Prader-Willi syndrome and Angelman syndrome: Visualisation of the molecular pathways for two chromosomal disorders

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ABSTRACT

Objectives: Prader-Willi syndrome (PWS) and Angelman syndrome (AS) are two syndromes that are caused by the same chromosomal deletion on 15q11.2-q13. Due to methylation patterns, different genes are responsible for the two distinct phenotypes resulting in the disorders. Patients of both disorders exhibit hypotonia in neonatal stage, delay in development and hypopigmentation. Typical features for PWS include hyperphagia, which leads to obesity, the major cause of mortality, and hypogonadism. In AS, patients suffer from a more severe developmental delay, they have a distinctive behaviour that is often described as unnaturally happy, and a tendency for epileptic seizures. For both syndromes, we identified and visualised molecular downstream pathways of the deleted genes that could give insight on the development of the clinical features.

Methods: This was done by consulting literature, genome browsers and pathway databases to identify molecular interactions and to construct downstream pathways.

Results: A pathway visualisation was created and uploaded to the open pathway database WikiPathways covering all molecular pathways that were found.

Conclusions: The visualisation of the downstream pathways of PWS- and AS-deleted genes shows that some of the typical symptoms are caused by multiple genes and reveals critical gaps in the current knowledge.

1. Introduction

Over 6,000 diseases that are caused by mutations in one or more genes are currently known and reported in the Online Mendelian Inheritance in Man (OMIM) database (OMIM 2017). Many of them are rare diseases, meaning that they occur in less than 1,500 (USA), 2,000 (EU) or 2,500 (Japan) individuals. However, there are also disorders that are caused by incorrect genomic imprinting, the epigenetic pattern of the DNA which is inherited by the parents (Cassidy and Schwartz 1998). A wide variety of health conditions are suspected to be regulated by such imprinting, including cancers, cognitive dysfunction, and respiratory, cardiovascular, reproductive, autoimmune, and neurobehavioral disorders (Weinhold 2006). Two interesting rare disorders that are subject to both (genetic variation and epigenetics) are Prader-Willi syndrome (PWS) and Angelman syndrome (AS). Both occur in approximately one in 10,000–15,000 individuals (Cassidy and Schwartz 1998). Both disorders are caused by a deletion in the range of 15q11.2-q13 (Driscoll et al. 1993; Duker et al. 2010) (or another defect which leads to the loss or defective change of imprinting) but due to epigenetic imprinting several genes in the region of the ‘healthy’ chromosome are silenced so the deletion on one chromosome leads to the total loss of the gene product. Due to difference in parental specific methylation patterns there is a different set of genes lost and PWS occurs if the deletion is on chromosome 15 from paternal origin, while AS occurs if it is on chromosome 15 of maternal origin.

Despite the chromosomal regions overlapping, both disorders have very different clinical features. Neonates with PWS exhibit hypotonia, resulting in poor suck and consequently a failure to thrive (Cassidy and Schwartz 1998). Lethargy, with decreased arousal and weak cry, are also prominent, leading to the...
necessity to wake the child to feed. At the age of 1–6 years, however, children with PWS start to eat excessively (hyperphagia). If this is not managed thoroughly, PWS patients become morbidly obese, and the consequences of obesity are a major cause of death in this disorder (Cassidy and Schwartz 1998; Einfeld et al. 2006). Besides, PWS patients also suffer from various complications including hypogonadism and infertility, growth hormone (GH) deficiency, delayed puberty, disturbance in circadian rhythm, hypopigmentation, osteoporosis, mild cognitive impairment, delay in motor and language development, and several characteristic behaviour types, facial features, and body habitus (Cassidy and Schwartz 1998).

Patients with AS have several consistent features. Neonates have slight hypotonia and problems with feeding, though less severe than in PWS (Cassidy and Schwartz 1998). They also exhibit sleep abnormalities and hypopigmentation (Cassidy and Schwartz 1998). Developmental delays are first noted at the age of 3–12 months, but the unique clinical features of the syndrome become manifest after the age of 1 year (Guerrini et al. 2003; Williams et al. 2010). Those features include severe developmental delay, speech impairment, typical facial features and a distinctive behavioural phenotype that includes a happy appearance, excessive laughter, hyperactivity and easy excitability (Cassidy and Schwartz 1998; Williams et al. 2010). On top of that, patients with AS exhibit gait ataxia, tremulousness of the limbs, hypotonia and seizures. They generally do not show hyperphagia, their overall health is good (Cassidy and Schwartz 1998), puberty is usually unaffected and fertility is possible, in contrast to PWS (Dagli et al. 1993).

