)

2.3 Angelman syndrome genes

The critical region on human chromosome 15 also contains paternally imprinted genes (UBE3A and ATP10C). Loss of their expression leads to another disorder, Angelman syndrome, which is characterised by developmental delay, intellectual disability, ataxia, hypotonia, seizures, limited speech, and distinctive behaviour with an apparent happy demeanour that includes inappropriate laughter and excitability. (reviewed by Buiting, 2010)

The UBE3A (ubiquitin-protein ligase E3A) is a paternally imprinted gene only in the brain, while in other tissues, it is expressed biallelically (Vu & Hoffman, 1997). Imprinting is maintained by the antisense UBE3A transcript, which represses paternal UBE3A expression in *cis* (Johnstone et al., 2006). As an E3 ubiquitin ligase, UBE3A influences many cellular processes (Scheffner et al., 1993). Primarily, its function involves the degradation of neuronal proteins critical for synaptic connection development and plasticity (Greer et al., 2010). Known targets are, e.g. the synaptic protein Arc, the circadian transcriptional factor BMAL1, and the guanine nucleotide exchange factor Ephexin5, whose activity is regulated through proteasomal degradation (Gossan et al., 2014; Greer et al., 2010; Margolis et al., 2010). Additionally, the SK2 potassium calcium-activated channel's cell surface levels are controlled differently through endocytosis (Sun et al., 2015).

The ATP10C (ATPase phospholipid transporting 10C) gene encodes a putative aminophospholipid translocase (Meguro et al. (2001). While its precise molecular function

remains unclear, studies have shown that the absence of this protein is associated with insulin resistance and obesity (Dhar et al., 2006; Hurst et al., 2012).

3 MOUSE MODELS

Mouse models of PWS can be broadly categorised into two types: larger deletion models, which encompass the entire PWS critical region (PWSCR) on mouse chromosome 7, and single-gene knockdown models, primarily targeting Snord116 or, less frequently, Magel2, Ndn, IC, and Snurf-Snrpn (reviewed by Bervini & Herzog, 2013). While complete deletion models may initially seem the most comprehensive, their neonatal lethality often limits their practicality for research (Gabriel et al., 1999). As a result, the Snord116$^{+/-}$ mouse is more commonly used due to its standard survival rate (Ding et al., 2008). However, its relatively mild PWS phenotype compared to human patients can complicate the assessment of potential treatments.

Interestingly, (Qi, Purtell, Fu, Sengmany, et al., 2017) have demonstrated that ambient temperature influences the manifestation of Snord116 loss. Mice housed at room temperature (22 °C) exhibited the expected PWS-like phenotype, whereas those maintained at thermoneutrality (30 °C) displayed only reduced body weight compared to wild-type (WT) controls. This finding suggests that environmental factors may modulate phenotypic outcomes in PWS models, potentially impacting preclinical research.

4 NEW THERAPEUTIC STRATEGIES

4.1 Hormonal therapy

Initially, it is important to mention the only possible treatments for PWS nowadays. Even though hormonal therapy cannot cure this disorder, it is currently the only possibility for managing PWS. While GH therapy is the standard treatment for improving growth, body composition, and motor function in PWS patients, emerging research has highlighted the potential for additional hormonal therapies. Oxytocin therapy, for instance, has shown promise in enhancing social cognition and regulating appetite, the area where GH therapy alone falls short. Likewise, GLP-1 receptor agonists, originally developed for type 2 diabetes, or diazoxide choline have demonstrated promising results in mitigating obesity. This chapter describes the mechanisms, clinical applications, and future potential of these hormone-based treatments.

4.1.1 Growth hormone

Growth hormone is a crucial peptide hormone produced by the anterior pituitary gland and is involved in regulating growth, metabolism, and body composition. A key mediator of GH's growth-promoting actions is insulin-like growth factor 1 (IGF-1) and IGF-2, which are primarily synthesised in the liver and epiphyseal plates in response to GH stimulation. IGF-1 and IGF-2 drive cell proliferation and differentiation, particularly in bone and muscle tissues, facilitating longitudinal growth and tissue repair. Beyond its role in growth, GH directly influences metabolism by promoting lipolysis, enhancing protein synthesis, and modulating glucose uptake, thereby regulating body composition and homeostasis. (reviewed by Ranke & Wit, 2018)

Insufficient GH production can lead to growth and metabolic disorders, necessitating therapeutic intervention (reviewed by Ayuk & Sheppard, 2006). Supplementation with GH has become an effective solution for these conditions with the development of recombinant safe GH production in the 1980s (Flodh, 1986).

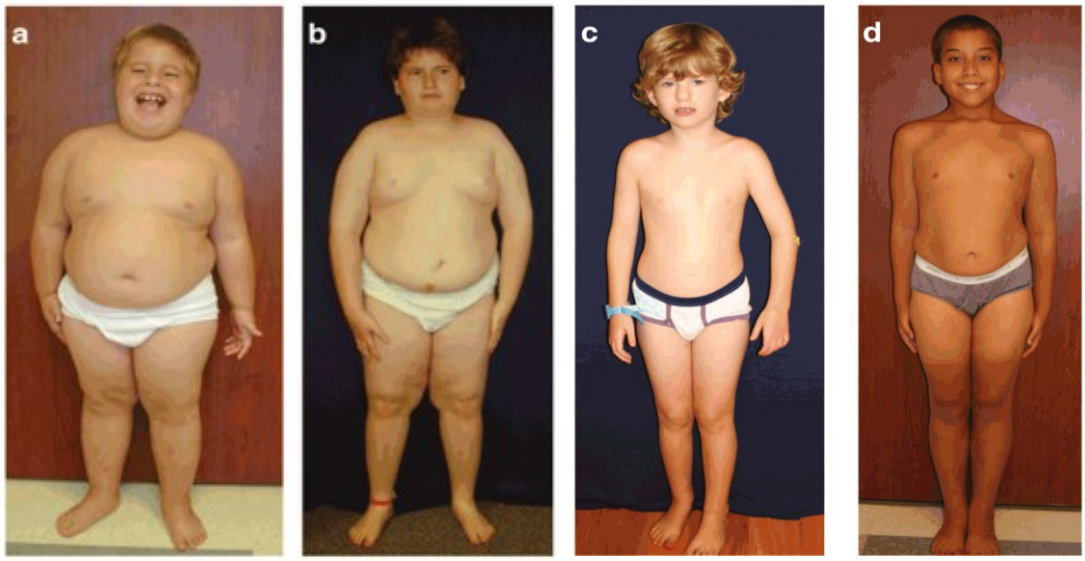


Figure 4: (**a** and **b**) 7- and 13-year-old children, respectively, not receiving growth hormone treatment. (**c** and **d**) 7- and 13-year-old children, respectively, who have had growth hormone treatment and good weight control. Informed consent was obtained for the publication of these photographs. (adapted from Cassidy et al. (2012), modified)

One of the endocrine deficiencies observed in PWS is impaired GH secretion due to hypothalamic dysfunction (Grugni et al., 2006). GH therapy has emerged as an effective approach to addressing this deficiency (see Figure 4). In adults with PWS, a comparative study by Frixou et al. (2021) has demonstrated that GH therapy significantly improved body composition by increasing lean body mass and reducing fat mass, even though changes in body

mass index and bone density were not observed. Additionally, GH therapy in adults were generally well tolerated, with only minor and transient adverse effects reported.

Results of the long-term study by Grootjen, Trueba-Timmermans, et al. (2022) comparing GH-treated and untreated children have emphasised the advantages of initiating GH therapy early. Beginning treatment within the first year of life led to a more favourable body composition and proportions development. Early treated children exhibited a more pronounced reduction in fat mass and a sustained increase in lean body mass index compared to those who began therapy later, between the ages of two and five. Furthermore, early GH intervention were linked to enhanced cognitive development, as evidenced by improved performance in language-related assessments, such as vocabulary scores (Donze et al., 2020; Grootjen, Trueba-Timmermans, et al., 2022). These findings suggest that GH therapy in infancy not only supports physical growth but also contributes to neurodevelopmental improvements, ultimately leading to better overall outcomes in children with PWS.

4.1.2 Oxytocin

Another possibility for addressing PWS lies in oxytocin therapy, a pharmacological approach aimed at targeting the syndrome's social and behavioural deficits and hyperphagia (reviewed by Althammer et al., 2022). While GH therapy is efficacious in improving physical growth and body composition, it does not sufficiently address social and appetite-related behaviour. Oxytocin, a neuropeptide crucial for social bonding and satiety regulation (reviewed by Lee et al., 2009; Olson et al., 1991), has emerged as a potential therapeutic candidate. Research indicates that the oxytocin system is impaired in PWS, characterised by a reduced number of oxytocin-producing neurons in the hypothalamic paraventricular nuclei and diminished expression of prohormone convertase 1 (PC1), leading to an accumulation of the less active pro-form of oxytocin (Burnett et al., 2016; Swaab et al., 1995). This dysregulation likely contributes to both the excessive hunger and the social difficulties observed in PWS.

In mouse models lacking Magel2, early postnatal oxytocin treatment has been shown to partially prevent social and learning deficits, suggesting that the critical period for intervention is a few days after birth. (Meziane et al., 2015; Schaller et al., 2010). Clinical trials investigating intranasal oxytocin application in PWS patients have yielded mixed results. A study conducted on individuals aged 12–30 years has not demonstrated significant improvements (Einfeld et al., 2014). However, trials focusing on younger children have reported some beneficial effects,

though the impact on behavioural parameters and hyperphagia varies across studies (Kuppens et al., 2016; Miller et al., 2017; Tauber et al., 2011; Valette et al., 2025).

To enhance treatment efficacy, Josselsohn et al. (2024) have proposed a dynamic dosing regimen combined with social interventions, rather than a fixed schedule, to better align with oxytocin's natural pulsatile secretion and reduce the risk of tolerance over time. Administering oxytocin during targeted therapies, such as speech or behavioural training, could enhance the brain's responsiveness to social cues, thereby maximising therapeutic benefits. By addressing hyperphagia and social dysfunction, oxytocin therapy offers a promising complement to existing interventions. Further research is necessary to refine dosing strategies and determine the optimal age for intervention.

4.1.3 Diazoxide choline

Uncontrolled appetite can lead to obesity, a significant risk factor for type 2 diabetes, cardiovascular diseases, and reduced mobility (Fletcher et al., 2002). Specially developed drugs that help manage hyperphagia can greatly ease the burden on carers, as once hyperphagia develops, controlling food-seeking behaviour becomes extremely challenging. Recently, the U.S. Food and Drug Administration (FDA) has approved a new drug called Vykat XR (diazoxide choline) (U.S. Food and Drug Administration, 2025), which functions as an activator of ATP-sensitive potassium channels on neuropeptide Y (NPY)/agouti-related protein (AgRP)/gamma-aminobutyric acid (GABA) (collectively referred to as NAG) neurons in the arcuate nucleus of the hypothalamus (Pocai et al., 2005; Secher et al., 2014). These neurons are implicated in appetite and endocrine regulation. Typically, leptin activates these potassium channels, leading to hyperpolarisation of the neuronal membrane and a reduction in the release of appetite-stimulating hormones (Spanswick et al., 1997; Van Den Top et al., 2004). Diazoxide choline mimics the function of leptin by acting as an agonist of the same channel. Owing to its strong ability to cross the blood-brain barrier, it can be effectively administered orally (Kishore et al., 2011).

The approval of diazoxide choline was preceded by several clinical studies utilising controlled-release tablets (DCCR). In a small Phase II pilot trial conducted by Kimonis et al. (2019), 13 overweight or obese adolescents and adults with PWS were administered DCCR for 10 weeks in an open-label phase, followed by a 4-week double-blind period in which a subset received a placebo. The study has found a notable reduction in appetite by the end of the open-label phase,

particularly among individuals who had reported more severe baseline hyperphagia or received higher doses. Additionally, reductions in aggressive behaviours and body fat, alongside increases in lean body mass, were reported, suggesting potential broader therapeutic benefits.

The larger Phase III DESTINY PWS trial, involving 127 participants aged four years and older, has not demonstrated a statistically significant overall reduction in appetite compared to placebo. However, participants with severe baseline hyperphagia experienced meaningful improvements, as did a subgroup assessed prior to disruptions caused by the COVID-19 pandemic. Other reported benefits are reduced body fat adipokines, acylated ghrelin, and insulin. (Miller et al., 2023)

Furthermore, a natural history comparison study by Strong et al. (2024) has found that DCCR-treated individuals exhibited significant reductions in hyperphagia at both six and twelve months, alongside improvements in behavioural symptoms such as aggression, anxiety, and compulsive behaviour. Reported side effects, most commonly peripheral oedema, mild hypertrichosis, and transient elevations in blood glucose, were generally manageable through dose adjustment or diuretic treatment (Kimonis et al., 2019; Woloschak et al., 2022). Collectively, these findings suggest that DCCR may represent a promising therapeutic option for individuals with PWS, particularly those experiencing more severe hyperphagia.

4.1.4 GLP-1 receptor agonists

Another promising approach for managing obesity in PWS is the use of glucagon-like peptide-1 receptor agonists (GLP-1 RAs), a class of drugs originally developed for type 2 diabetes (T2D), but recent research has shown potential in addressing the metabolic challenges of PWS. GLP-1 is an incretin hormone primarily secreted by the intestines in response to food intake and is involved in glucose metabolism and appetite regulation. It enhances insulin secretion, inhibits glucagon release, slows gastric emptying, and promotes satiety. GLP-1 receptor agonists, such as liraglutide and exenatide, mimic the effects of natural GLP-1 while providing prolonged activity compared to the endogenous hormone (reviewed by Barnett, 2007; reviewed by Müller et al., 2019; Secher et al., 2014)

Clinical reports have highlighted their potential benefits. In one case, a 17-year-old girl with PWS and T2D treated with liraglutide experienced a 3.1 kg weight loss and reduced appetite within two months, though weight regain occurred after 1.5 years (Duje et al., 2021). Similarly, an adult patient with PWS achieved a remarkable 56.7 kg weight loss over a decade using

exenatide and liraglutide, sustained by a low-calorie diet, with no significant side effects (Ahmed et al., 2023).

