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# Prader–Willi syndrome and early-onset morbid obesity NIH rare disease consortium: A review of natural history study

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## Abstract

We describe the National Institutes of Health rare disease consortium for Prader-Willi syndrome (PWS) developed to address concerns regarding medical care, diagnosis, growth and development, awareness, and natural history. PWS results from errors in genomic imprinting leading to loss of paternally expressed genes due to 15q11-q13 deletion, maternal disomy 15 or imprinting defects. The 8 year study was conducted at four national sites on individuals with genetically confirmed PWS and early-onset morbid obesity (EMO) with data accumulated to gain a better understanding of the natural history, cause and treatment of PWS. Enrollment of 355 subjects with PWS and 36 subjects with EMO began in September 2006 with study completion in July 2014. Clinical, genetic, cognitive, behavior, and natural history data were systematically collected along with PWS genetic subtypes, pregnancy and birth history, mortality, obesity, and cognitive status with study details as important endpoints in both subject groups. Of the 355 individuals with PWS, 217 (61%) had the 15q11-q13 deletion, 127 (36%) had maternal disomy 15, and 11 (3%) had imprinting defects. Six deaths were reported in our PWS cohort with 598 cumulative years of study exposure and one death in the EMO group with 42 years of exposure. To our knowledge, this description of a longitudinal study in PWS represents the largest and most comprehensive cohort useful for investigators in planning comparable studies in other rare disorders. Ongoing studies utilizing this database should have a direct impact on care and services, diagnosis, treatment, genotype–phenotype correlations, and clinical outcomes in PWS.

### Keywords

genotype–phenotype correlations; longitudinal natural history study; mortality; Prader–Willi syndrome; PWS genetic subtypes; rare disease consortium

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## 1 | INTRODUCTION

Rare diseases affect about 200,000 people or nearly 1 in 1,500 people in the United States but present a significant and growing health care concern nationwide (Rare Disease Act of 2002-PL 107–280). There are approximately 7,000 different rare diseases and disorders that affect more than 300 million individuals worldwide (www.globalgenes.org/rarelist). Rare diseases can vary in occurrence between populations as a disorder in some populations may be rare but more common elsewhere. For example, cystic fibrosis which is relatively common in Europe and European descendants is rare in most Asian populations.

A classic example of a rare disease is Prader–Willi syndrome (PWS), which presents in infancy with the combination of severe hypotonia, a poor suck with feeding problems, failure to thrive, developmental delay, and hypogonadism/hypogenitalism. Growth and other hormone deficiencies leading to short stature and small hands and feet are present in childhood with obesity due to food seeking and hyperphagia. Mild learning impairment and behavioral problems are also noted including self-injury, OCD, and temper tantrums (Angulo, Butler, & Cataletto, 2015; Bittel & Butler, 2005; Butler, 1990, 2016a; Butler et al., 2002; Cassidy et al., 2012). PWS is due to errors in genomic imprinting with loss of paternally expressed genes generally from a de novo paternally derived chromosome 15q11-q13 deletion (Bittel & Butler, 2005; Butler, 1990; Butler et al., 2002; Butler, Lee, & Whitman, 2006; Cassidy, Schwartz, Miller, & Driscoll, 2012).

Patients with rare diseases are frequently misdiagnosed or undiagnosed. In addition, direct medical costs are higher as evident in Prader–Willi syndrome when compared with commercially insured patient counterparts without PWS at similar ages. Recently, the total annual direct medical costs were reported to be 8.8 times greater than for patients without PWS (Shoffstall et al., 2016). However, few drug companies conduct research into the cause or treatment of rare diseases as it is difficult for these companies to recover costs of developing treatments for such small, geographically dispersed populations. Therefore, advances in medical research and knowledge on rare diseases are fraught with delays and problems. To address these concerns, the National Institutes of Health (NIH) in 2002 proposed the Rare Diseases Act of 2002 (PL 107–280) for development of a research network to facilitate collaboration, patient recruitment and enrollment in research studies and clinical trials. This act established the Rare Diseases Clinical Research Network (RDCRN) to meet those needs. The RDCRN was developed to enable collaboration among scientists and address the unique challenges of rare disease research utilizing multiple disciplines. Furthermore, this approach would provide shared access to geographically distributed research resources and patient populations from throughout the United States and consisted of 10 research consortia.