As mentioned above, in the majority of patients PWS and AS are both caused by a deletion of the same region on chromosome 15: 15q11.2-q13 (Driscoll et al. 1993; Duker et al. 2010). This region contains several genes, depicted in Figure 1 (Driscoll et al. 1993), which contribute to the phenotypic appearance of the patients. The PWS region includes paternally expressed genes, of which five encoded polypeptides (MKRN3, MAGEL2, NDN and SNURF-SNRPN). As for AS, only two genes seem to be responsible for causing the syndrome: UBE3A and ATP10A. UBE3A encodes an ubiquitin-protein ligase, which is involved i.a. in cell-cycle regulation (Gamell et al. 2017). The function of ATP10A is not yet understood (Driscoll et al. 1993), although there are some hints that it may be involved in body fat generation in mice (Dhar et al. 2000) and chromosome 15-related autism (Herzing et al. 2001). Two other genes, that are described to be relevant in both PWS and AS, are GABRB3 and OCA2 (Delahanty et al. 2016).

With advancing medical and scientific knowledge, researchers have more data, information and tools to decipher the cause for diseases. Nevertheless, a wealth of information is still hidden, and revealing interesting clues and their solutions is essential. Known molecular interactions can be visualised through graphical biological pathways, which can give an accessible overview of important cellular events that take place. This is beneficial for the field of rare genetic disorders as little is known of many causative genes, and molecular interaction information about their normal function is the first step to understand which (disturbed) metabolic or signalling pathways lead to the disorder phenotype. A pathway visualising the downstream effects of a causative gene was already made for, e.g., Rett syndrome (Ehrhart et al. 2016). Online pathway databases like KEGG, Reactome and WikiPathways provide this information and allow use of these pathways to analyse high-throughput transcriptomics, proteomics or metabolomics data (Pico et al. 2008). WikiPathways, is a user-curated database that allows the collection, visualisation and publishing of new biological pathways by both (bio)medical professionals and bioinformaticians. In WikiPathways, a newly created pathway can be shared and accessed by other researchers in a quick and easy manner.

The aim of this review was to collect and visualise molecular interaction data of the genes and gene clusters deleted in PWS and AS, to determine in what way the deletion of these genes is involved in the development of both syndromes.

2. Selection of information sources

General information on PWS and AS, the involved genes and their molecular interactions was obtained through literature research using PubMed. The studies were selected if they contained information about...
molecular interactions of the selected gene, ideally in a human PWS- or AS-related study (e.g., cell models), but also animal cell models or other disease context were investigated. DisGeNET (Pinero et al. 2017) and OMIM (Hamosh 1985–2017) provided collections of human disorders and phenotypes with their associated genes and variants. Through these two databases, an overview of the genes most often associated with either of the syndromes was obtained. UniProt, a protein database (The UniProt Consortium 2017), provides functional information about proteins and information to determine differences between, e.g., prohormones and active hormones. Ensembl (Yates et al. 2016) is a genome browser for vertebrate genomes, which was used to annotate genes and gene products in the genetic pathway, and it provided detailed information about gene transcripts and homologues in other species. Entrez Gene (Maglott et al. 2007) was used to find information and annotations for gene clusters, e.g., the SNORD116 gene cluster. WikiPathways (Pico et al. 2008) and Reactome (Milacic et al. 2012; Fabregat et al. 2016), two pathway databases, were used to find existing downstream pathways.