Beyond individual cases, systematic evidence supports the broader applicability of GLP-1 agonists in PWS. Ng et al. (2022) have evaluated the results of ten studies, including 23 PWS patients aged 13–37 years, treated with either exenatide or liraglutide for durations ranging from 14 weeks to 4 years, found that 16 had T2D, and 19 showed HbA1c improvements (0.3% to 7.5%), while 10 experienced BMI reductions (1.5 to 16.0 kg/m²). Mechanistically, GLP-1 agonists may suppress ghrelin, an orexigenic hormone implicated in PWS hyperphagia (Purtell et al., 2011). However, evidence remains inconsistent: One study has reported a significant decrease in serum ghrelin levels after a year of liraglutide treatment (Senda et al., 2012), while another study has found no significant change with exenatide (Salehi et al., 2017).

The drugs' safety profile appears favourable, with transient nausea as the primary side effect and no reports of severe complications like gastric rupture despite theoretical risks from delayed gastric emptying (Arenz et al., 2010; Ng et al., 2022; Paisey et al., 2011; Salehi et al., 2017). These findings position GLP-1 agonists as a promising adjunctive therapy, potentially complementing dietary interventions to mitigate obesity, though their long-term impact on weight maintenance requires further investigation.

4.2 Activation of expression from the maternal chromosome

One potential strategy for treating PWS involves reactivating the silent maternal copy to compensate for the missing paternal expression. This can be achieved theoretically using modern technologies such as histone methyltransferase inhibitors, CRISPR/dCas9-based epigenetic effectors or activators of transcription. Unlike hormone therapy, which primarily mitigates symptoms, this approach has the potential to address the underlying cause of PWS. However, challenges remain, including the need for repeated treatments, specificity, and the risk of activating UBE3A-ATS, which could lead to UBE3A silencing.

Horsthemke & Wagstaff (2008) and Xin et al. (2003) have suggested that DNA methylation is essential for PWS imprint establishment, but later in development, histone modifications play a more significant role in maintaining gene silencing. This is one of the reasons why research has primarily focused on removing repressive histone methylation marks from critical genes (Cruvinel et al., 2014; Kim et al., 2017; Langouët et al., 2020). Various epigenetic histone marks can dynamically regulate gene expression levels. Typically, methylation of Lys4 on

histone H3 (H3K4) and acetylation of Lys9 on histone H3 (H3K9) are associated with chromatin relaxation, allowing better transcriptional access. In contrast, methylation of H3K9 is linked to transcriptional repression. (reviewed by Horsthemke & Buiting, 2008)

4.2.1 Methyltransferases inhibitors

Saitoh & Wada (2000) were the first, who have demonstrated the possibility of reactivating imprinted genes through drug treatment. They have shown that treating cells derived from PWS patients with the DNA methyltransferase inhibitor 5-azadeoxycytidine led to increased expression of SNURF-SNRPN. This effect was associated with the SNURF-SNRPN CpG island demethylation and increased histone H3 acetylation. However, despite these promising *in vitro* findings, the approach using DNA methyltransferase inhibitors has never been successfully replicated *in vivo*.

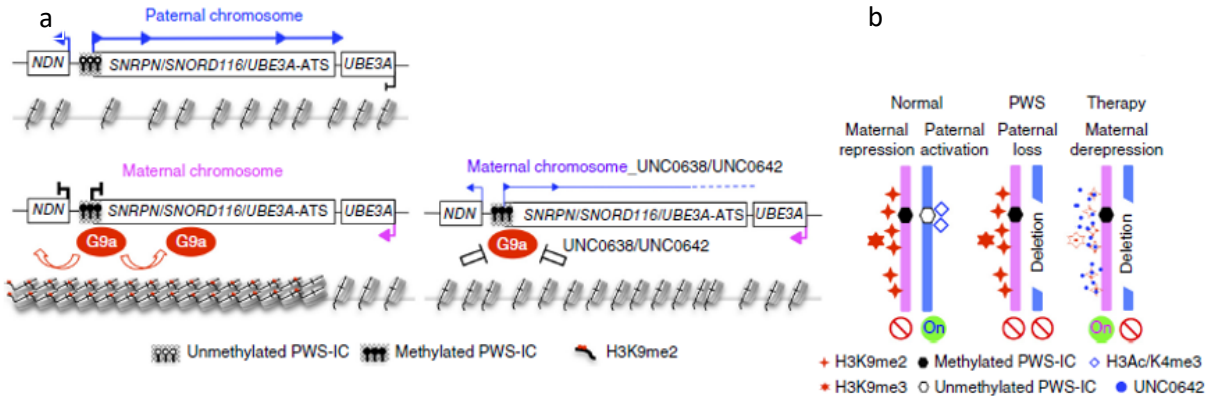


Figure 5: (a) A schematic chromatin-spreading model for maternal activation of the candidate PWS-associated genes in response to G9a inhibitor. Top, on the paternal chromosome, a widely open chromatin structure allows for gene transcription (for example, NDN, SNRPN and SNORD116). Bottom left, on the maternal chromosome, G9a-mediated methylation of H3K9 can propagate in a bidirectional manner along the PWS-associated genes. A compact, closed chromatin structure suppresses gene transcription of paternally expressed genes. Bottom right, G9a inhibitors induce the opening of chromatin structure through the reduction of H3K9 methylation, which derepresses the expression of PWS-associated genes. (b) Principle of epigenetic therapy for PWS via the G9a inhibitor. UNC0638 and UNC0642 (blue dots) directly reduce H3K9 methylation (red stars) but do not change methylated PWS-IC (black hexagon). The reduction of H3K9 methylation would be sufficient to activate PWS-associated genes and thereby offer therapeutic benefits. (adapted from Kim et al. (2017); modified)

Almost two decades later, Kim et al. (2017) took a different approach by targeting the histone methyltransferase G9a, building on the findings of Xin et al. (2003), to reverse the silencing of the maternal allele. They have demonstrated that selective inhibition of G9a with small-molecule inhibitors UNC0638 and UNC0642 specifically reduced H3K9 dimethylation (H3K9me2) at the PWS imprinting centre while leaving DNA methylation unchanged. This

epigenetic modification resulted in increased chromatin accessibility and reactivation of Ndn, Snurf-Snrpn and Snord116 gene expression. (see Figure 5)

These findings have been validated in both human PWS patient-derived fibroblasts and a PWS mouse model carrying a deletion from Snrpn to Ube3a ($m^{+/p\Delta S-U)}$. This mouse model offers a valuable balance between mimicking the progression of PWS in humans and allowing for long-term experiments, as some individuals can survive for extended periods (3 months). Despite the high neonatal mortality and the presence of the PWS phenotype, the model's ability to survive up to three months provides a significant advantage over the complete deletion model, enabling the study of longer-term effects. Notably, in the mouse model, UNC0642 treatment significantly improved the survival and growth of newborn pups, providing a compelling proof-of-concept for an epigenetics-based therapeutic strategy for PWS.

However, the underlying molecular mechanisms remain unclear. One intriguing question is how treatment activates Snord116 expression without influencing the Ube3a ATS, given that both arise from the same long transcript, Snhg14. This suggests the involvement of distinct regulatory mechanisms. One possible explanation is that the expression of the ATS transcript may initiate from alternative transcriptional start sites (TSSs) within this region. Galiveti et al. (2014) observed no correlation between UBE3A ATS and snoRNA expression profiles, proposing two alternative TSSs: one located between the SNORD116 and SNORD115 snoRNA clusters and another upstream of the UBE3A ATS. Another explanation could be that the effect of the inhibitor is localised to the IC, with distant regions remaining transcriptionally inaccessible (Kim et al., 2017). Interestingly, Wu et al. (2019) have investigated different G9a inhibitors (A366, UNC0638, and UNC0642) in PWS neural progenitor cells (NPCs) and observed upregulation of UBE3A ATS and, surprisingly, even UBE3A itself. This unexpected result suggests that G9a inhibition may have broader effects on chromatin accessibility and gene regulation.

A further question concerns the specificity of the treatment. G9a is a crucial histone methyltransferase involved in histone modifications and also can act on non-histone targets (Rathert et al., 2008; Tachibana et al., 2001), and while the authors claim that their small molecules specifically target the PWS region, the exact molecular mechanism remains unknown. Furthermore, the authors did not provide evidence, such as bulk mRNA sequencing, to show that their treatment does not affect the expression of other potentially unintended genes. On the other hand, wild-type mice treated with UNC0642 did not exhibit any signs of

health or neurological impairments, suggesting that the treatment does not cause broad adverse effects. Finally, the positive effect of the treatment has been shown to diminish over time after the drug injection, implying the need for repeated administration to maintain its therapeutic efficacy.

4.2.2 Further promising targets

Recent research into the epigenetic mechanisms of PWS has highlighted ZNF274 and SETDB1, in addition to G9a, as key regulators of maternal gene silencing. These findings open exciting possibilities for therapeutic intervention. ZNF274, a zinc finger protein (ZFP), binds to specific sites within the maternal SNORD116 cluster and recruits SETDB1, a histone methyltransferase. SETDB1 then deposits repressive H3K9me3 marks, locking the maternal allele in a silent state. Targeting the ZNF274-SETDB1 interaction represents a promising strategy for reactivating these silenced genes. (Cruvinel et al., 2014; Frietze et al., 2010; Langouët et al., 2018)

Studies have demonstrated the feasibility of this approach using distinct methods. Cruvinel et al. (2014) have shown that SETDB1 knockdown in induced pluripotent stem cells (iPSCs) derived from PWS patients reduced H3K9me3 levels at the SNORD116 locus, leading to partial reactivation of maternal SNURF-SNRPN and SNORD116. This suggests that SETDB1 inhibition could reverse epigenetic silencing; however, its broad activity across the genome raises concerns about off-target effects. A more precise approach, such as localised SETDB1 inhibition at SNORD116, could enhance specificity and therapeutic potential.

Alternatively, Langouët et al. (2020) utilised CRISPR/Cas9 to edit ZNF274 binding sites within the SNORD116 cluster in PWS iPSCs. By deleting or mutating these sites, they disrupted ZNF274 binding, leading to the reactivation of maternal SNORD116 and SNURF-SNRPN expression in PWS-derived neurons, reaching 50% of paternal expression levels. Unlike SETDB1 knockdown, this method avoids altering ZNF274 or SETDB1 function elsewhere in the genome (Valle-García et al., 2016), offering a targeted solution with reduced risk of unintended consequences.

4.2.3 Delivery systems

Previously mentioned drugs, due to their small size and simplicity, do not require specialised delivery systems. However, the advent of precise gene therapy introduced the need for more

sophisticated delivery methods, as these therapies often consist of multiple subunits that must be targeted to specific locations within the organism. To address this challenge, viral-based delivery systems were developed to leverage the natural ability of viruses to transfer genetic material into cells efficiently. For the treatment of PWS, AAV and lentiviral vector systems have been utilised, offering distinct advantages in terms of target specificity, efficiency, and long-term gene expression.

4.2.3.1 AAV Vectors

AAV vectors, derived from adeno-associated viruses (*Parvoviridae*), have become a cornerstone of gene therapy due to their ability to target specific tissues with minimal systemic toxicity efficiently. These vectors are engineered to be non-replicating and primarily maintain gene expression through episomal persistence, thus they rarely integrate into the host genome, thereby reducing the risk of unintended genetic disruptions (reviewed by McCarty et al., 2004). A pivotal advantage of AAV vectors lies in their diverse serotypes, which enable tissue-specific targeting. For instance, AAV9 efficiently transduces the heart and brain, while AAV8 exhibits a strong tropism for the liver (Deverman et al., 2016; Gao et al., 2002; reviewed by Zhao et al., 2024). However, their packaging capacity is limited to approximately 5 kb for single-stranded DNA (Z. Wu et al., 2010), which poses challenges for delivering larger genes. To overcome this limitation were developed innovative solutions such as dual-vector systems and engineered mini genes (Senís et al., 2014; Swiech et al., 2015; B. Wang et al., 2000). This precision has led to the successful application of AAV vectors in FDA-approved therapies for inherited retinal diseases and spinal muscular atrophy while also demonstrating the potential for treating neurological disorders, metabolic conditions, and even certain cancers (reviewed by Wang et al., 2024). Despite their advantages, AAV-based therapies face challenges, including immune responses at high doses, potential liver toxicity, and scalability issues in large-scale production therapy (Manno et al., 2006; Nathwani et al., 2014).

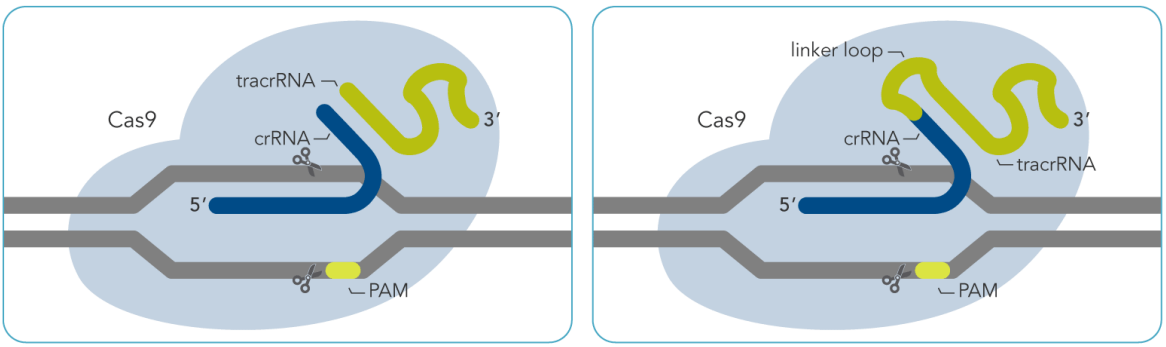
4.2.3.2 Lentiviral Vectors

Lentiviral vectors derived from human immunodeficiency viruses (HIV) offer an alternative to AAV-based gene therapies. Unlike AAV, they integrate into the host genome, ensuring stable and long-lasting gene expression, even in dividing cells (reviewed by Wang et al., 2021). This makes them particularly well-suited for applications such as chimeric antigen receptors T-cell therapies for blood cancers, where they have demonstrated considerable clinical success (Kochenderfer et al., 2014; Maude et al., 2014). Beyond oncology, lentiviral vectors have

proven effective in correcting genetic disorders, including β-thalassemia, X-linked adrenoleukodystrophy, metachromatic leukodystrophy, and Wiskott-Aldrich syndrome by modifying hematopoietic stem cells *ex vivo* (Aiuti et al., 2013; Biffi et al., 2013; Cartier et al., 2009; Imren et al., 2002). Their larger packaging capacity of approximately 8–9 kb allows for the delivery of bigger genes or multiple genetic elements, making them ideal for more complex therapeutic strategies (Kumar et al., 2001; Tiscornia et al., 2006). Advancements in safety have significantly reduced risks associated with lentiviral vectors. The last third generation of self-inactivating vectors minimises the likelihood of generating replication-competent viruses (Dull et al., 1998). However, challenges persist, including a small but inherent risk of insertional mutagenesis and the need for rigorous manufacturing protocols and long-term patient monitoring (Babaei et al., 2015; Lewinski et al., 2006). Despite these hurdles, lentiviral vectors remain an indispensable tool for therapies requiring permanent genetic modifications, offering a robust platform for a wide range of clinical applications.