A central Data and Technology Coordinating Center (DTCC) was also established and supported by NIH funding of $71 million through five-year grants. The goal of the DTCC was to develop and introduce novel technologies including data collection and information sharing supporting performance and productivity of the investigators at multiple national sites. The RDCRN would lead the effort by incorporating standard data elements in the rare diseases selected for study and enhance research with informatics and statistical support

from the DTCC staff. These efforts would allow RDCRN researchers and consortia study coordinators and staff to integrate their data with other clinical networks and scientists with input from parent support associations enabling the development of new diagnostic tools, treatments, and preventive strategies for better understanding of each rare disease under study in order to improve quality of life of the affected individual. The ten approved research consortia conducted more than 20 studies in greater than 50 geographical sites in the United States and other countries. Each consortium studied a group of related rare diseases which individually may be fatal such as bone marrow failure conditions, thrombotic disorders, lung diseases, genetic steroid disorders, neurologic conditions, urea cycle defects, and other metabolic disorders.

Each consortium in the RDCRN included active participation by patient advocacy groups. The RDCRN Coalition of Patient Advocacy Groups (CPAG) represented more than 30 of these national patient advocacy groups instrumental in outreach to the affected populations and provided a patient perspective to the development of studies helpful in support, subject recruitment and continuity of studies at each participating site. The RDCRN developed a unique web-based contact registry for patients who wish to learn about their disease and for participation in clinical studies providing a useful source of key information about rare diseases for physicians, investigators, patients, and the general public. Through the coordinated international efforts of RDCRN investigators, new approaches to the diagnosis, treatment, and prevention of rare diseases will be developed to reduce burden of these diseases. Additionally, the collaboration of physicians, investigators and patient support groups is critical for dissemination of knowledge and adopt research productivity to translate to the clinical setting. By studying the genetic component of these rare diseases, better predictions of the course of the illness and more effective, personalized treatments should be developed. The next generation of rare disease investigators trained in the RDCRN should ensure that the needs of families and patients with rare diseases will be addressed now and in the future, key to improve the quality of life of affected individuals. Our multi-site consortium was initially organized and administered at The Baylor College of Medicine, Houston, TX, and entitled Angelman, Rett, & Prader-Willi Syndromes Consortium which was focused around these rare genetic disorders which have in common errors of DNA methylation or disturbances in epigenetics.

Specific goals of this NIH funded rare disease consortium will be discussed in this report and the study design relating to PWS. The primary study objective included the determination of natural history with respect to birth data, medical problems, age of onset of obesity and description of nutritional phases, mortality and cumulative amount of time in study, cognition, psychiatric/behavioral problems, frequency of PWS genetic subtypes, physical features, and body composition measures with bone density over time (8 years to the time of enrollment of the first individual with PWS until end of study). Interventions included growth hormone replacement, treatment for other endocrine disturbances and therapeutic responses were recorded and analyzed during the time of study from initiation to closure covering approximately 8 years of natural history in PWS.

## 2 | MATERIALS AND METHODS

### 2.1 | Description, study population, and main eligibility/exclusion criteria

The methodology used consisted of systematic recruitment and enrollment of individuals with Prader–Willi syndrome (PWS) and those with non-PWS early-onset morbid obesity (EMO); and collection of data (clinical, cognitive, behavior, PWS genetic subtypes, physical, and body composition measures) utilizing approved data collection forms leading to an 8 year multicenter longitudinal observational natural history study conducted at four different sites by investigators who are experts in the study of PWS. The EMO subject group consisted of obese patients with childhood onset of obesity and with behavioral, cognitive, and medical issues similar to those seen in individuals with PWS and who were referred for genetics evaluation.