3. The construction of the PWS and AS pathway

The PWS/AS pathway was constructed using PathVisio software (version 3.2.4) (van Iersel et al. 2008; Kutmon et al. 2015). First, all genes involved in PWS and AS were visualised as data nodes and annotated with their database identifiers. Their annotations were derived primarily from Ensembl or Entrez Gene. BridgeDb for Homo sapiens genes and gene products (version Ensembl_85) was used to map the gene identifiers from one database to others (van Iersel et al. 2010). Then, the pathway was gradually built up by adding downstream molecular interactions. Additionally, literature references for these interactions were added in the annotations. For annotation of gene clusters (e.g., SNORD116(6)) Entrez Gene identifiers were used. If information about a potential downstream pathway was available only for an animal model it was investigated whether this gene exists homologously in humans and, if yes, the human gene identifier was used (which was true for all genes in this pathway). Reference and information about the animal model was integrated as annotations in the interactions. For metabolites, ChEBI (Hastings et al. 2013), a database collecting information on small chemical compounds, was used. The metabolite identifiers were mapped between databases using the BridgeDb for metabolites (version 20160108). To link the genes, gene products and metabolites properly with each other Molecular Interaction Maps (MIM) standardised interactions were used as edges (Kohn 1999; Luna et al. 2011). The MIM interactions give information about whether a molecular interaction is a stimulation, conversion, inhibition, catalysis or others. In this newly created pathway, 91 interactions were integrated. For some interactions, however (six in this pathway), the literature did not reveal which exact interaction occurred. Therefore, a basic interaction arrow was used on those occasions. There also remained some gaps in the pathways, which were indicated with a dashed line, in combination with a basic interaction arrow or a MIM gap. An overview of all interaction annotations and their meaning can be found in the legend of Figure 2. The completed pathway was labelled for species Homo sapiens and uploaded to WikiPathways using the WikiPathways plugin of PathVisio, and is now openly available http://www.wikipathways.org/instance/WP3998 (Pico et al. 2008; Janssen et al. 2017).

4. The involved genes and their downstream pathways in detail

4.1. Prader-Willi syndrome

MKRN3 is the first gene in the PWS region at chromosome 15. It is involved in controlling the onset of puberty (Abreu et al. 2015). Figure 3 shows how MKRN3 inhibits expression of the gonadotropin-releasing hormone (GnRH1), either directly or via the neurokinin B (NKB) pathway (Navarro et al. 2011). This causes luteinizing hormone (LH) and follicle-stimulating hormone levels to decrease causing downstream effects, which are not displayed here. The exact mechanism by which MKRN3 inhibits either NKB or GnRH1 is unknown. If MKRN3 suffers from loss of function by either a mutation or a deletion, puberty occurs early in life (Abreu et al. 2015). In PWS patients, however, pubic and axillary hair may develop early or normally, but the other features of puberty occur late and incomplete or not at all (Cassidy and Schwartz 1998). With the information that is now known about MKRN3, there is no explanation that can be given for this result. Any hypotheses about involvement of distal regulators within the PWS region, DNA loops or microRNA remain speculative.

MAGEL2 and NDN are involved in various processes (Figure 4). The gene products can bind together to the complex of FEZ1 and FEZ2 (called FEZ1/2), to inhibit the effect of the proteasome degradation pathway on the latter (Lee et al. 2005). FEZ1 is involved in
Figure 2. Prader-Willi syndrome and Angelman syndrome pathway. The complete pathway consists of seven sections, clustered using different colours. At the top, the different genes that are involved in PWS and AS are mapped. The genes in the PWS region are only expressed on the paternally derived chromosome, whereas the genes in the AS region are only expressed on the maternally derived chromosome. The genes in both non-imprinted regions are expressed on the paternally as well as the maternally inherited chromosome. There are three breakpoints indicated; in PWS and AS, the chromosome section is deleted from either breakpoint 1 or 2, up to breakpoint 3. Please find a high-resolution figure in the supplementary data and the online pathway with more interactive functions at http://www.wikipathways.org/instance/WP3998.
downstream effects on neurons. Studies on Ndntm2Stw mice showed that FEZ1 stimulates neurite and axonal outgrowth. In PC12 cells (rat pheochromocytoma cells), NDN enhances neurite outgrowth after stimulation by nerve growth factor (Tcherpakov et al. 2002), whereas FEZ1 causes neurite outgrowth after being phosphorylated by PRKCZ (Kuroda et al. 1999). Furthermore, the FEZ1 orthologue UNC-76 in Drosophila melanogaster interacts with the molecular motor kinesin, which is essential for axonal transport (Kuroda et al. 1999; Lee et al. 2005). MAGEL2 and NDN share another downstream effect, both interact with BBS4, although in what manner is not known (Lee et al. 2005). BBS4 is thought to interact with the dynein microtubule-based molecular motor, in order to transport the scaffold protein PCM1 to centrosomal satellites, which enables the formation of the centrosomal microtubule organising centre. Apart from the processes mentioned above, MAGEL2 alone is also thought to be involved in leptin-mediated depolarisation of proopiomelanocortin (POMC) neurons (Colmers and Wevrick 2013; Mercer et al. 2013), and in the development of hypothalamic anorexigenic circuits (Maillard et al. 2016). The effect of MAGEL2 in either process has been proved in mouse studies, but an explicit pathway could not be defined from these data. In another mouse study, NDN was found to be able to upregulate GNRH1 transcription (Miller et al. 2009). NDN may bind to MSX1, thereby preventing its repression of GNRH1 transcription. The exact manner in which this happens is currently unknown.