4.2.4 CRISPR/dCas9-based epigenetic effectors

The invention of CRISPR technology has revolutionised genetic and epigenetic research, providing precise tools to manipulate gene expression and explore complex regulatory mechanisms. Originally derived from a bacterial immune system, CRISPR (clustered regularly interspaced short palindromic repeats) utilises the Cas9 nuclease to introduce targeted double-strand breaks in DNA, guided by a small RNA molecule known as a guide RNA (gRNA). This gRNA is composed of two components: the CRISPR RNA (crRNA), which includes a sequence complementary to the target DNA and recognises the protospacer adjacent motif (PAM) necessary for cleavage, and the trans-activating CRISPR RNA (tracrRNA), which binds to Cas9 and is essential for its activation. For convenience and efficiency in genome editing applications, these two RNA elements are often fused into a single guide RNA (sgRNA) (see Figure 6). (Jinek et al., 2012, 2014)



A. 2-part guide RNA B. Single guide RNA

Figure 6: (**A**) The two-part guide RNA consists of a duplex of a tracrRNA (green, upper right) and a crRNA (dark blue). (**B**) In sgRNA, the linker sequence forms a hairpin loop-like structure that fuses the crRNA and tracrRNA into a single RNA oligo that functions as the guide RNA. (adapted from Turk & Spencer (2024))

A major advancement in this system came with the development of a catalytically inactive version of Cas9, known as dCas9 (deactivated Cas9), which retains its DNA-binding ability but lacks nuclease activity. This modification transformed dCas9 into a versatile platform for epigenetic editing by allowing fusion with various effector domains, enabling precise modulation of gene expression without altering the underlying genetic sequence. These CRISPR/dCas9-based epigenetic effectors have opened new avenues for investigating gene regulation and hold immense promise for therapeutic applications, particularly in disorders caused by epigenetic dysregulation, such as PWS. (Cano-Rodriguez et al., 2016; Hilton et al., 2015; X. S. Liu et al., 2016)

A recent study by Rohm et al. (2025) has employed CRISPR/dCas9-based epigenetic effectors to achieve precise activation of maternally silenced genes. The researchers have used dCas9 fused to different effector domains delivered by lentiviral particles to target and manipulate the epigenetic marks of the PWS locus in human iPSCs. Specifically, they have used two distinct approaches: transcriptional activation with a VP64-fused dCas9 (^{VP64}dCas9V^{P64}) and DNA demethylation with a Tet1-fused dCas9 (^{Tet1}dCas9). These effectors target specific regulatory elements within the PWS locus, identified through CRISPR activation (CRISPRa) and interference (CRISPRi) screens (reviewed by Kampmann, 2018), to reactivate maternally silenced genes, including SNURF-SNRPN and downstream transcripts such as SNORD116.

The ^{VP64}dCas9^{VP64} system involves fusing the VP64 transcriptional activator, a tetramer of the herpes simplex virus VP16's minimal activation domain, to both ends of dCas9, enhancing its ability to recruit transcriptional machinery and activate gene expression (Beerli et al., 1998; Chakraborty et al., 2014). In the study, this effector was used in a CRISPRa screen with a

gRNA library tiling the 15q11-13 region, revealing two clusters of regulatory elements (mat1 and mat2) approximately 100 kb upstream of the PWS-IC. Targeting these regions with ^{VP64}dCas9^{VP64} significantly increased maternal SNURF-SNRPN expression, although it reached only ~7% of wild-type levels in PWS iPSCs.

In contrast, the ^{Tet1}dCas9 approach focuses on reversing DNA methylation. The Tet1 enzyme, part of the ten-eleven translocation family, catalyses the conversion of 5-methylcytosine to 5-hydroxymethylcytosine, initiating active DNA demethylation (Tahiliani et al., 2009). The study tested multiple Tet1 constructs, ultimately optimising with ^{Tet1v4}dCas9, which features an extended linker for improved efficacy. A targeted screen with this effector identified a regulatory region overlapping the PWS-IC (mat3), distinct from the VP64 targets, where demethylation activated maternal SNURD-SNRPN to over 25% of wild-type levels. Remarkably, transient expression of ^{Tet1v4}dCas9 led to stable, long-term expression of maternal SNURF-SNRPN, suggesting a heritable epigenetic reprogramming event.

This study provides insights into the epigenetic regulation of PWS and highlights the potential of CRISPR/dCas9-based effectors for therapeutic applications. While ^{VP64}dCas9^{VP64} and ^{Tet1v4}dCas9 partially reactivated SNURF-SNRPN, future work should explore their effects on other PWS-related genes to achieve broader epigenetic correction. Additionally, further *in viv*o studies in mouse models are necessary to assess the long-term stability, specificity, and therapeutic feasibility of these approaches in a physiological context.

4.2.5 Zinc finger protein-based transcriptional reprogramming

Zinc finger proteins (ZFPs) are a class of naturally occurring transcription factors prevalent in eukaryotic cells, known for their ability to bind specific DNA sequences through modular zinc finger domains (Nagai et al., 1988). These proteins can be engineered to target precise genomic loci, making them powerful tools for genome editing and transcriptional regulation (reviewed by Thakore et al., 2016). In the context of PWS, ZFP-based transcriptional reprogramming offers a promising therapeutic strategy. Unlike AS, where the goal is often to activate the repressed paternal UBE3A allele (Meng et al., 2015; Schmid et al., 2021; Wolter et al., 2020), PWS requires the reactivation of silenced maternal alleles. The study by O'Geen et al. (2023) provides valuable insights into how ZFP technology, combined with AAV delivery, can be adapted for such purposes.

In the referenced study, an artificial transcription factor (ATF), termed ATF-S1K, has been designed to target the Snurf-Snrpn TSS in a mouse model of AS. This ATF consists of a ZFP fused to a Krüppel-associated box (KRAB) repressor domain (Beerli et al., 1998; Margolin et al., 1994), which silences the long non-coding RNA Ube3a-ATS. By downregulating Ube3a-ATS, the paternal Ube3a allele is unsilenced, restoring UBE3A protein expression to approximately 25% of wild-type levels in neurons across the brain. The delivery mechanism has utilised an AAV vector, administered via a single tail vein injection, achieving brain-wide transduction in adult AS mice. This approach has demonstrated high specificity, minimal off-target effects, and no detectable inflammatory response five weeks post-administration, highlighting the tolerability and efficacy of AAV-delivered ZFPs.

For PWS, a similar ZFP-based strategy could be employed, but with a modification: replacing the repressive KRAB domain with an activation domain, such as VP64 or p65 (Ji et al., 2014; P.-Q. Liu et al., 2001). By targeting the maternal SNURF-SNRPN promoter or regulatory elements within the PWS-IC, an activating ZFP could potentially overcome the epigenetic silencing imposed by maternal imprinting, reactivating genes like SNURF-SNRPN and SNHG14. The small size of ZFPs (e.g., ATF-S1K is ~0.8 kb) compared to CRISPR-Cas9 systems (Senís et al., 2014; Swiech et al., 2015) makes them particularly suitable for AAV packaging, which has a capacity limit of approximately 5 kb (Z. Wu et al., 2010). This size advantage, combined with the neurotropic properties of AAV serotypes like AAV9 or AAV-PHP.B, enables efficient delivery across the blood-brain barrier, a critical requirement for addressing the neurological deficits in PWS (Cearley & Wolfe, 2006; Deverman et al., 2016).

4.3 Gene augmentation therapy

The most straightforward approach to addressing the PWS is to deliver the missing genes into the affected individual. In contrast to AS, where a single gene, UBE3A, is the primary contributor, PWS is more complex. The large size of the missing genetic information (several Mbp) makes complete delivery with current tools impossible. For this reason, scientists have primarily focused on delivering a functional SNORD116.

4.3.1 SNORD116

Since the SNORD116 gene cluster is considered the primary contributor to Prader-Willi syndrome (PWS) in humans, scientists have primarily focused on restoring its expression

(Coulson et al., 2018; Qi, Purtell, Fu, Zhang, et al., 2017). However, this approach presents several challenges. Although a single SNORD116 copy is relatively small (~100 bp) and suitable for AAV delivery, research using interaction predictors suggests that each copy (or rather, each class) may have a unique set of targets (Baldini et al., 2022; Bazeley et al., 2008; Deschamps-Francoeur et al., 2022; Gilmore et al., 2024; Kehr et al., 2011). This implies that using just one copy may not be sufficient for complete functional restoration. Moreover, constructing a larger functional SNORD116 cluster is complex. The transcribed RNA undergoes neuron-specific splicing, which requires the Rbfox3 factor and an unknown element from the SNHG14 for the proper formation of snoRNAs and the 116HG transcript (Coulson et al., 2018). The following paragraphs summarise previous experiments, which, despite being unsuccessful, have provided valuable insights into the molecular biology of SNORD116.

Qi, Purtell, Fu, Zhang, et al. (2017) have investigated the potential of using an AAV-based approach to reintroduce a single copy of SNORD116 into the hypothalamus of Snord116$^{-/-}$ mice. These mice exhibit PWS-like phenotypes, including low postnatal body weight and increased food intake (Qi et al., 2016). However, they do not develop obesity due to elevated energy expenditure. The study has found that reintroducing SNORD116 specifically into the mid-hypothalamus of 6-week-old mice housed at 20 °C led to a slight reduction in body weight and weight gain, an effect attributed to increased energy expenditure. In contrast, injections into other hypothalamic regions did not produce any significant changes. Additionally, older mice treated with viral particles showed no improvement, suggesting that the therapeutic window for SNORD116 restoration may be limited to early developmental stages.

Unfortunately, this study has several methodological flaws that undermine the validity of its conclusions. The most critical issue is the absence of WT controls. Instead of comparing treated mice to a proper WT group, authors only compared Snord116$^{-/-}$ mice treated with an empty AAV vector to those receiving SNORD116, making it difficult to assess the actual impact of the intervention. One possible reason for this omission is that the used Snord116$^{-/-}$ model exhibits only a mild PWS phenotype (Qi et al., 2016). Notably, when housed at 30 °C, these mice closely resemble WT animals, further complicating the interpretation of results (Qi, Purtell, Fu, Sengmany, et al., 2017). Additionally, for unclear reasons, the authors used the human SNORD116 sequence rather than the mouse counterpart, which could have affected the functional outcome of the experiment. These limitations highlight the need for more rigorous methodological design in future studies exploring AAV-SNORD116 therapeutic strategies.

4.3.2 BDNF

Among the promising therapeutic approaches for PWS, gene therapy targeting brain-derived neurotrophic factor (BDNF) has emerged as a potential strategy to mitigate disease manifestations. BDNF, a neurotrophin essential for synaptic plasticity, neuronal survival, and growth, plays a crucial role in PWS pathophysiology, as its expression is reduced in the hypothalamus of affected individuals (Bochukova et al., 2018; Snider, 1994). Recent research by Queen et al. (2022) and Queen et al. (2023) has examined the use of AAV-mediated BDNF gene therapy in the Magel2-null mouse model, which recapitulates typical PWS features, including increased adiposity and impaired metabolic functions (Kozlov et al., 2007; Mercer & Wevrick, 2009). These findings highlight the potential of BDNF-based interventions for addressing metabolic and neurological deficits in PWS.

Queen et al. (2022) have demonstrated that hypothalamic AAV-BDNF gene therapy significantly improved metabolic function and behaviour in female Magel2-null mice. The study has found that BDNF treatment effectively prevented excessive weight gain, reduced fat mass, increased lean mass, and enhanced energy expenditure over 23 weeks, all without inducing adverse behavioural effects. Additionally, treated mice exhibited improved glucose metabolism and insulin sensitivity, along with normalised levels of circulating leptin. Similar metabolic benefits were observed in male Magel2-null mice, including improved body composition and glycaemic control. Behavioural assessments in females further indicated comparable performance to WT mice in repetitive and exploratory behaviour tests, suggesting that BDNF supplementation may help address both metabolic and neurological deficits associated with Magel2 deficiency (Mercer et al., 2009).

Building on this foundation, Queen et al. (2023) have explored the molecular mechanisms underlying these improvements, focusing on the hypothalamic microenvironment. Through mRNA sequencing, they identified a neuroinflammatory signature in Magel2-null mice, characterised by upregulated inflammatory genes and microglial activation markers, consistent with observations in human PWS patients (Bochukova et al., 2018). Remarkably, AAV-BDNF gene therapy reversed this neuroinflammatory profile, downregulating inflammatory pathways (reviewed by Cai and Khor, 2019) and microglial activation markers. This suggests that BDNF may exert its therapeutic effects by modulating obesity-related neuroinflammation (De Souza et al., 2005; Zhang et al., 2008), either directly or indirectly, potentially through interactions with hypothalamic microglia or systemic metabolic feedback. These findings validate the

Magel2-null mouse as a model for studying PWS-related neuroinflammation and highlight AAV-BDNF as a promising intervention for mitigating both metabolic and inflammatory aspects of the syndrome.