This 5202 NIH rare disease consortium protocol was approved by the local IRB human subjects committees at each of the 4 study sites and consisted of screening, baseline, and follow-up forms for each subject. Informed consent was obtained on all subjects. The data collection process required forms to be completed prior to data entry in the computer generated database. The primary variables included the protocol for historical, physical, cognitive, behavioral, PWS genetic subtype data, cumulative time in study in years and mortality.

Our collection forms consisted of a screening form; 15 baseline forms; 12 two year, 12 four year, 12 six year, and 12 eight year follow-up forms. The Baseline History form consisted of 20 pages and the Return History form was 15 pages utilized for PWS study participants with 16 pages for the EMO group. The Physical Examination form was 10 pages in length and the Impression form was 1 page in length for patients with PWS and 2 pages for those with EMO. These forms were used to collect information on screening eligibility, demographics and diagnosis, pregnancy and birth history, medication history, and concomitant medications, childhood and weigh history, education history, behavior history, medical history, family history, physical examination, nutritional phases and impressions, diet history, photographs and DEXA for body composition, Kaufman Brief Intelligence Test, Second Edition (KBIT-2) testing and Behavioral Assessment System for Children, Second Edition (BASC-2) behavioral assessment for those 22 years of age and younger.

The first subject was enrolled on September 7, 2006. The study ended on July 31, 2014. The sites were the University of Florida (lead site), University of Kansas Medical Center, University of California at Irvine, and Vanderbilt University. Table 1 shows important study dates regarding protocol approval, enrollment, amendments generated, and study closure. This project was funded through the Rare Disease Clinical Research Network (RDCRN) by the National Institutes of Health (NIH)/National Institute of Child Health and Human Development (NICHD). Data were entered and stored at the Data Management Coordinating Center (DMCC) at the University of South Florida in Tampa, Florida. The DMCC facilitated the generation of electronic forms for data entry, data retrieval, and statistical analyses. Monthly conference calls were held to include principal investigators and study coordinators at the four sites, along with staff and interested parties from the DMCC, NICHD, and the Prader–Willi Syndrome Association [PWSA (USA)]. The screening, enrollment and

evaluation of 391 subjects included 355 individuals with genetically confirmed Prader-Willi syndrome, and 36 individuals with early-onset morbid obesity, but without PWS, fragile X syndrome or other known syndromic causes of obesity were evaluated and recruited from the four participating clinical sites. The number of individuals with EMO was small in relationship to PWS due to enhanced recruitment effort at the four nationally recognized sites focused on PWS. However, the recruitment of those with early-onset morbid obesity/ “Prader-Willi-like” without fragile X syndrome or other recognized obesity-related disorders was undertaken to collect a subset of subjects for determination of data with respect to birth history, medical problems, age of onset of obesity, mortality, cognition, psychiatric/ behavioral problems, and physical/clinical features and laboratory measures. Comparisons and contrasts of data were made between the PWS and the EMO subject group as goals of our study to learn more about those with genetically confirmed PWS and those with overlapping features or clinical misdiagnoses without genetic confirmation of PWS. Array CGH, high-resolution SNP microarrays and/or MS-MLPA assays (Bittel, Kibiryeva, & Butler, 2007; Butler, Fischer, Kibiryeva, & Bittel, 2008; Papenhausen et al., 2011) were used when available to identify small deletions within the 15q11.2-q13 region, as well as in the entire genome (e.g., at least 370 obesity related genes are now recognized (Butler, McGuire, & Manzardo, 2015) which could contribute to the clinical similarity to those with PWS, an obesity-related genetic disorder (Butler, 2016a). The information gathered and learned during the longitudinal natural history study of 355 subjects with PWS and shared with the academic medical community should have direct impact on the care, delivery of services, treatment approaches and diagnosis of PWS (Angulo et al., 2015; Butler et al., 2006; Butler, Manzardo, & Forster, 2016; Cassidy et al., 2012) thereby enhancing the quality of life for affected individuals and their families.