If MAGEL2 and NDN are lost, most of the problems that arise involve the development of neurons (Figure 4). Although it is not exactly defined in what way components or functions of the neurons are disturbed, the defective development itself does make sense. PWS patients tend to have aggressive behaviour, obsessive-compulsive characteristics, and psychiatric problems (Cassidy and Schwartz 1998; Swaab 2003). On top of that they also often exhibit mild cognitive impairment and a delay in motor and language development. These symptoms are most likely caused by defects in the hypothalamus, but how they emerge remains unclear (Cassidy and Schwartz 1998; Myers et al. 2000; Swaab 2003). Hyperphagia is also believed

**Figure 3.** MKRN3 pathway section. MKRN3 inhibits the expression of gonadotropin-releasing hormone (GNRH1), either via NKB and its downstream factors, or directly. By inhibiting GNRH1 expression, luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels decrease. The dashed lines indicate that the exact mechanism is not clear; there might be more steps involved.

**Figure 4.** MAGEL2/NDN pathway section. MAGEL2 and NDN have a shared effect. Proteosomal degradation of the FEZ1/2 complex is prevented by MAGEL2 and NDN binding to it. FEZ1 is then thought to regulate neurite axonal outgrowth and axonal transport. This has been found in studies in different cell types, which is why there are three subsections describing the process. MAGEL2 and NDN interact through an unknown mechanism with BBS4, which then facilitates the formation of the centrosomal microtubule organising centre. MAGEL2 alone is also found to influence leptin-mediated depolarisation of POMC neurons and the development of hypothalamic anorexigenic circuits. NDN is responsible for upregulation of GNRH1 transcription. It binds to MSX1, thereby preventing repression of the GNRH1 gene by MSX1. The exact mechanism through which this occurs is unknown.
to originate from a defect in the hypothalamus. A study on PWS patients has pointed out the paraventricular nucleus as a possible control centre for food intake and body weight. Although the exact mechanism remains unclear, the volume of the oxytocin-secreting paraventricular nucleus cells was severely reduced, suggesting that the problem might lie there (Swaab 2003).

The arcuate nucleus of the hypothalamus is a major site for leptin action (Mercer et al. 2013). Neurons expressing neuropeptide Y (NPY) and agouti-related peptide (AgRP), as well as neurons expressing POMC are located there. Upon activation, NPY/AgRP neurons stimulate food intake, whereas POMC neurons reduce food intake. Leptin is secreted by adipose tissue in order to regulate fat storage (Myers et al. 2000). It is capable of stimulating POMC neurons, but Varela and Horvath (2012) found that the leptin-mediated depolarisation of POMC neurons is disturbed when MAGEL2 is lost, meaning that food intake is being less repressed. This could be another explanation for hyperphagia. Research by Maillard et al. (2016) stated that loss of MAGEL2 in mice leads to a disruption of hypothalamic feeding circuits in general, which is in line with the results of Varela and Horvath. Furthermore, after POMC neurons would be depolarised, neuropeptide precursor POMC is cleaved to α-melanocyte stimulating hormone (Belgardt et al. 2009). This peptide activates receptors on neuron populations that are located in the PVN. In this way, the reduced volume of the PVN, and the reduced activation of and secretion by POMC neurons, might have a relation.

Miller et al. (2009) observed a 25% reduction in number of GNRH-positive neurons in the medial preoptic area, another nucleus of the hypothalamus. The stimulation of the transcription by NDN, as well as the number of neurons that can secrete GNRH1, are disturbed in PWS. The lack of NDN activity might be a major cause in this, but this cannot be confirmed by the current literature.

The hypothalamus, and in particular the paraventricular nucleus, arcuate nucleus and the medial preoptic area, would be an interesting starting point for further investigation on the effect of MAGEL2 and NDN on hyperphagia and psychiatric and behavioural problems in PWS.