Despite its potential, AAV-BDNF therapy faces several challenges in clinical translation. The deep location of the hypothalamus complicates direct injections, highlighting the need for alternative, less invasive delivery methods (Queen et al., 2023). Moreover, as the therapy focuses solely on BDNF augmentation rather than restoring the full spectrum of PWS genes, its impact is limited to symptom management rather than addressing the root genetic cause of the disorder. Future research should explore combinatorial approaches, integrating BDNF therapy with strategies aimed at reactivating the maternal allele to provide a more comprehensive treatment for PWS. Nevertheless, AAV-BDNF gene therapy represents a significant advancement, offering a promising avenue to mitigate the metabolic and inflammatory complications associated with PWS.

5 CONCLUSION

Prader-Willi syndrome remains a complex neurodevelopmental disorder with no curative treatment currently available. As outlined in this bachelor thesis, PWS arises from genetic abnormalities affecting the 15q11-q13 chromosomal region, leading to severe metabolic, endocrine, cognitive, and behavioural symptoms. Current treatment strategies primarily focus on symptom management, with growth hormone therapy playing a central role in improving growth and body composition. However, GH therapy does not address all aspects of the disorder, particularly hyperphagia and social-behavioural deficits.

Recent advancements in molecular and pharmacological research have opened new possibilities for PWS treatment. Emerging hormone-based therapies, such as oxytocin, GLP-1 receptor agonists, and diazoxide choline, show promise in tackling appetite dysregulation and social impairments, complementing GH therapy to provide a more comprehensive treatment approach. Additionally, gene therapy strategies, including epigenetic reprogramming, CRISPR/dCas9-based interventions, and gene augmentation therapy, hold potential for addressing the genetic cause of PWS. Studies targeting the reactivation of the silenced maternal allele or introducing missing genes, such as SNORD116, represent a shift toward disease-modifying treatments that could significantly alter the course of PWS management in the future.

Despite these promising developments, numerous challenges remain. Gene therapy approaches must overcome delivery limitations, safety concerns, and potential off-target effects before becoming viable clinical treatments. Similarly, long-term studies are required to assess the efficacy and safety of novel pharmacological interventions, particularly in young patients with developing nervous and endocrine systems. Ethical considerations also arise in the context of genetic modifications, necessitating careful evaluation of risks and benefits before widespread clinical application. As research progresses, a multidisciplinary approach integrating endocrinology, genetics, and neuroscience will be essential for developing personalised and effective therapies for PWS. The transition from symptom management to curative treatments will require continued innovation, collaboration, and clinical validation. Although PWS remains a challenging condition, the advancements discussed in this bachelor's thesis bring hope for improved patient outcomes and a future where individuals with PWS can achieve a higher quality of life through targeted, effective treatments.

6 DECLARATION REGARDING THE USE OF AI TOOLS

During the preparation of this bachelor's thesis, AI-based tools (ChatGPT; Grammarly) were used to assist with grammar correction and stylistic improvements. All outputs generated with the help of these tools were thoroughly reviewed and verified by the author. The author assumes full responsibility for the final content of the thesis.

7 BIBLIOGRAPHY

Secondary sources are marked with an asterisk (*)

Ahmed, S., Naz, A., & K, M. (2023). Weight loss of over 100 lbs in a patient of Prader-Willi syndrome treated with glucagon-like peptide-1 (GLP-1) agonists. *Cureus*, *15*(2), e35102. https://doi.org/10.7759/cureus.35102

Aiuti, A., Biasco, L., Scaramuzza, S., Ferrua, F., Cicalese, M. P., Baricordi, C., Dionisio, F., Calabria, A., Giannelli, S., Castiello, M. C., Bosticardo, M., Evangelio, C., Assanelli, A., Casiraghi, M., Di Nunzio, S., Callegaro, L., Benati, C., Rizzardi, P., Pellin, D., … Naldini, L. (2013). Lentiviral hematopoietic stem cell gene therapy in patients with wiskott-aldrich syndrome. *Science*, *341*(6148), 1233151. https://doi.org/10.1126/science.1233151

*Althammer, F., Muscatelli, F., Grinevich, V., & Schaaf, C. P. (2022). Oxytocin-based therapies for treatment of Prader-Willi and Schaaf-Yang syndromes: Evidence, disappointments, and future research strategies. *Translational Psychiatry*, *12*(1), 318. https://doi.org/10.1038/s41398-022-02054-1

Amos-Landgraf, J. M., Ji, Y., Gottlieb, W., Depinet, T., Wandstrat, A. E., Cassidy, S. B., Driscoll, D. J., Rogan, P. K., Schwartz, S., & Nicholls, R. D. (1999). Chromosome breakage in the Prader-Willi and Angelman syndromes involves recombination between large, transcribed repeats at proximal and distal breakpoints. *The American Journal of Human Genetics*, *65*(2), 370–386. https://doi.org/10.1086/302510

Arenz, T., Schwarzer, A., Pfluger, Th., Koletzko, S., & Schmidt, H. (2010). Delayed gastric emptying in patients with prader willi syndrome. *Journal of Pediatric Endocrinology and Metabolism*, *23*(9), 867–871. https://doi.org/10.1515/jpem.2010.140

*Ayuk, J., & Sheppard, M. C. (2006). Growth hormone and its disorders. *Postgraduate Medical Journal*, *82*(963), 24–30. https://doi.org/10.1136/pgmj.2005.036087

Babaei, S., Akhtar, W., De Jong, J., Reinders, M., & De Ridder, J. (2015). 3D hotspots of recurrent retroviral insertions reveal long-range interactions with cancer genes. *Nature Communications*, *6*(1), 6381. https://doi.org/10.1038/ncomms7381

Baldini, L., Robert, A., Charpentier, B., & Labialle, S. (2022). Phylogenetic and molecular analyses identify SNORD116 targets involved in the Prader–Willi syndrome. *Molecular Biology and Evolution*, *39*(1), msab348. https://doi.org/10.1093/molbev/msab348

*Barnett, A. (2007). Exenatide. *Expert Opinion on Pharmacotherapy*, *8*(15), 2593–2608. https://doi.org/10.1517/14656566.8.15.2593

Bazeley, P. S., Shepelev, V., Talebizadeh, Z., Butler, M. G., Fedorova, L., Filatov, V., & Fedorov, A. (2008). snoTARGET shows that human orphan snoRNA targets locate close to alternative splice junctions. *Gene*, *408*(1–2), 172–179. https://doi.org/10.1016/j.gene.2007.10.037

Beerli, R. R., Segal, D. J., Dreier, B., & Barbas, C. F. (1998). Toward controlling gene expression at will: Specific regulation of the erbB-2/HER-2 promoter by using polydactyl zinc finger proteins constructed from modular building blocks. *Proceedings of the National Academy of Sciences*, *95*(25), 14628–14633. https://doi.org/10.1073/pnas.95.25.14628

*Bervini, S., & Herzog, H. (2013). Mouse models of Prader–Willi Syndrome: A systematic review. *Frontiers in Neuroendocrinology*, *34*(2), 107–119. https://doi.org/10.1016/j.yfrne.2013.01.002

Bestor, T., Laudano, A., Mattaliano, R., & Ingram, V. (1988). Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. *Journal of Molecular Biology*, *203*(4), 971–983. https://doi.org/10.1016/0022-2836(88)90122-2

Biffi, A., Montini, E., Lorioli, L., Cesani, M., Fumagalli, F., Plati, T., Baldoli, C., Martino, S., Calabria, A., Canale, S., Benedicenti, F., Vallanti, G., Biasco, L., Leo, S., Kabbara, N., Zanetti, G., Rizzo, W. B., Mehta, N. A. L., Cicalese, M. P., … Naldini, L. (2013). Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy. *Science*, *341*(6148), 1233158. https://doi.org/10.1126/science.1233158

Boccaccio, I., Glatt-Deeley, H., Watrin, F., Roeckel, N., Lalande, M., & Muscatelli, F. (1999). The human MAGEL2 gene and its mouse homologue are paternally expressed and mapped to the Prader-Willi region. *Human Molecular Genetics*, *8*(13), 2497–2505. https://doi.org/10.1093/hmg/8.13.2497

Bochukova, E. G., Lawler, K., Croizier, S., Keogh, J. M., Patel, N., Strohbehn, G., Lo, K. K., Humphrey, J., Hokken-Koelega, A., Damen, L., Donze, S., Bouret, S. G., Plagnol, V., & Farooqi, I. S. (2018). A transcriptomic signature of the hypothalamic response to fasting and BDNF deficiency in prader-willi syndrome. *Cell Reports*, *22*(13), 3401–3408. https://doi.org/10.1016/j.celrep.2018.03.018

Bohonowych, J., Miller, J., McCandless, S. E., & Strong, T. V. (2019). The global Prader-Willi syndrome registry: Development, launch, and early demographics. *Genes*, *10*(9), 713. https://doi.org/10.3390/genes10090713

*Buiting, K. (2010). Prader–Willi syndrome and Angelman syndrome. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*, *154C*(3), 365–376. https://doi.org/10.1002/ajmg.c.30273

Buiting, K., Groß, S., Lich, C., Gillessen-Kaesbach, G., El-Maarri, O., & Horsthemke, B. (2003). Epimutations in Prader-Willi and Angelman syndromes: A molecular study of 136 patients with an imprinting defect. *The American Journal of Human Genetics*, *72*(3), 571–577. https://doi.org/10.1086/367926

Buiting, K., Nazlican, H., Galetzka, D., Wawrzik, M., Groβ, S., & Horsthemke, B. (2007). C15orf2 and a novel noncoding transcript from the Prader–Willi/Angelman syndrome region show monoallelic expression in fetal brain. *Genomics*, *89*(5), 588–595. https://doi.org/10.1016/j.ygeno.2006.12.008

Bürger, J., Horn, D., Tönnies, H., Neitzel, H., & Reis, A. (2002). Familial interstitial 570 kbp deletion of the UBE3A gene region causing Angelman syndrome but not Prader-Willi syndrome. *American Journal of Medical Genetics*, *111*(3), 233–237. https://doi.org/10.1002/ajmg.10498

Burnett, L. C., LeDuc, C. A., Sulsona, C. R., Paull, D., Rausch, R., Eddiry, S., Carli, J. F. M., Morabito, M. V., Skowronski, A. A., Hubner, G., Zimmer, M., Wang, L., Day, R., Levy, B., Fennoy, I., Dubern, B., Poitou, C., Clement, K., Butler, M. G., … Leibel, R. L. (2016). Deficiency in prohormone convertase PC1 impairs prohormone processing in Prader-Willi syndrome. *Journal of Clinical Investigation*, *127*(1), 293–305. https://doi.org/10.1172/JCI88648

Butler, M. G., Fischer, W., Kibiryeva, N., & Bittel, D. C. (2008). Array comparative genomic hybridization (aCGH) analysis in Prader–Willi syndrome. *American Journal of Medical Genetics Part A*, *146A*(7), 854–860. https://doi.org/10.1002/ajmg.a.32249

Butler, M. G., & Palmer, C. G. (1983). Parental origin of chromosome 15 deletion in Prader-Willi syndrome. *The Lancet*, *321*(8336), 1285–1286. https://doi.org/10.1016/S0140-6736(83)92745-9

Butler, M. G., & Thompson, T. (2000). Prader-Willi syndrome: Clinical and genetic findings. *The Endocrinologist*, *10*(4 Supplement 1), 3S-16S. https://doi.org/10.1097/00019616-200010041-00002

*Cai, D., & Khor, S. (2019). "Hypothalamic microinflammation" paradigm in aging and metabolic diseases. *Cell Metabolism*, *30*(1), 19–35. https://doi.org/10.1016/j.cmet.2019.05.021

Cano-Rodriguez, D., Gjaltema, R. A. F., Jilderda, L. J., Jellema, P., Dokter-Fokkens, J., Ruiters, M. H. J., & Rots, M. G. (2016). Writing of H3K4Me3 overcomes epigenetic silencing in a sustained but context-dependent manner. *Nature Communications*, *7*(1), 12284. https://doi.org/10.1038/ncomms12284

Cao, Y., AlHumaidi, S. S., Faqeih, E. A., Pitel, B. A., Lundquist, P., & Aypar, U. (2017). A novel deletion of SNURF/SNRPN exon 1 in a patient with Prader-Willi-like phenotype. *European Journal of Medical Genetics*, *60*(8), 416–420. https://doi.org/10.1016/j.ejmg.2017.05.003

Carrel, A. L., Moerchen, V., Myers, S. E., Bekx, M. T., Whitman, B. Y., & Allen, D. B. (2004). Growth hormone improves mobility and body composition in infants and toddlers with Prader-Willi syndrome. *The Journal of Pediatrics*, *145*(6), 744–749. https://doi.org/10.1016/j.jpeds.2004.08.002

Cartier, N., Hacein-Bey-Abina, S., Bartholomae, C. C., Veres, G., Schmidt, M., Kutschera, I., Vidaud, M., Abel, U., Dal-Cortivo, L., Caccavelli, L., Mahlaoui, N., Kiermer, V., Mittelstaedt, D., Bellesme, C., Lahlou, N., Lefrère, F., Blanche, S., Audit, M., Payen, E., … Aubourg, P. (2009). Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy. *Science*, *326*(5954), 818–823. https://doi.org/10.1126/science.1171242

*Cassidy, S. B., Schwartz, S., Miller, J. L., & Driscoll, D. J. (2012). Prader-Willi syndrome. *Genetics in Medicine*, *14*(1), 10–26. https://doi.org/10.1038/gim.0b013e31822bead0