The **PWS group** had appropriate molecular and cytogenetic testing to confirm a diagnosis of PWS (i.e., chromosomes, FISH analysis of chromosome 15q11-q13 probes, DNA methylation status, and where necessary, chromosome 15 DNA polymorphism studies utilizing parental DNA), and were categorized into the appropriate molecular group (i.e., deletion, uniparental disomy, and imprinting defect) (Angulo et al., 2015; Bittel & Butler, 2005; Butler, 2016a; Butler et al., 2006; Cassidy et al., 2012). It should be noted that appropriate genetic testing of DNA methylation can correctly identify >99% of all patients with PWS (Butler et al., 2006; Driscoll et al., 1992; Kubota et al., 1996). Patients with imprinting defects will be included, but the low frequency in this group will make statistical comparisons with its two molecular classes (i.e., small microdeletions and epimutations) challenging to detect. Participants were excluded from this part of the study if they were not genetically confirmed to have PWS using approved genetic testing methodology or were not classified as having a 15q11-q13 deletion, maternal disomy 15 or imprinting defect.

The **EMO group** was selected solely based on a documented medical chart history of their weight having exceeded 150% of Ideal Body Weight (IBW) or a Body Mass Index (BMI, $kg/m^2$) of greater than the 97 percentile before 4 years of age. Participants were excluded from the study if they had a known chromosomal aneuploidy, toxin exposure, CNS condition or other syndromes (e.g., fragile X syndrome). All EMO participants had a chromosomal

study or a high resolution microarray analysis that would exclude chromosomal aneuploidy or deletions/duplications at the genome level prior to entry in the study.

### 2.2 | Outcome assurances or study endpoints

The primary outcome measures or primary endpoints in this study were phenotypic assessments of the participants by clinicians with expertise in PWS and included cognitive level assessments, behavioral analysis, physical features, co-morbidities (e.g., skin picking, psychiatric history, seizures, autistic behavior), medications required for treatment, body measurements, body composition [physical exam, skin-folds, dual-energy X-ray absorptiometry (DEXA) scans], and further comparison with the underlying molecular diagnosis or PWS genetic subtype for each subject. In addition, PWS and EMO participants were compared to each other.

Secondary endpoints included the analysis of longitudinal patterns of progression over 8 years when available to assess the natural history of clinical features of this cohort, with assessment of cognition, behavior and body composition. In addition, the age that growth hormone (or other hormone) treatment was begun in the PWS participants was correlated with physical features, body composition, cognition, behavior, developmental milestones, pubertal issues, and the onset of the nutritional phases (Miller et al., 2011).

## 3 | RESULTS AND DISCUSSION

Our historical NIH rare disease consortium for Prader–Willi syndrome is the largest PWS cohort studied to date. The reporting of data from this consortium and individual projects with analysis of datasets generated manuscripts and peer-reviewed publications and ongoing projects are underway stimulated by the limited knowledge on natural history, detailed genotype–phenotype correlations, morbidity, and mortality in this rare syndrome (Butler, 2011; Butler et al., 2009, 2011, 2016; Butler, Manzardo, Heinemann, Loker, & Loker, 2017; Gold et al., 2014; Henkhaus et al., 2012; Manzardo, Loker, Heinemann, Loker, & Butler, 2017; Miller et al., 2011; Scheimann et al., 2012; Scheimann et al., 2015).

The 15q11-q13 deletion was found in 217 study participants with PWS (61%), 127 had maternal disomy 15 (36%), and 11 had imprinting defects (3%). The deletion molecular class was further subtyped into the larger typical 15q11-q13 Type I deletion involving chromosome 15 breakpoints BP1 and BP3 ($N = 79$; 36.4%), the smaller typical 15q11-q13 Type II deletion involving breakpoints BP2 and BP3 ($N = 120$; 55.3%) and Atypical deletions that are smaller or larger than the typical deletions ($N = 18$; 8.3%). Analysis of the maternal disomy 15 (UPD) subclasses and other molecular classes and current cognitive and behavioral data is currently ongoing and will be reported in separate publications. Table 2 shows the study subject characteristics at the time of enrollment or entry along with age category, BMI, full-scale IQ scores obtained from educational records, testing and molecular class data. Subjects in our study less than 3 years of age required annual evaluations per protocol and those greater than 3 years of age were followed biennially. The age range of participants was 10 weeks of age to 62 years at the time of enrollment. Assessments on each participant were repeated for each follow-up visit except for screening eligibility and demographics. Table 3 shows the study exposure of 598 cumulative years for the 355