SNURF-SNRPN is a bicistronic gene, encoding two different proteins (Driscoll et al. 1993; Duker et al. 2010). One of those is the small nucleolar ribonucleoprotein polypeptide N (SNRPN) upstream reading frame, or SNURF. The function of SNURF is currently unclear, hence the gap annotation in the PWS pathway (Figure 5). SNRPN encodes a protein called SmN, but this is presented according to HGNC (Human Gene Nomenclature) as SNRPN in the PWS pathway. SNRPN is involved in the formation of the spliceosomal A complex, which is in turn an important component in the major splicing pathway of mRNA processing (mRNA_splicing_pathway 2017). Looking at the expression pattern, one could argue that SNRPN has something to do with the development of the brain or the remaining nervous system (Petryszak et al. 2016; SNRPN_Expression_pattern 2017). However, there is no evidence of how SNRPN would play a role in any pathway concerning this process. All in all, despite the fact that SNRPN was long thought to be the most important gene causing the clinical appearance of PWS (as it is part of the local imprinting centre and methylation analysis of its promoter correctly reveals PWS with high accurateness) (Glenn et al. 1996), very little information on its mechanism of action is available.

Research in mice revealed that loss of the SNORD116 gene cluster (annotated as SNORD116@), without interruption of any other genes, causes a reduction of NHLH2 and prohormone convertase PC1 (PCSK1) expression (Burnett et al. 2017) (Figure 6). The way in which this happens is not known. NHLH2 stimulates PCSK1 expression, and PCSK1 itself binds to a Ca\(^{2+}\) cofactor. POMC, ghrelin, GHRH and insulin are converted by PCSK1 to their active form (Brange and Langkjoer 1993; Burnett et al. 2017). Oxytocin, BDNF and GNRH1 are mentioned as prohormone candidates. It is very likely that they are also processed by PCSK1, but strong evidence for that is lacking. This results in impaired prohormone processing, and as such a decrease in active hormone levels (Brange and
Langkjoer 1993; Burnett et al. 2017). The hormone processing furthermore seems to undergo a switch from early childhood (with low appetite) to later childhood (with high appetite), possibly due to ghrelin modifications (Beauloye et al. 2016). Burnett et al. (2017) suggest that the major neuroendocrine features of PWS are due to PCSK1 deficiency. They connected the affected hormones to symptoms of PWS, and covered a lot of them (Figure 7).

A decrease in POMC, oxytocin and BDNF processing would be responsible for hyperphagia and body weight aberrations. A decreased processing of proghrelin to ghrelin leads to a higher blood level of proghrelin and total ghrelin, increasing the appetite (Klok et al. 2007). Ghrelin is also involved in the secretion of growth hormone (GH), which will then be lower as well (Dimaraki and Jaffe 2006). ProGNRH1 is not converted to GNRH1, resulting in a low level of gonadotropin and consequent hypogonadism. A lack of GHRH, and consequently low GH levels, might be responsible for the short stature seen in PWS patients, and finally a low insulin level could cause type 2 diabetic features. Although the results are derived from mice studies, it is likely that these processes occur in a similar manner in humans. Hypotonia and developmental delay were also found to be caused by a deletion of SNORD116@, without interruption of other genes (de Smith et al. 2009; Duker et al. 2010). However, those two features are not explained by the processes that are pointed out here (Figure 6, Figure 7).

SNORD115@ is another gene cluster that is located in the PWS region (Figure 8). Little is known, but Garfield et al. (2016) found that SNORD115@ plays a role in alternate splicing of HTR2C in mice. SNORD115@ binds to a specific sequence in exon Va of the HTR2C pre-RNA. This promotes the production of full-length 5HT2C-receptors. Absence of SNORD115@ would cause more alternate splicing and adenosine-to-inosine RNA editing, resulting in truncated and dysfunctional receptors (Canton et al. 1996; Burns et al. 1997; Garfield et al. 2016). It is known that disturbance of the central serotonin system, specifically a reduction in serotonin availability or efficacy, can cause hyperphagia (Garfield and Heisler 2009). 5HT2C receptors play the most important role in the anorectic action of serotonin (Lam et al. 2010). Garfield et al. (2016) showed that SNORD115@ is involved in the processing of pre-RNA of this receptor. It promotes the production of full-length 5HT2C, and, when it is lost, more truncated pre-RNA will be produced and thus more dysfunctional receptors. This is yet another gene located in the PWS region, the loss of which can cause hyperphagia.