Cavaillé, J., Buiting, K., Kiefmann, M., Lalande, M., Brannan, C. I., Horsthemke, B., Bachellerie, J.-P., Brosius, J., & Hüttenhofer, A. (2000). Identification of brain-specific and imprinted small nucleolar RNA genes exhibiting an unusual genomic organization. *Proceedings of the National Academy of Sciences*, *97*(26), 14311–14316. https://doi.org/10.1073/pnas.250426397

Cearley, C. N., & Wolfe, J. H. (2006). Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. *Molecular Therapy*, *13*(3), 528–537. https://doi.org/10.1016/j.ymthe.2005.11.015

Chakraborty, S., Ji, H., Kabadi, A. M., Gersbach, C. A., Christoforou, N., & Leong, K. W. (2014). A CRISPR/Cas9-based system for reprogramming cell lineage specification. *Stem Cell Reports*, *3*(6), 940–947. https://doi.org/10.1016/j.stemcr.2014.09.013

ChatGPT. (2025). https://chatgpt.com

Chen, H., Victor, A. K., Klein, J., Tacer, K. F., Tai, D. J. C., De Esch, C., Nuttle, A., Temirov, J., Burnett, L. C., Rosenbaum, M., Zhang, Y., Ding, L., Moresco, J. J., Diedrich, J. K., Yates, J. R., Tillman, H. S., Leibel, R. L., Talkowski, M. E., Billadeau, D. D., … Potts, P. R. (2020). Loss of MAGEL2 in Prader-Willi syndrome leads to decreased secretory granule and neuropeptide production. *JCI Insight*, *5*(17), e138576. https://doi.org/10.1172/jci.insight.138576

Christian, S. L., Bhatt, N. K., Martin, S. A., Sutcliffe, J. S., Kubota, T., Huang, B., Mutirangura, A., Chinault, A. C., Beaudet, A. L., & Ledbetter, D. H. (1998). Integrated YAC contig map of the Prader–Willi/Angelman region on chromosome 15q11–q13 with average STS spacing of 35 kb. *Genome Research*, *8*(2), 146–157. https://doi.org/10.1101/gr.8.2.146

Coulson, R. L., Powell, W. T., Yasui, D. H., Dileep, G., Resnick, J., & LaSalle, J. M. (2018). Prader–Willi locus Snord116 RNA processing requires an active endogenous allele and neuron-specific splicing by Rbfox3/NeuN. *Human Molecular Genetics*, *27*(31), 4051–4060. https://doi.org/10.1093/hmg/ddy296

Cruvinel, E., Budinetz, T., Germain, N., Chamberlain, S., Lalande, M., & Martins-Taylor, K. (2014). Reactivation of maternal SNORD116 cluster via SETDB1 knockdown in Prader-Willi syndrome iPSCs. *Human Molecular Genetics*, *23*(17), 4674–4685. https://doi.org/10.1093/hmg/ddu187

De Smith, A. J., Purmann, C., Walters, R. G., Ellis, R. J., Holder, S. E., Van Haelst, M. M., Brady, A. F., Fairbrother, U. L., Dattani, M., Keogh, J. M., Henning, E., Yeo, G. S. H., O'Rahilly, S., Froguel, P., Farooqi, I. S., & Blakemore, A. I. F. (2009). A deletion of the HBII-85 class of small nucleolar RNAs (snoRNAs) is associated with hyperphagia, obesity and hypogonadism. *Human Molecular Genetics*, *18*(17), 3257–3265. https://doi.org/10.1093/hmg/ddp263

De Souza, C. T., Araujo, E. P., Bordin, S., Ashimine, R., Zollner, R. L., Boschero, A. C., Saad, M. J. A., & Velloso, L. A. (2005). Consumption of a fat-rich diet activates a proinflammatory response and induces insulin resistance in the hypothalamus. *Endocrinology*, *146*(10), 4192–4199. https://doi.org/10.1210/en.2004-1520

Deschamps-Francoeur, G., Couture, S., Abou-Elela, S., & Scott, M. S. (2022). The snoGloBe interaction predictor reveals a broad spectrum of C/D snoRNA RNA targets. *Nucleic Acids Research*, *50*(11), 6067–6083. https://doi.org/10.1093/nar/gkac475

Deverman, B. E., Pravdo, P. L., Simpson, B. P., Kumar, S. R., Chan, K. Y., Banerjee, A., Wu, W.-L., Yang, B., Huber, N., Pasca, S. P., & Gradinaru, V. (2016). Cre-dependent selection yields AAV variants for widespread gene transfer to the adult brain. *Nature Biotechnology*, *34*(2), 204–209. https://doi.org/10.1038/nbt.3440

Dhar, M. S., Yuan, J. S., Elliott, S. B., & Sommardahl, C. (2006). A type IV P-type ATPase affects insulin-mediated glucose uptake in adipose tissue and skeletal muscle in mice. *The Journal of Nutritional Biochemistry*, *17*(12), 811–820. https://doi.org/10.1016/j.jnutbio.2006.01.002

Ding, F., Li, H. H., Zhang, S., Solomon, N. M., Camper, S. A., Cohen, P., & Francke, U. (2008). SnoRNA Snord116 (Pwcr1/MBII-85) deletion causes growth deficiency and hyperphagia in mice. *PLoS ONE*, *3*(3), e1709. https://doi.org/10.1371/journal.pone.0001709

Donze, S. H., Damen, L., Mahabier, E. F., & Hokken-Koelega, A. C. S. (2020). Cognitive functioning in children with Prader–Willi syndrome during 8 years of growth hormone treatment. *European Journal of Endocrinology*, *182*(4), 405–411. https://doi.org/10.1530/EJE-19-0479

Duje, B., Nevena, K., Katja, D. K., Anita, Š. U., Igor, M., & Jurica, V. (2021). 208 Use of GLP-1 analog in a patient with prader-willi syndrome. *Archives of Disease in Childhood*, *106*(Suppl 2), A88. https://doi.org/10.1136/archdischild-2021-europaediatrics.208

Dull, T., Zufferey, R., Kelly, M., Mandel, R. J., Nguyen, M., Trono, D., & Naldini, L. (1998). A third-generation lentivirus vector with a conditional packaging system. *Journal of Virology*, *72*(11), 8463–8471. https://doi.org/10.1128/JVI.72.11.8463-8471.1998

Eiholzer, U., & l'Allemand, D. (2000). Growth hormone normalises height, prediction of final height and hand length in children with Prader-Willi syndrome after 4 years of therapy. *Hormone Research in Paediatrics*, *53*(4), 185–192. https://doi.org/10.1159/000023565

Einfeld, S. L., Smith, E., McGregor, I. S., Steinbeck, K., Taffe, J., Rice, L. J., Horstead, S. K., Rogers, N., Hodge, M. A., & Guastella, A. J. (2014). A double-blind randomized controlled trial of oxytocin nasal spray in Prader Willi syndrome. *American Journal of Medical Genetics Part A*, *164*(9), 2232–2239. https://doi.org/10.1002/ajmg.a.36653

Falaleeva, M., Pages, A., Matuszek, Z., Hidmi, S., Agranat-Tamir, L., Korotkov, K., Nevo, Y., Eyras, E., Sperling, R., & Stamm, S. (2016). Dual function of C/D box small nucleolar RNAs in rRNA modification and alternative pre-mRNA splicing. *Proceedings of the National Academy of Sciences*, *113*(12). https://doi.org/10.1073/pnas.1519292113

Färber, C., Groß, S., Neesen, J., Buiting, K., & Horsthemke, B. (2000). Identification of a testis-specific gene (C15orf2) in the Prader–Willi syndrome region on chromosome 15. *Genomics*, *65*(2), 174–183. https://doi.org/10.1006/geno.2000.6158

Fletcher, B., Gulanick, M., & Lamendola, C. (2002). Risk factors for type 2 diabetes mellitus. *The Journal of Cardiovascular Nursing*, *16*(2), 17–23. https://doi.org/10.1097/00005082-200201000-00003

Flodh, H. (1986). Human growth hormone produced with recombinant DNA technology: Development and production. *Acta Paediatrica*, *75*(s325), 1–9. https://doi.org/10.1111/j.1651-2227.1986.tb10356.x

Frietze, S., O'Geen, H., Blahnik, K. R., Jin, V. X., & Farnham, P. J. (2010). ZNF274 recruits the histone methyltransferase SETDB1 to the 3′ ends of ZNF genes. *PLoS ONE*, *5*(12), e15082. https://doi.org/10.1371/journal.pone.0015082

Frixou, M., Vlek, D., Lucas-Herald, A. K., Keir, L., Kyriakou, A., & Shaikh, M. G. (2021). The use of growth hormone therapy in adults with Prader-Willi syndrome: A systematic review. *Clinical Endocrinology*, *94*(4), 645–655. https://doi.org/10.1111/cen.14372

Gabriel, J. M., Merchant, M., Ohta, T., Ji, Y., Caldwell, R. G., Ramsey, M. J., Tucker, J. D., Longnecker, R., & Nicholls, R. D. (1999). A transgene insertion creating a heritable chromosome deletion mouse model of Prader-Willi and Angelman syndromes. *Proceedings of the National Academy of Sciences*, *96*(16), 9258–9263. https://doi.org/10.1073/pnas.96.16.9258

Galiveti, C. R., Raabe, C. A., Konthur, Z., & Rozhdestvensky, T. S. (2014). Differential regulation of non-protein coding RNAs from Prader-Willi Syndrome locus. *Scientific Reports*, *4*(1), 6445. https://doi.org/10.1038/srep06445

Ganot, P., Bortolin, M.-L., & Kiss, T. (1997). Site-specific pseudouridine formation in preribosomal RNA is guided by small nucleolar RNAs. *Cell*, *89*(5), 799–809. https://doi.org/10.1016/S0092-8674(00)80263-9

Gao, G.-P., Alvira, M. R., Wang, L., Calcedo, R., Johnston, J., & Wilson, J. M. (2002). Novel adeno-associated viruses from rhesus monkeys as vectors for human gene therapy. *Proceedings of the National Academy of Sciences*, *99*(18), 11854–11859. https://doi.org/10.1073/pnas.182412299

Gilmore, R. B., Liu, Y., Stoddard, C. E., Chung, M. S., Carmichael, G. G., & Cotney, J. (2024). Identifying key underlying regulatory networks and predicting targets of orphan C/D box SNORD116 snoRNAs in Prader–Willi syndrome. *Nucleic Acids Research*, *52*(22), 13757–13774. https://doi.org/10.1093/nar/gkae1129

Gossan, N. C., Zhang, F., Guo, B., Jin, D., Yoshitane, H., Yao, A., Glossop, N., Zhang, Y. Q., Fukada, Y., & Meng, Q.-J. (2014). The E3 ubiquitin ligase UBE3A is an integral component of the molecular circadian clock through regulating the BMAL1 transcription factor. *Nucleic Acids Research*, *42*(9), 5765–5775. https://doi.org/10.1093/nar/gku225

Gowher, H., Liebert, K., Hermann, A., Xu, G., & Jeltsch, A. (2005). Mechanism of stimulation of catalytic activity of Dnmt3A and Dnmt3B DNA-(cytosine-C5)-methyltransferases by Dnmt3L. *Journal of Biological Chemistry*, *280*(14), 13341–13348. https://doi.org/10.1074/jbc.M413412200

Grammarly. (2025). https://app.grammarly.com/

Greer, P. L., Hanayama, R., Bloodgood, B. L., Mardinly, A. R., Lipton, D. M., Flavell, S. W., Kim, T.-K., Griffith, E. C., Waldon, Z., Maehr, R., Ploegh, H. L., Chowdhury, S., Worley, P. F., Steen, J., & Greenberg, M. E. (2010). The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating Arc. *Cell*, *140*(5), 704–716. https://doi.org/10.1016/j.cell.2010.01.026

Grootjen, L. N., Juriaans, A. F., Kerkhof, G. F., & Hokken-Koelega, A. C. S. (2022). Atypical 15q11.2-q13 deletions and the Prader-Willi phenotype. *Journal of Clinical Medicine*, *11*(15), 4636. https://doi.org/10.3390/jcm11154636

Grootjen, L. N., Trueba-Timmermans, D. J., Damen, L., Mahabier, E. F., Kerkhof, G. F., & Hokken-Koelega, A. C. S. (2022). Long-term growth hormone treatment of children with PWS: The earlier the start, the better the outcomes? *Journal of Clinical Medicine*, *11*(9), 2496. https://doi.org/10.3390/jcm11092496

Grugni, G., Marzullo, P., Ragusa, L., Sartorio, A., Trifirò, G., Liuzzi, A., Crinò, A., & on behalf of the Genetic Obesity Study Group of the Italian Society of Pediatric Endocrinology and Diabetology. (2006). Impairment of GH responsiveness to combined GH-releasing hormone and arginine administration in adult patients with Prader-Willi syndrome. *Clinical Endocrinology*, *65*(4), 492–499. https://doi.org/10.1111/j.1365-2265.2006.02621.x

Hamabe, J., Kuroki, Y., Imaizumi, K., Sugimoto, T., Fukushima, Y., Yamaguchi, A., Izumikawa, Y., & Niikawa, N. (1991). DNA deletion and its parental origin in Angelman syndrome patients. *American Journal of Medical Genetics*, *41*(1), 64–68. https://doi.org/10.1002/ajmg.1320410117

Hao, Y.-H., Doyle, J. M., Ramanathan, S., Gomez, T. S., Jia, D., Xu, M., Chen, Z. J., Billadeau, D. D., Rosen, M. K., & Potts, P. R. (2013). Regulation of WASH-dependent actin polymerization and protein trafficking by ubiquitination. *Cell*, *152*(5), 1051–1064. https://doi.org/10.1016/j.cell.2013.01.051

Hilton, I. B., D'Ippolito, A. M., Vockley, C. M., Thakore, P. I., Crawford, G. E., Reddy, T. E., & Gersbach, C. A. (2015). Epigenome editing by a CRISPR-Cas9-based acetyltransferase activates genes from promoters and enhancers. *Nature Biotechnology*, *33*(5), 510–517. https://doi.org/10.1038/nbt.3199