individuals with PWS and 42 years for the 36 EMO subjects. Seven observed deaths were recorded while on study for the PWS and EMO subject groups. Table 4 lists additional information for the seven observed deaths during the study. The number of deaths was considered low and no formal statistical tests were done because of the incidence rate. However, this study did not yield evidence that the mortality was higher in the PWS group than in the EMO group (mortality incidence rates of 1.0 and 2.4 per 100 years exposure for PWS and EMO, respectively).

A summary list (previously described in the section 2) of data points was created. As an example of data points accumulated from this natural history dataset, 11,206 observations from 376 individuals or approximately 30 per subject with PWS and recorded by February 28, 2013 with 3,368 variables obtained using 22 separate forms and file names listed in a table of contents. More data points would be available on subjects enrolled early and with follow-up visits to the study sites. Therefore, not all 376 individuals would have had the same number of observations. Despite that, this study and the number of subjects with genetically-confirmed PWS with the genetic subtype recorded represents the largest cohort by far of its kind in the world.

Two assessments were considered in order to examine the completeness of the dataset generated in this rare disease consortium. These included the patterns of early discontinuation and data collection compliance rate (percentage of forms filled out completely). The majority of those discontinuing early did so in the first 2 years after enrollment, with death of participating subjects, voluntary withdrawal, becoming lost to follow-up secondary to moving away from the study sites or disinterest in continuing in the study relevant factors. There is a potential for bias in our dataset if subjects who discontinued early had more health issues than those completing the study. However, this concern may be attenuated to some degree by the fact that 1) a wide age range of PWS subjects enrolled and 2) the data collected at baseline was virtually complete for all subjects. Bias caused by early drop out would have been more of a concern if all subjects enrolled in the longitudinal study at baseline were of the same age. In addition, not all races were well represented in our study as 93% of participants with PWS were Caucasian. The natural history and clinical problems unique to patients with PWS in specific racial/ethnic backgrounds may not be identified or recognized in our study.

When examining the completeness of the forms collected, all study data were captured from 14 forms, 11 of which were taken at each visit. Each form had certain required elements to be collected and if all required elements were obtained then the form was considered complete. The percent of complete forms submitted for baseline, 2 year, and 4 year time points were calculated for each of the forms. At these times, all subjects regardless of their age should have a completed visit. The percentage of complete forms ranged from 94–100% at baseline, 71–100% at 2 years, and 79–100% at 4 years. We consider the compliance rate to be adequate for a study of this size and duration.

As experts in the field, the principal investigators and their research partners are addressing several ongoing projects related to the natural history and better delineation of this rare syndrome with genotype-phenotype correlations. During this interim, we have published

review articles on PWS (Butler, 2011, 2016a, 2016b) Cassidy et al., 2012), characterization of the nutritional phases of PWS (Miller et al., 2011), development of syndrome-specific standardized growth charts for PWS with and without growth hormone treatment (Butler et al., 2011, 2015, 2016), clinical reports of atypical findings in PWS (Butler, Bittel, Kibiryeva, Cooley, & Yu, 2010; Dang et al., 2016; Hassan & Butler, 2016), study of neuropeptides (Butler, Nelson, Driscoll, & Manzardo, 2015a, 2015b; Johnson, Manzardo, Miller, Driscoll, & Butler, 2016; Manzardo, Johnson, Miller, Driscoll, & Butler, 2016) and cytokines (Butler, Hossain, Sulsona, Driscoll, & Manzardo, 2015), reproductive issues (Butler et al., 2009; Gold et al., 2014) and causes of death with survival trends utilizing an existing PWS specific mortality database (Butler et al., 2017; Manzardo et al., 2017). Our study of the mortality incidence rate in PWS per 100 years of exposure is novel and showed a low rate, not significantly different than that observed in the EMO group. The opportunity to address new questions and analyze this extensive clinical and genetic database is unique to this consortium and vital to gain a better understanding of the cause and diagnosis of PWS, the most common syndromic cause of morbid obesity in children (Butler, 1990).