4.2. Angelman syndrome

The molecular pathway constructed for UBE3A, a protein responsible for ubiquitination and therefore targeted degradation of other proteins, is actually a pathway described in such detail only in cancer cell
Figure 7. Schematic representation of the effects of impaired hormone processing. Many PWS features are connected to a decrease of a certain hormone level. All of the level decreases seen here are caused by a PCSK1, which in turn caused by a loss of the SNORD116 gene cluster. Figure modified after Burnett et al. (2017).

Figure 8. SNORD115 cluster pathway section. SNORD115 gene cluster, annotated as SNORD115@, binds to a specific sequence in exon Va of the HTR2C pre-RNA. By doing so, it promotes the inclusion of exon Vb and thus the production of full-length 5HT2C receptors. In the absence of SNORD115 complex, more alternate splicing and adenosine-to-inosine RNA editing takes place, resulting in the production of more truncated splice variants and thus more dysfunctional receptors.
model systems as this gene is mainly involved in regulation of cell cycle. UBE3A was found to suppress cancer by promoting the expression of tumour-suppressor genes located on the INK4/ARF locus (Figure 9). Gamell et al. (2017) found that the expression of several tumour-suppressor genes was decreased in UBE3A-deficient mouse fibroblasts. In other words, UBE3A stimulates the expression of those genes. Once bound to E2F1, UBE3A stimulates transcription of the INK4/ARF locus containing p16 INK4a. This then forms a complex with CDKN2B and CDKN2C, which can inhibit two other complexes. The ultimate result of UBE3A action here is the inhibition of E2F1 expression, and thus G1/S progression. As E2F1 is also at the top of the pathway, it might provide a feedback system. The INK4/ARF locus also encodes ARF, which can cause apoptosis and G2/M arrest. MDM4 might provide a regulatory function here, so that cells are not in permanent arrest or apoptosis.

As there are many ubiquitination targets, UBE3A may have many more, yet unknown, effects. Judson et al. (2016) identified a specific downstream effect that may explain the AS phenotype. The specific loss of UBE3A from GABAergic neurons causes AS-like EEG patterns, which could be due to a specific ubiquitination activity on the protein ARC (Greer et al. 2010). ARC is a synaptic protein which is responsible for the trafficking of a GABA receptor subtype. Uncontrolled accumulation of ARC results in increased internalisation of the GABA receptor and impairs normal synapsis function. UBE3A mutations or dysregulations were observed in several intellectual disorders, neurodevelopmental delay, epilepsy and autism spectrum disorders (Zhang et al. 2017). A recent publication indicates that CBLN1 might also be a linking pin, although the molecular pathway is not known (Krishnan et al. 2017).

4.3. Both PWS and AS

The last pathway section contains four genes that are involved in PWS as well as in AS (Figure 10).
Although, they are not imprinted in the same way as the PWS- and AS-causing genes, which would lead to a complete loss of the gene product, the gene doses are reduced. GABRB3 is the main actor here, as it stimulates the transcription of GABRA5, GABRG3 and OCA2 (Delahanty et al. 2016). GABRB3, GABRA5 and GABRG3 all encode a subunit of the GABA(A) receptor. Expression of GABRB3 was found in embryonic stem cells and neural crest stem cells (Delahanty et al. 2016). These cells are known to give rise to various cells, including melanocytes. What role GABRB3 plays in the differentiation of those stem cells is unknown, visualised by dashed lines in the pathway. A lack of subunit β-3 impairs the function of the GABA(A) receptor, causing problems in rapid inhibitory synaptic transmission in the central nervous system (Homanics et al. 1997). This can lead to epilepsy, cleft palate and hypersensitive behaviour, especially in the case of AS together with the loss of UBE3A induced dysfunction of the GABAergic neurons (Greer et al. 2010; Judson et al. 2016). Loss of GABRA5 and GABRG3 also impair GABA(A) receptor function (and there is recent evidence that the GABA levels are also decreased in PWS patients (Rice et al. 2016)). This mechanism could also play a role in the development of these disorders in humans, but this has not yet been proven. OCA2 encodes the P-protein, which is known to be important in the production of melanin (Delahanty et al. 2016). The loss of GABRB3 alone causes expression of OCA2 to be impaired, leading to hypopigmentation. In PWS and AS, both genes are deleted, probably enhancing that effect.