*Horsthemke, B., & Buiting, K. (2008). Chapter 8 Genomic imprinting and imprinting defects in humans. In *Advances in Genetics* (Vol. 61, pp. 225–246). Elsevier. https://doi.org/10.1016/S0065-2660(07)00008-9

Horsthemke, B., & Wagstaff, J. (2008). Mechanisms of imprinting of the Prader–Willi/Angelman region. *American Journal of Medical Genetics Part A*, *146A*(16), 2041–2052. https://doi.org/10.1002/ajmg.a.32364

Hurst, S. E., Minkin, S. C., Biggerstaff, J., & Dhar, M. S. (2012). Transient silencing of a type IV P-type ATPase, Atp10c , results in decreased glucose uptake in C2C12 myotubes. *Journal of Nutrition and Metabolism*, *2012*, 1–9. https://doi.org/10.1155/2012/152902

Imren, S., Payen, E., Westerman, K. A., Pawliuk, R., Fabry, M. E., Eaves, C. J., Cavilla, B., Wadsworth, L. D., Beuzard, Y., Bouhassira, E. E., Russell, R., London, I. M., Nagel, R. L., Leboulch, P., & Humphries, R. K. (2002). Permanent and panerythroid correction of murine β thalassemia by multiple lentiviral integration in hematopoietic stem cells. *Proceedings of the National Academy of Sciences*, *99*(22), 14380–14385. https://doi.org/10.1073/pnas.212507099

Jay, P., Rougeulle, C., Massacrier, A., Moncla, A., Mattei, M.-G., Malzac, P., Roëckel, N., Taviaux, S., Bergé Lefranc, J.-L., Cau, P., Berta, P., Lalande, M., & Muscatelli, F. (1997). The human necdin gene, NDN, is maternally imprinted and located in the Prader-Willi syndrome chromosomal region. *Nature Genetics*, *17*(3), 357–361. https://doi.org/10.1038/ng1197-357

Ji, Q., Fischer, A. L., Brown, C. R., Eastlund, E. R., Dvash, T., Zhong, B., Gerber, M. A., Lyons, I., Knight, S. W., & Kreader, C. A. (2014). Engineered zinc-finger transcription factors activate OCT4 (POU5F1), SOX2, KLF4, c-MYC (MYC) and miR302/367. *Nucleic Acids Research*, *42*(10), 6158–6167. https://doi.org/10.1093/nar/gku243

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, *337*(6096), 816–821. https://doi.org/10.1126/science.1225829

Jinek, M., Jiang, F., Taylor, D. W., Sternberg, S. H., Kaya, E., Ma, E., Anders, C., Hauer, M., Zhou, K., Lin, S., Kaplan, M., Iavarone, A. T., Charpentier, E., Nogales, E., & Doudna, J. A. (2014). Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science*, *343*(6176), 1247997. https://doi.org/10.1126/science.1247997

Johnstone, K. A., DuBose, A. J., Futtner, C. R., Elmore, M. D., Brannan, C. I., & Resnick, J. L. (2006). A human imprinting centre demonstrates conserved acquisition but diverged maintenance of imprinting in a mouse model for Angelman syndrome imprinting defects. *Human Molecular Genetics*, *15*(3), 393–404. https://doi.org/10.1093/hmg/ddi456

Jong, M. T. C., Gray, T. A., Ji, Y., Glenn, C. C., Saitoh, S., Driscoll, D. J., & Nicholls, R. D. (1999). A novel imprinted gene, encoding a RING zinc-finger protein, and overlapping antisense transcript in the Prader-Willi syndrome critical region. *Human Molecular Genetics*, *8*(5), 783–793. https://doi.org/10.1093/hmg/8.5.783

*Kampmann, M. (2018). CRISPRi and CRISPRa screens in mammalian cells for precision biology and medicine. *ACS Chemical Biology*, *13*(2), 406–416. https://doi.org/10.1021/acschembio.7b00657

Känsäkoski, J., Raivio, T., Juul, A., & Tommiska, J. (2015). A missense mutation in MKRN3 in a Danish girl with central precocious puberty and her brother with early puberty. *Pediatric Research*, *78*(6), 709–711. https://doi.org/10.1038/pr.2015.159

Kehr, S., Bartschat, S., Stadler, P. F., & Tafer, H. (2011). PLEXY: Efficient target prediction for box C/D snoRNAs. *Bioinformatics*, *27*(2), 279–280. https://doi.org/10.1093/bioinformatics/btq642

Kim, Y., Lee, H.-M., Xiong, Y., Sciaky, N., Hulbert, S. W., Cao, X., Everitt, J. I., Jin, J., Roth, B. L., & Jiang, Y. (2017). Targeting the histone methyltransferase G9a activates imprinted genes and improves survival of a mouse model of Prader–Willi syndrome. *Nature Medicine*, *23*(2), 213–222. https://doi.org/10.1038/nm.4257

Kimonis, V., Surampalli, A., Wencel, M., Gold, J.-A., & Cowen, N. M. (2019). A randomized pilot efficacy and safety trial of diazoxide choline controlled-release in patients with Prader-Willi syndrome. *PLOS ONE*, *14*(9), e0221615. https://doi.org/10.1371/journal.pone.0221615

Kishino, T., Lalande, M., & Wagstaff, J. (1997). UBE3A/E6-AP mutations cause Angelman syndrome. *Nature Genetics*, *15*(1), 70–73. https://doi.org/10.1038/ng0197-70

Kishore, P., Boucai, L., Zhang, K., Li, W., Koppaka, S., Kehlenbrink, S., Schiwek, A., Esterson, Y. B., Mehta, D., Bursheh, S., Su, Y., Gutierrez-Juarez, R., Muzumdar, R., Schwartz, G. J., &

Hawkins, M. (2011). Activation of KATP channels suppresses glucose production in humans. *Journal of Clinical Investigation*, *121*(12), 4916–4920. https://doi.org/10.1172/JCI58035

Kiss-Laszlo, Z., Henry, I., & Kiss, T. (1998). Sequence and structural elements of methylation guide snoRNAs essential for site-specific ribose methylation of pre-rRNA. *The EMBO Journal*, *17*(3), 797–807. https://doi.org/10.1093/emboj/17.3.797

Kochenderfer, J. N., Somerville, R., Lu, L., Iwamoto, A., Yang, J. C., Klebanoff, C., Kammula, U., Sherry, R. M., Victoria, S., Yuan, C., Feldman, S., Feldman, T., Goy, A., Morton, K. E., Toomey, M. A., & Rosenberg, S. A. (2014). Anti-CD19 CAR T-cells administered after low-dose chemotherapy can induce remissions of chemotherapy-refractory diffuse large B-cell lymphoma. *Blood*, *124*(21), 550–550. https://doi.org/10.1182/blood.V124.21.550.550

Kozlov, S. V., Bogenpohl, J. W., Howell, M. P., Wevrick, R., Panda, S., Hogenesch, J. B., Muglia, L. J., Van Gelder, R. N., Herzog, E. D., & Stewart, C. L. (2007). The imprinted gene Magel2 regulates normal circadian output. *Nature Genetics*, *39*(10), 1266–1272. https://doi.org/10.1038/ng2114

Kumar, M., Keller, B., Makalou, N., & Sutton, R. E. (2001). Systematic determination of the packaging limit of lentiviral vectors. *Human Gene Therapy*, *12*(15), 1893–1905. https://doi.org/10.1089/104303401753153947

Kuppens, R. J., Donze, S. H., & Hokken-Koelega, A. C. S. (2016). Promising effects of oxytocin on social and food-related behaviour in young children with Prader–Willi syndrome: A randomized, double-blind, controlled crossover trial. *Clinical Endocrinology*, *85*(6), 979–987. https://doi.org/10.1111/cen.13169

Kurihara, Y., Kawamura, Y., Uchijima, Y., Amamo, T., Kobayashi, H., Asano, T., & Kurihara, H. (2008). Maintenance of genomic methylation patterns during preimplantation development requires the somatic form of DNA methyltransferase 1. *Developmental Biology*, *313*(1), 335–346. https://doi.org/10.1016/j.ydbio.2007.10.033

Kuslich, C. D., Kobori, J. A., Mohapatra, G., Gregorio-King, C., & Donlon, T. A. (1999). Prader-Willi Syndrome is caused by disruption of the SNRPN gene. *The American Journal of Human Genetics*, *64*(1), 70–76. https://doi.org/10.1086/302177

Langouët, M., Glatt-Deeley, H. R., Chung, M. S., Dupont-Thibert, C. M., Mathieux, E., Banda, E. C., Stoddard, C. E., Crandall, L., & Lalande, M. (2018). Zinc finger protein 274 regulates imprinted expression of transcripts in Prader-Willi syndrome neurons. *Human Molecular Genetics*, *27*(3), 505–515. https://doi.org/10.1093/hmg/ddx420

Langouët, M., Gorka, D., Orniacki, C., Dupont-Thibert, C. M., Chung, M. S., Glatt-Deeley, H. R., Germain, N., Crandall, L. J., Cotney, J. L., Stoddard, C. E., Lalande, M., & Chamberlain, S. J. (2020). Specific ZNF274 binding interference at SNORD116 activates the maternal transcripts in Prader-Willi syndrome neurons. *Human Molecular Genetics*, *29*(19), 3285–3295. https://doi.org/10.1093/hmg/ddaa210

*Lee, H.-J., Macbeth, A. H., Pagani, J., & Young, W. S. (2009). Oxytocin: The great facilitator of life. *Progress in Neurobiology*, *88*(2), 127–151. https://doi.org/10.1016/j.pneurobio.2009.04.001

Leff, S. E., Brannan, C. I., Reed, M. L., Özçelik, T., Francke, U., Copeland, N. G., & Jenkins, N. A. (1992). Maternal imprinting of the mouse Snrpn gene and conserved linkage homology with the human Prader–Willi syndrome region. *Nature Genetics*, *2*(4), 259–264. https://doi.org/10.1038/ng1292-259

Lei, M., Mitsuhashi, S., Miyake, N., Ohta, T., Liang, D., Wu, L., & Matsumoto, N. (2019). Translocation breakpoint disrupting the host SNHG14 gene but not coding genes or snoRNAs in typical Prader-Willi syndrome. *Journal of Human Genetics*, *64*(7), 647–652. https://doi.org/10.1038/s10038-019-0596-2

Lewinski, M. K., Yamashita, M., Emerman, M., Ciuffi, A., Marshall, H., Crawford, G., Collins, F., Shinn, P., Leipzig, J., Hannenhalli, S., Berry, C. C., Ecker, J. R., & Bushman, F. D. (2006). Retroviral DNA integration: Viral and cellular determinants of target-site selection. *PLoS Pathogens*, *2*(6), e60. https://doi.org/10.1371/journal.ppat.0020060

Li, X., Ito, M., Zhou, F., Youngson, N., Zuo, X., Leder, P., & Ferguson-Smith, A. C. (2008). A maternal-zygotic effect gene, Zfp57, maintains both maternal and paternal imprints. *Developmental Cell*, *15*(4), 547–557. https://doi.org/10.1016/j.devcel.2008.08.014

*Li, Y., & Sasaki, H. (2011). Genomic imprinting in mammals: Its life cycle, molecular mechanisms and reprogramming. *Cell Research*, *21*(3), 466–473. https://doi.org/10.1038/cr.2011.15

Lionti, T., Reid, S. M., White, S. M., & Rowell, M. M. (2015). A population-based profile of 160 Australians with Prader-Willi syndrome: Trends in diagnosis, birth prevalence and birth characteristics. *American Journal of Medical Genetics Part A*, *167*(2), 371–378. https://doi.org/10.1002/ajmg.a.36845

Liu, P.-Q., Rebar, E. J., Zhang, L., Liu, Q., Jamieson, A. C., Liang, Y., Qi, H., Li, P.-X., Chen, B., Mendel, M. C., Zhong, X., Lee, Y.-L., Eisenberg, S. P., Spratt, S. K., Case, C. C., & Wolffe, A. P. (2001). Regulation of an endogenous locus using a panel of designed zinc finger proteins targeted to accessible chromatin regions. *Journal of Biological Chemistry*, *276*(14), 11323–11334. https://doi.org/10.1074/jbc.M011172200

Liu, X. S., Wu, H., Ji, X., Stelzer, Y., Wu, X., Czauderna, S., Shu, J., Dadon, D., Young, R. A., & Jaenisch, R. (2016). Editing DNA methylation in the mammalian genome. *Cell*, *167*(1), 233-247.e17. https://doi.org/10.1016/j.cell.2016.08.056

Macedo, D. B., França, M. M., Montenegro, L. R., Cunha-Silva, M., Bessa, D. S., Abreu, A. P., Kaiser, U. B., Mendonca, B. B., Jorge, A. A. L., Brito, V. N., & Latronico, A. C. (2018). Central precocious puberty caused by a heterozygous deletion in the MKRN3 promoter region. *Neuroendocrinology*, *107*(2), 127–132. https://doi.org/10.1159/000490059

Margolin, J. F., Friedman, J. R., Meyer, W. K., Vissing, H., Thiesen, H. J., & Rauscher, F. J. (1994). Krüppel-associated boxes are potent transcriptional repression domains. *Proceedings of the National Academy of Sciences*, *91*(10), 4509–4513. https://doi.org/10.1073/pnas.91.10.4509

Margolis, S. S., Salogiannis, J., Lipton, D. M., Mandel-Brehm, C., Wills, Z. P., Mardinly, A. R., Hu, L., Greer, P. L., Bikoff, J. B., Ho, H.-Y. H., Soskis, M. J., Sahin, M., & Greenberg, M. E. (2010). EphB-mediated degradation of the RhoA GEF ephexin5 relieves a developmental brake on excitatory synapse formation. *Cell*, *143*(3), 442–455. https://doi.org/10.1016/j.cell.2010.09.038

Maude, S. L., Frey, N., Shaw, P. A., Aplenc, R., Barrett, D. M., Bunin, N. J., Chew, A., Gonzalez, V. E., Zheng, Z., Lacey, S. F., Mahnke, Y. D., Melenhorst, J. J., Rheingold, S. R., Shen, A., Teachey, D. T., Levine, B. L., June, C. H., Porter, D. L., & Grupp, S. A. (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *New England Journal of Medicine*, *371*(16), 1507–1517. https://doi.org/10.1056/NEJMoa1407222