Funding from the NIH for the rare disease consortium for PWS and EMO ended on July 31, 2014. Funding from PWSA (USA) has been obtained in the interim and continued access to the services of the DMCC will be pursued by the members of the PWS-EMO consortium. The principal investigators involved with the PWS and EMO consortium continue to pursue funding for long-term ongoing access to the services and statistical support of the DMCC staff. All data will be released to the NIH.

A viable long-term access to this existing, *one-of-a-kind* valuable dataset is important to generate reports and manuscripts to investigate and better delineate the natural history and comorbidities (recognized, unrecognized or understudied) related to PWS such as diabetes and other endocrine disturbances; growth hormone measures, treatment and outcomes; cognitive and behavioral problems in relationship to PWS genetic subtypes; mortality rates and causes of death (see Table 4) surgery records; family history; medications (dosage, indication, length of treatment); thrombotic and embolic events and medical problems unique to PWS in relationship to other rare genetic disorders or shared with others having similar features such as non-syndromic obesity; obesity (onset, degree, fluctuations), hyperphagia (onset, level), and nutritional status and osteoporosis. Going forward, this dataset will undoubtedly prove invaluable to academic researchers and patient advocacy groups, as well as pharmaceutical companies planning or conducting clinical trials to address problems related to PWS including hyperphagia and obesity. Special attention will be given to the more life-threatening aspects of the complex syndrome in order to find answers. However, the number of EMO participants in our study was relatively small and limits our ability to make meaningful comparisons with the PWS study group participants. A larger number of obese subjects should be recruited from different sources for genetic evaluation to compare with PWS.

Secondary study objectives included comparisons of the three PWS main molecular classes (deletion, uniparental disomy, and imprinting defect) from data collected at the various centers which was particularly crucial for identification of those with imprinting defects since these are rare. In addition, comparisons were done within the deletion class to compare

those with a larger typical deletion (i.e., type I), smaller typical deletion (i.e., type II) and those with rarer atypical deletions, either smaller or larger than the typical type I or type II deletion. Study participants were recruited and enrolled from infancy through adulthood and array comparative genomic hybridization (CGH), high resolution SNP arrays, genotyping of proband, and parental DNA using chromosome 15 markers and/or MS-MLPA protocols were used when available to confirm the DNA methylation status and/or distinguish type I and type II deletions from atypical deletions or maternal disomy 15 in our PWS subjects.

Our rare disease consortium consisting of four national sites involved with standardized collection of longitudinal clinical, medical, cognitive, behavior and genetic data represents the largest PWS cohort studied to date and consisting of 355 subjects with genetic confirmation of PWS and identified genetic subtypes. We found that 61% showed a 15q11-q13 deletion, 36% had maternal disomy 15 and 3% with an imprinting defect. The percentage of individuals with PWS in our consortium showed a lower number of deletions (61%) and a higher number for maternal disomy 15 (36%) than generally quoted in the literature, possibly due to a change in frequency related to improved genetic testing, older age of parents with delayed child bearing and better awareness and recognition of PWS.