GABRB3 therefore appears to play a role in the hypopigmentation that is seen in PWS as well as AS. It plays a role in the differentiation of melanocytes (Delahanty et al. 2016). This was concluded due to the fact that wild-type mice had far more melanocytes in the last two out of four maturation stages than mice lacking one or two copies of GABRB3. GABRB3 also influences pigmentation via OCA2. Deletion of GABRB3 causes the expression of OCA2 to drop significantly. In PWS and AS, both genes are deleted, resulting in an impaired melanin synthesis pathway. However, one non-imprinted copy remains, preventing the affected individuals from having no pigment at all. As GABRB3 encodes a subunit of the GABA(A) receptor, and stimulates transcription of two other subunits (GABRA5 and GABRG3), loss of it will interfere with the function of this receptor. This was found to cause several disorders in mice, including epilepsy, cleft palate and hyperactive behaviour. It is plausible that this mechanism also plays a role in the development of these disorders in humans. Epilepsy features might be related to the seizures that are seen in AS, yet they are not reported in PWS. The relation of the cleft palate and hyperactive behaviour to these two syndromes remains open to debate. However, it is an effect caused by GABRB3, a PWS/AS related gene, and therefore it is depicted here.

Figure 10. GABRB3, GABRA5, GABRG3 and OCA2 pathway section. GABRB3 stimulates the expression of GABRA5 and GABRG3. All three encode a subunit of the GABA(A) receptor. GABRB3 itself is involved in stem cell differentiation into melanocytes. When GABRB3 is lost, the GABA(A) receptor is defective and epilepsy, cleft palate and hypersensitive behaviour are three disorders that can arise. Decreased expression of GABRA5 and GABRG3 also interferes with normal GABA(A) receptor functioning. Expression of OCA2 is also stimulated by GABRB3. When expression of OCA2 decreases, the melanin biosynthesis pathway is disturbed, leading to hypopigmentation.
5. Conclusion and limitations

The visualisation of the molecular pathways of PWS and AS demonstrates that several PWS and AS symptoms can be linked to more than one gene and that their downstream effects, which are pointed out here, may be additive. Incorrect development of the brain, and possibly the hypothalamus, find an origin in the loss of both MAGEL2 and NDN. This can have many consequences, as we have discussed in previous paragraphs. MAGEL2, SNORD116 and SNORD115 are all thought to contribute to hyperphagia via different pathways: hormones (ghrelin, leptin, insulin, etc.) and dysregulation in the hypothalamus. Hyperphagia is considered the most important symptom of PWS due to its consequence of obesity, which leads to early death. This is probably also a reason why there is extensive information available on hyperphagia. GABRB3 and OCA2 are both able to cause hypopigmentation in PWS as well as in AS.

However, there remains missing knowledge that should be filled by future research. The reason for hypogonadism and delayed puberty in PWS, for example, is still unclear. Disturbed GNRH1 expression is an important factor, and both NDN and SNORD116 could be contributing to the delay of development as their downstream pathways interfere with these pathways. Yet, both processes are not confirmed with certainty. It is also not clear whether these two impairments would be sufficient to cause hypogonadism of this kind. SNORD116 is found to be sufficient to elicit hypotonia in neonates, as well as developmental delay in a later stage, but the mechanism of action has yet to be found. Many of the other symptoms, such as lethargy, a disturbed circadian rhythm, cognitive impairment and the typical behaviour, could have their origin in a disturbed development of the hypothalamus, but there is no evidence for that (Cassidy and Schwartz 1998; Myers et al. 2000; Swaab 2003).

Furthermore, the effect of MKRN3 loss is completely contradictory to the delayed puberty seen in PWS. It would be interesting to see how this effect is influenced by other pathways, so that puberty is suppressed. The effect of SNRPN on symptom level is unknown, which is notable, because this gene was long believed to be causing most of the symptoms. As for SNURF, there is nothing to be displayed in a pathway.

According to the currently available literature, it seems like there are many more processes regulated by UBE3A, because this appears to be the most important gene out of the two causing AS. Cassidy and Schwartz (1998) mentioned that, in healthy individuals, UBE3A is imprinted in some parts of the brain, but both copies are expressed in lymphocytes and fibroblasts, as well as other organs.

To conclude, in this study a collection and presentation of currently available knowledge of the molecular interactions and downstream pathways of genes that are involved in PWS and AS is presented. This pathway shows for the first time that several of the symptoms may have their molecular origin in more than one gene (cluster) and reveals gaps of knowledge which should be closed in future research.

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Statement of interest

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