McAllister, G., Roby-Shemkovitz, A., Amara, S. G., & Lerner, M. R. (1989). cDNA sequence of the rat U snRNP-associated protein N: Description of a potential Sm epitope. *The EMBO Journal*, *8*(4), 1177–1181. https://doi.org/10.1002/j.1460-2075.1989.tb03489.x

McCarthy, J. M., McCann-Crosby, B. M., Rech, M. E., Yin, J., Chen, C.-A., Ali, M. A., Nguyen, H. N., Miller, J. L., & Schaaf, C. P. (2018). Hormonal, metabolic and skeletal phenotype of Schaaf-Yang syndrome: A comparison to Prader-Willi syndrome. *Journal of Medical Genetics*, *55*(5), 307–315. https://doi.org/10.1136/jmedgenet-2017-105024

*McCarty, D. M., Young, S. M., & Samulski, R. J. (2004). Integration of adeno-associated virus (AAV) and recombinant AAV Vectors. *Annual Review of Genetics*, *38*(1), 819–845. https://doi.org/10.1146/annurev.genet.37.110801.143717

McGrath, J., & Solter, D. (1984). Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell*, *37*(1), 179–183. https://doi.org/10.1016/0092-8674(84)90313-1

Meguro, M., Kashiwagi, A., Mitsuya, K., Nakao, M., Kondo, I., Saitoh, S., & Oshimura, M. (2001). A novel maternally expressed gene, ATP10C, encodes a putative aminophospholipid translocase associated with Angelman syndrome. *Nature Genetics*, *28*(1), 19–20. https://doi.org/10.1038/ng0501-19

Meng, L., Ward, A. J., Chun, S., Bennett, C. F., Beaudet, A. L., & Rigo, F. (2015). Towards a therapy for Angelman syndrome by targeting a long non-coding RNA. *Nature*, *518*(7539), 409–412. https://doi.org/10.1038/nature13975

Mercer, R. E., Kwolek, E. M., Bischof, J. M., Van Eede, M., Henkelman, R. M., & Wevrick, R. (2009). Regionally reduced brain volume, altered serotonin neurochemistry, and abnormal behavior in mice null for the circadian rhythm output gene Magel2. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, *150B*(8), 1085–1099.

https://doi.org/10.1002/ajmg.b.30934
Mercer, R. E., & Wevrick, R. (2009). Loss of magel2, a candidate gene for features of Prader-Willi syndrome, impairs reproductive function in mice. *PloS One*, *4*(1), e4291. https://doi.org/10.1371/journal.pone.0004291
Meziane, H., Schaller, F., Bauer, S., Villard, C., Matarazzo, V., Riet, F., Guillon, G., Lafitte, D., Desarmenien, M. G., Tauber, M., & Muscatelli, F. (2015). An early postnatal oxytocin treatment prevents social and learning deficits in adult mice deficient for magel2, a gene involved in Prader-Willi syndrome and autism. *Biological Psychiatry*, *78*(2), 85–94. https://doi.org/10.1016/j.biopsych.2014.11.010
Miller, J. L., Gevers, E., Bridges, N., Yanovski, J. A., Salehi, P., Obrynba, K. S., Felner, E. I., Bird, L. M., Shoemaker, A. H., Angulo, M., Butler, M. G., Stevenson, D., Abuzzahab, J., Barrett, T., Lah, M., Littlejohn, E., Mathew, V., Cowen, N. M., Bhatnagar, A., … Wilding, P. J. (2023). Diazoxide choline extended-release tablet in people with Prader-Willi syndrome: A double-blind, placebo-controlled trial. *The Journal of Clinical Endocrinology & Metabolism*, *108*(7), 1676–1685. https://doi.org/10.1210/clinem/dgad014
Miller, J. L., Tamura, R., Butler, M. G., Kimonis, V., Sulsona, C., Gold, J., & Driscoll, D. J. (2017). Oxytocin treatment in children with Prader–Willi syndrome: A double-blind, placebo-controlled, crossover study. *American Journal of Medical Genetics Part A*, *173*(5), 1243–1250. https://doi.org/10.1002/ajmg.a.38160
Moore, T., & Haid, D. (1991). Genomic imprinting in mammalian development: A parental tug-of-war. *Trends in Genetics*, *7*(2), 45–49. https://doi.org/10.1016/0168-9525(91)90230-N
Müller, T. D., Finan, B., Bloom, S. R., D'Alessio, D., Drucker, D. J., Flatt, P. R., Fritsche, A., Gribble, F., Grill, H. J., Habener, J. F., Holst, J. J., Langhans, W., Meier, J. J., Nauck, M. A., Perez-Tilve, D., Pocai, A., Reimann, F., Sandoval, D. A., Schwartz, T. W., … Tschöp, M. H. (2019). Glucagon-like peptide 1 (GLP-1). *Molecular Metabolism*, *30*, 72–130. https://doi.org/10.1016/j.molmet.2019.09.010
Nagai, K., Nakaseko, Y., Nasmyth, K., & Rhodes, D. (1988). Zinc-finger motifs expressed in E. coli and folded *in vitro* direct specific binding to DNA. *Nature*, *332*(6161), 284–286. https://doi.org/10.1038/332284a0
National Institutes of Health. (2021). *What are the treatments for Prader-Willi syndrome (PWS)?* https://www.nichd.nih.gov/health/topics/prader-willi/conditioninfo/treatments
Neumann, L. C., Markaki, Y., Mladenov, E., Hoffmann, D., Buiting, K., & Horsthemke, B. (2012). The imprinted NPAP1/C15orf2 gene in the Prader–Willi syndrome region encodes a nuclear pore complex associated protein. *Human Molecular Genetics*, *21*(18), 4038–4048. https://doi.org/10.1093/hmg/dds228
Ng, N. B. H., Low, Y. W., Rajgor, D. D., Low, J. M., Lim, Y. Y., Loke, K. Y., & Lee, Y. S. (2022). The effects of glucagon-like peptide (GLP)-1 receptor agonists on weight and glycaemic control in Prader–Willi syndrome: A systematic review. *Clinical Endocrinology*, *96*(2), 144–154. https://doi.org/10.1111/cen.14583
O'Geen, H., Beitnere, U., Garcia, M. S., Adhikari, A., Cameron, D. L., Fenton, T. A., Copping, N. A., Deng, P., Lock, S., Halmai, J. A. N. M., Villegas, I. J., Liu, J., Wang, D., Fink, K. D., Silverman, J. L., & Segal, D. J. (2023). Transcriptional reprogramming restores UBE3A brain-wide and rescues behavioral phenotypes in an Angelman syndrome mouse model. *Molecular Therapy*, *31*(4), 1088–1105. https://doi.org/10.1016/j.ymthe.2023.01.013
Okano, M., Bell, D. W., Haber, D. A., & Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for *de novo* methylation and mammalian development. *Cell*, *99*(3), 247–257. https://doi.org/10.1016/S0092-8674(00)81656-6
Olson, B. R., Drutarosky, M. D., Chow, M.-S., Hruby, V. J., Stricker, E. M., & Verbalis, J. G. (1991). Oxytocin and an oxytocin agonist administered centrally decrease food intake in rats. *Peptides*, *12*(1), 113–118. https://doi.org/10.1016/0196-9781(91)90176-P
Özçelik, T., Leff, S., Robinson, W., Donlon, T., Lalande, M., Sanjines, E., Schinzel, A., & Francke, U. (1992). Small nuclear ribonucleoprotein polypeptide N (SNRPN), an expressed gene in the Prader–Willi syndrome critical region. *Nature Genetics*, *2*(4), 265–269. https://doi.org/10.1038/ng1292-265

Paisey, R., Bower, L., Rosindale, S., & Lawrence, C. (2011). Successful treatment of obesity and diabetes with incretin analogue over four years in an adult with Prader–Willi syndrome. *Practical Diabetes*, *28*(7), 306–307. https://doi.org/10.1002/pdi.1621

Perk, J., Makedonski, K., Lande, L., Cedar, H., Razin, A., & Shemer, R. (2002). The imprinting mechanism of the Prader-Willi/Angelman regional control center. *The EMBO Journal*, *21*(21), 5807–5814. https://doi.org/10.1093/emboj/cdf570

Pocai, A., Lam, T. K. T., Gutierrez-Juarez, R., Obici, S., Schwartz, G. J., Bryan, J., Aguilar-Bryan, L., & Rossetti, L. (2005). Hypothalamic KATP channels control hepatic glucose production. *Nature*, *434*(7036), 1026–1031. https://doi.org/10.1038/nature03439

Powell, W. T., Coulson, R. L., Crary, F. K., Wong, S. S., Ach, R. A., Tsang, P., Alice Yamada, N., Yasui, D. H., & LaSalle, J. M. (2013). A Prader–Willi locus lncRNA cloud modulates diurnal genes and energy expenditure. *Human Molecular Genetics*, *22*(21), 4318–4328. https://doi.org/10.1093/hmg/ddt281

Purtell, L., Sze, L., Loughnan, G., Smith, E., Herzog, H., Sainsbury, A., Steinbeck, K., Campbell, L. V., & Viardot, A. (2011). In adults with Prader–Willi syndrome, elevated ghrelin levels are more consistent with hyperphagia than high PYY and GLP-1 levels. *Neuropeptides*, *45*(4), 301–307. https://doi.org/10.1016/j.npep.2011.06.001

Qi, Y., Purtell, L., Fu, M., Lee, N. J., Aepler, J., Zhang, L., Loh, K., Enriquez, R. F., Baldock, P. A., Zolotukhin, S., Campbell, L. V., & Herzog, H. (2016). Snord116 is critical in the regulation of food intake and body weight. *Scientific Reports*, *6*(1), 18614. https://doi.org/10.1038/srep18614

Qi, Y., Purtell, L., Fu, M., Sengmany, K., Loh, K., Zhang, L., Zolotukhin, S., Sainsbury, A., Campbell, L., & Herzog, H. (2017). Ambient temperature modulates the effects of the Prader-Willi syndrome candidate gene Snord116 on energy homeostasis. *Neuropeptides*, *61*, 87–93. https://doi.org/10.1016/j.npep.2016.10.006

Qi, Y., Purtell, L., Fu, M., Zhang, L., Zolotukhin, S., Campbell, L., & Herzog, H. (2017). Hypothalamus specific re-introduction of SNORD116 into otherwise snord116 deficient mice increased energy expenditure. *Journal of Neuroendocrinology*, *29*(10), e12457. https://doi.org/10.1111/jne.12457

Queen, N. J., Huang, W., Zou, X., Mo, X., & Cao, L. (2023). AAV-BDNF gene therapy ameliorates a hypothalamic neuroinflammatory signature in the Magel2-null model of Prader-Willi syndrome. *Molecular Therapy - Methods & Clinical Development*, *31*, 101108. https://doi.org/10.1016/j.omtm.2023.09.004

Queen, N. J., Zou, X., Anderson, J. M., Huang, W., Appana, B., Komatineni, S., Wevrick, R., & Cao, L. (2022). Hypothalamic AAV-BDNF gene therapy improves metabolic function and behavior in the Magel2-null mouse model of Prader-Willi syndrome. *Molecular Therapy - Methods & Clinical Development*, *27*, 131–148. https://doi.org/10.1016/j.omtm.2022.09.012

Ranke, M. B., & Wit, J. M. (2018). Growth hormone—Past, present and future. *Nature Reviews Endocrinology*, *14*(5), 285–300. https://doi.org/10.1038/nrendo.2018.22

Rathert, P., Dhayalan, A., Murakami, M., Zhang, X., Tamas, R., Jurkowska, R., Komatsu, Y., Shinkai, Y., Cheng, X., & Jeltsch, A. (2008). Protein lysine methyltransferase G9a acts on non-histone targets. *Nature Chemical Biology*, *4*(6), 344–346. https://doi.org/10.1038/nchembio.88

Rohm, D., Black, J. B., McCutcheon, S. R., Barrera, A., Berry, S. S., Morone, D. J., Nuttle, X., De Esch, C. E., Tai, D. J. C., Talkowski, M. E., Iglesias, N., & Gersbach, C. A. (2025). Activation of the imprinted Prader-Willi syndrome locus by CRISPR-based epigenome editing. *Cell Genomics*, *5*(2), 100770. https://doi.org/10.1016/j.xgen.2025.100770

Rolf Turk & Netanya Y Spencer. (2024). *Difference between crRNA and sgRNA | IDT*. Integrated DNA Technologies. https://eu.idtdna.com/pages/education/decoded/article/a-high-degree-of-similarity-in-crispr-cas9-editing-efficiency-is-found-between-2-part-guide-rnas-and-single-guide-rnas

Runte, M., Hüttenhofer, A., Groß, S., Kiefmann, M., Horsthemke, B., & Buiting, K. (2001). The IC-SNURF-SNRPN transcript serves as a host for multiple small nucleolar RNA species and as an antisense RNA for UBE3A. *Human Molecular Genetics*, *10*(23), 2687–2700. https://doi.org/10.1093/hmg/10.23.2687

Sahoo, T., Bacino, C. A., German, J. R., Shaw, C. A., Bird, L. M., Kimonis, V., Anselm, I., Waisbren, S., Beaudet, A. L., & Peters, S. U. (2007). Identification of novel deletions of 15q11q13 in Angelman syndrome by array-CGH: Molecular characterization and genotype–phenotype correlations. *European Journal of Human Genetics*, *15*(9), 943–949. https://doi.org/10.1038/sj.ejhg.5201859

Sahoo, T., Del Gaudio, D., German, J. R., Shinawi, M., Peters, S. U., Person, R. E., Garnica, A., Cheung, S. W., & Beaudet, A. L. (2008). Prader-Willi phenotype caused by paternal deficiency for the HBII-85 C/D box small nucleolar RNA cluster. *Nature Genetics*, *40*(6), 719–721. https://doi.org/10.1038/ng.158

Saitoh, S., & Wada, T. (2000). Parent-of-origin specific histone acetylation and reactivation of a key imprinted gene locus in Prader-Willi syndrome. *The American Journal of Human Genetics*, *66*(6), 1958–1962. https://doi.org/10.1086/302917

Salehi, P., Hsu, I., Azen, C. G., Mittelman, S. D., Geffner, M. E., & Jeandron, D. (2017). Effects of exenatide on weight and appetite in overweight adolescents and young adults with Prader-Willi syndrome. *Pediatric Obesity*, *12*(3), 221–228. https://doi.org/10.1111/ijpo.12131

Schaaf, C. P., Gonzalez-Garay, M. L., Xia, F., Potocki, L., Gripp, K. W., Zhang, B., Peters, B. A., McElwain, M. A., Drmanac, R., Beaudet, A. L., Caskey, C. T., & Yang, Y. (2013). Truncating mutations of MAGEL2 cause Prader-Willi phenotypes and autism. *Nature Genetics*, *45*(11), 1405–1408. https://doi.org/10.1038/ng.2776

Schaller, F., Watrin, F., Sturny, R., Massacrier, A., Szepetowski, P., & Muscatelli, F. (2010). A single postnatal injection of oxytocin rescues the lethal feeding behaviour in mouse newborns deficient for the imprinted Magel2 gene. *Human Molecular Genetics*, *19*(24), 4895–4905. https://doi.org/10.1093/hmg/ddq424

Scheffner, M., Huibregtse, J. M., Vierstra, R. D., & Howley, P. M. (1993). The HPV-16 E6 and E6-AP complex functions as a ubiquitin-protein ligase in the ubiquitination of p53. *Cell*, *75*(3), 495–505. https://doi.org/10.1016/0092-8674(93)90384-3

Schmid, R. S., Deng, X., Panikker, P., Msackyi, M., Breton, C., & Wilson, J. M. (2021). CRISPR/Cas9 directed to the Ube3a antisense transcript improves Angelman syndrome phenotype in mice. *Journal of Clinical Investigation*, *131*(5), e142574. https://doi.org/10.1172/JCI142574

Secher, A., Jelsing, J., Baquero, A. F., Hecksher-Sørensen, J., Cowley, M. A., Dalbøge, L. S., Hansen, G., Grove, K. L., Pyke, C., Raun, K., Schäffer, L., Tang-Christensen, M., Verma, S., Witgen, B. M., Vrang, N., & Bjerre Knudsen, L. (2014). The arcuate nucleus mediates GLP-1 receptor agonist liraglutide-dependent weight loss. *The Journal of Clinical Investigation*, *124*(10), 4473–4488. https://doi.org/10.1172/JCI75276

Senda, M., Ogawa, S., Nako, K., Okamura, M., Sakamoto, T., & Ito, S. (2012). The glucagon-like peptide-1 analog liraglutide suppresses ghrelin and controls diabetes in a patient with Prader-Willi syndrome. *Endocrine Journal*, *59*(10), 889–894. https://doi.org/10.1507/endocrj.EJ12-0074

Senís, E., Fatouros, C., Große, S., Wiedtke, E., Niopek, D., Mueller, A., Börner, K., & Grimm, D. (2014). CRISPR/Cas9-mediated genome engineering: An adeno-associated viral (AAV) vector toolbox. *Biotechnology Journal*, *9*(11), 1402–1412. https://doi.org/10.1002/biot.201400046

Shemer, R., Birger, Y., Riggs, A. D., & Razin, A. (1997). Structure of the imprinted mouse Snrpn gene and establishment of its parental-specific methylation pattern. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(19), 10267–10272. https://doi.org/10.1073/pnas.94.19.10267

Snider, W. D. (1994). Functions of the neurotrophins during nervous system development: What the knockouts are teaching us. *Cell*, *77*(5), 627–638. https://doi.org/10.1016/0092-8674(94)90048-5

Spanswick, D., Smith, M. A., Groppi, V. E., Logan, S. D., & Ashford, M. L. J. (1997). Leptin inhibits hypothalamic neurons by activation of ATP-sensitive potassium channels. *Nature*, *390*(6659), 521–525. https://doi.org/10.1038/37379

Sripathy, S. P., Stevens, J., & Schultz, D. C. (2006). The KAP1 corepressor functions to coordinate the assembly of *de novo* HP1-demarcated microenvironments of heterochromatin required for

KRAB zinc finger protein-mediated transcriptional repression. *Molecular and Cellular Biology*, *26*(22), 8623–8638. https://doi.org/10.1128/MCB.00487-06

Strong, T. V., Miller, J. L., McCandless, S. E., Gevers, E., Yanovski, J. A., Matesevac, L., Bohonowych, J., Ballal, S., Yen, K., Hirano, P., Cowen, N. M., & Bhatnagar, A. (2024). Behavioral changes in patients with Prader-Willi syndrome receiving diazoxide choline extended-release tablets compared to the PATH for PWS natural history study. *Journal of Neurodevelopmental Disorders*, *16*(1), 22. https://doi.org/10.1186/s11689-024-09536-x

Sun, J., Zhu, G., Liu, Y., Standley, S., Ji, A., Tunuguntla, R., Wang, Y., Claus, C., Luo, Y., Baudry, M., & Bi, X. (2015). UBE3A regulates synaptic plasticity and learning and memory by controlling SK2 channel endocytosis. *Cell Reports*, *12*(3), 449–461. https://doi.org/10.1016/j.celrep.2015.06.023

Swaab, D. F., Purba, J. S., & Hofman, M. A. (1995). Alterations in the hypothalamic paraventricular nucleus and its oxytocin neurons (putative satiety cells) in Prader-Willi syndrome: A study of five cases. *The Journal of Clinical Endocrinology and Metabolism*, *80*(2), 573–579. https://doi.org/10.1210/jcem.80.2.7852523

Swiech, L., Heidenreich, M., Banerjee, A., Habib, N., Li, Y., Trombetta, J., Sur, M., & Zhang, F. (2015). In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. *Nature Biotechnology*, *33*(1), 102–106. https://doi.org/10.1038/nbt.3055

Tachibana, M., Sugimoto, K., Fukushima, T., & Shinkai, Y. (2001). SET Domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *Journal of Biological Chemistry*, *276*(27), 25309–25317. https://doi.org/10.1074/jbc.M101914200

Tahiliani, M., Koh, K. P., Shen, Y., Pastor, W. A., Bandukwala, H., Brudno, Y., Agarwal, S., Iyer, L. M., Liu, D. R., Aravind, L., & Rao, A. (2009). Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*, *324*(5929), 930–935. https://doi.org/10.1126/science.1170116

Taniura, H., Matsumoto, K., & Yoshikawa, K. (1999). Physical and functional interactions of neuronal growth suppressor necdin with p53. *Journal of Biological Chemistry*, *274*(23), 16242–16248. https://doi.org/10.1074/jbc.274.23.16242

Tauber, M., Mantoulan, C., Copet, P., Jauregui, J., Demeer, G., Diene, G., Rogé, B., Laurier, V., Ehlinger, V., Arnaud, C., Molinas, C., & Thuilleaux, D. (2011). Oxytocin may be useful to increase trust in others and decrease disruptive behaviours in patients with Prader-Willi syndrome: A randomised placebo-controlled trial in 24 patients. *Orphanet Journal of Rare Diseases*, *6*(1), 47. https://doi.org/10.1186/1750-1172-6-47

*Thakore, P. I., Black, J. B., Hilton, I. B., & Gersbach, C. A. (2016). Editing the epigenome: Technologies for programmable transcription and epigenetic modulation. *Nature Methods*, *13*(2), 127–137. https://doi.org/10.1038/nmeth.3733

Tiscornia, G., Singer, O., & Verma, I. M. (2006). Production and purification of lentiviral vectors. *Nature Protocols*, *1*(1), 241–245. https://doi.org/10.1038/nprot.2006.37

Tucci, V., Isles, A. R., Kelsey, G., Ferguson-Smith, A. C., Bartolomei, M. S., Benvenisty, N., Bourc'his, D., Charalambous, M., Dulac, C., Feil, R., Glaser, J., Huelsmann, L., John, R. M., McNamara, G. I., Moorwood, K., Muscatelli, F., Sasaki, H., Strassmann, B. I., Vincenz, C., & Wilkins, J. (2019). Genomic imprinting and physiological processes in mammals. *Cell*, *176*(5), 952–965. https://doi.org/10.1016/j.cell.2019.01.043

Uetsuki, T., Takagi, K., Sugiura, H., & Yoshikawa, K. (1996). Structure and expression of the mouse necdin gene. *Journal of Biological Chemistry*, *271*(2), 918–924. https://doi.org/10.1074/jbc.271.2.918

U.S. Food and Drug Administration. (2025). *Drugs@FDA: FDA-approved drugs*. https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=BasicSearch.process

Valette, M., Diene, G., Glattard, M., Cortadellas, J., Molinas, C., Faye, S., Benvegnu, G., Boulanouar, K., Payoux, P., Salles, J.-P., Arnaud, C., Çabal, S., & Tauber, M. (2025). Early oxytocin treatment in infants with Prader–Willi syndrome is safe and is associated with better endocrine, metabolic and behavioral outcomes. *Orphanet Journal of Rare Diseases*, *20*(1), 96. https://doi.org/10.1186/s13023-025-03560-3

Valle-García, D., Qadeer, Z. A., McHugh, D. S., Ghiraldini, F. G., Chowdhury, A. H., Hasson, D., Dyer, M. A., Recillas-Targa, F., & Bernstein, E. (2016). ATRX binds to atypical chromatin domains at the 3′ exons of zinc finger genes to preserve H3K9me3 enrichment. *Epigenetics*, *11*(6), 398–414. https://doi.org/10.1080/15592294.2016.1169351

Van Den Top, M., Lee, K., Whyment, A. D., Blanks, A. M., & Spanswick, D. (2004). Orexigen-sensitive NPY/AgRP pacemaker neurons in the hypothalamic arcuate nucleus. *Nature Neuroscience*, *7*(5), 493–494. https://doi.org/10.1038/nn1226

Vu, T. H., & Hoffman, A. R. (1997). Imprinting of the Angelman syndrome gene, UBE3A, is restricted to brain. *Nature Genetics*, *17*(1), 12–13. https://doi.org/10.1038/ng0997-12

Wang, B., Li, J., & Xiao, X. (2000). Adeno-associated virus vector carrying human minidystrophin genes effectively ameliorates muscular dystrophy in mdx mouse model. *Proceedings of the National Academy of Sciences*, *97*(25), 13714–13719. https://doi.org/10.1073/pnas.240335297

*Wang, J.-H., Gessler, D. J., Zhan, W., Gallagher, T. L., & Gao, G. (2024). Adeno-associated virus as a delivery vector for gene therapy of human diseases. *Signal Transduction and Targeted Therapy*, *9*(1), 78. https://doi.org/10.1038/s41392-024-01780-w

Wang, X., Ma, C., Rodríguez Labrada, R., Qin, Z., Xu, T., He, Z., & Wei, Y. (2021). Recent advances in lentiviral vectors for gene therapy. *Science China Life Sciences*, *64*(11), 1842–1857. https://doi.org/10.1007/s11427-021-1952-5

Woloschak, M., Miller, J., Felner, E., Bird, L., Angulo, M., Mejia-Corletto, J., Gevers, E., Shoemaker, A., Yanovski, J., Butler, M., Salehi, P., Stevenson, D., Wilding, J., Abuzzahab, J., Konczal, L., Guftar Shaikh, M., Viskochil, D., Lah, M., Mathew, V., … Obrynba, K. (2022). RF24 | PSUN94 Long-term safety of diazoxide choline extended-release (DCCR) tablets in patients with Prader-Willi syndrome. *Journal of the Endocrine Society*, *6*(Supplement_1), A35–A36. https://doi.org/10.1210/jendso/bvac150.074

Wolter, J. M., Mao, H., Fragola, G., Simon, J. M., Krantz, J. L., Bazick, H. O., Oztemiz, B., Stein, J. L., & Zylka, M. J. (2020). Cas9 gene therapy for Angelman syndrome traps Ube3a-ATS long non-coding RNA. *Nature*, *587*(7833), 281–284. https://doi.org/10.1038/s41586-020-2835-2

Wu, H., Ng, C., Villegas, V., Chamberlain, S., Cacace, A., & Wallace, O. (2019). *Small molecule inhibitors of G9a reactivate the maternal PWS genes in Prader-Willi-Syndrome patient derived neural stem cells and differentiated neurons*. https://doi.org/10.1101/640938

Wu, Z., Yang, H., & Colosi, P. (2010). Effect of genome size on AAV vector packaging. *Molecular Therapy: The Journal of the American Society of Gene Therapy*, *18*(1), 80–86. https://doi.org/10.1038/mt.2009.255

Xin, Z., Tachibana, M., Guggiari, M., Heard, E., Shinkai, Y., & Wagstaff, J. (2003). Role of histone methyltransferase G9a in CpG methylation of the Prader-Willi syndrome imprinting center. *Journal of Biological Chemistry*, *278*(17), 14996–15000. https://doi.org/10.1074/jbc.M211753200

*Yau, R., & Rape, M. (2016). The increasing complexity of the ubiquitin code. *Nature Cell Biology*, *18*(6), 579–586. https://doi.org/10.1038/ncb3358

Zhang, X., Zhang, G., Zhang, H., Karin, M., Bai, H., & Cai, D. (2008). Hypothalamic IKKβ/NF-κB and ER stress link overnutrition to energy imbalance and obesity. *Cell*, *135*(1), 61–73. https://doi.org/10.1016/j.cell.2008.07.043

*Zhao, L., Yang, Z., Zheng, M., Shi, L., Gu, M., Liu, G., Miao, F., Chang, Y., Huang, F., & Tang, N. (2024). Recombinant adeno-associated virus 8 vector in gene therapy: Opportunities and challenges. *Genes & Diseases*, *11*(1), 283–293. https://doi.org/10.1016/j.gendis.2023.02.010