Other clinical, psychiatric and genetic databases in PWS do exist that glean information on diagnosis, genetic subtype-phenotypic correlations, psychiatric/behavior, treatment and natural history with mortality and survival trends for this rare disorder. The following studies or surveys represent examples of such databases involving PWS with specific focused themes: intellectual characteristics of Prader–Willi syndrome and comparison of genetic subtypes (Roof et al., 2000) intellectual abilities and behavioral features in Prader–Willi syndrome by genetic subtype (Milner et al., 2005) growth hormone treatment and adverse events in Prader–Willi syndrome utilizing the KIGS (the Pfizer International Growth Database) (Craig et al., 2006); the relationship between compulsive behavior and academic achievement across the three genetic subtypes of Prader–Willi syndrome (Zarcone et al., 2007); the European Prader–Willi Syndrome Clinical Research Database (Holland et al., 2009); cognitive profiling in a large French cohort of adults with Prader–Willi syndrome (Copet et al., 2010) and causes of death with survival trends in Prader–Willi syndrome utilizing the Prader–Willi Syndrome Association (USA) 40-year mortality survey (Butler et al., 2017; Manzardo et al., 2017).

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## REFERENCES


Angulo MA, Butler MG, & Cataletto ME (2015). Prader-Willi syndrome: A review of clinical, genetic, and endocrine findings. Journal of Endocrinological Investigation, 38(12), 1249–1263.26062517

Bittel DC, & Butler MG (2005). Prader-Willi syndrome: Clinical genetics, cytogenetics and molecular biology. Expert Reviews in Molecular Medicine, 7, 1–20.

Bittel DC, Kibiryeva N, & Butler MG (2007). Methylation-specific multiplex ligation-dependent probe amplification analysis of subjects with chromosome 15 abnormalities. Genetic Testing, 11(4), 467–475.18294067

Butler JV, Whittington JE, Holland AJ, Boer H, Clarke D, & Webb T (2002). Prevalence of, and risk factors for, physical ill-health in people with Prader-Willi syndrome: a population-based study. Developmental Medicine & Child Neurology, 44(4), 248–255.11995893

Butler MG (1990). Prader-Willi syndrome: Current understanding of cause and diagnosis. American Journal of Medical Genetics, 35(3), 319–332.2309779

Butler MG (2011). Prader-Willi syndrome: Obesity due to genomic imprinting. Current Genomics, 12(3), 204–215.22043168

Butler MG (2016a). Single gene and syndromic causes of obesity: Illustrative examples. Progress in Molecular Biology and Translational Science, 140, 1–45.27288824

Butler MG (2016b). Benefits and limitations of prenatal screening for Prader-Willi syndrome. Prenatal Diagnosis, 36, 1–14.

Butler MG, Bittel DC, Kibiryeva N, Cooley LD, & Yu S (2010). An interstitial 15q11-q14 deletion: expanded Prader-Willi syndrome phenotype. American Journal of Medical Genetics Part A, 152A(2), 404–408.20082457

Butler MG, Fischer W, Kibiryeva N, & Bittel DC (2008). Array comparative genomic hybridization (aCGH) analysis in Prader-Willi syndrome. American Journal of Medical Genetics Part A, 146A(7), 854–860.18266248

Butler MG, Hossain W, Sulsona C, Driscoll DJ, & Manzardo AM (2015). Increased plasma chemokine levels in children with Prader-Willi syndrome. American Journal of Medical Genetics Part A, 167A(3), 563–571.25691409

Butler MG, Lee J, Cox DM, Manzardo AM, Gold JA, Miller JL, … Driscoll DJ (2016b). Growth charts for Prader-Willi syndrome during growth hormone treatment. Clin Pediatr (Phila), 55(10), 957–974.26842920

Butler MG, Lee J, Manzardo AM, Gold JA, Kimonis V, Miller JL, & Driscoll DJ (2015). Growth charts for non-growth hormone treated Prader-Willi syndrome. Pediatrics, 135(1), 126–135.25548332

Butler MG, Lee PDK, & Whitman BY (2006). Management of Prader-Willi Syndrome. New York: Springer-Verlag.

Butler MG, Manzardo AM, & Forster JL (2016a). Prader-Willi syndrome: Clinical genetics and diagnostic aspects with treatment approaches. Current Pediatric Reviews, 12(2), 136–166.26592417

Butler MG, Manzardo AM, Heinemann J, Loker C, & Loker J (2017). Causes of death in Prader-Willi syndrome: Prader-Willi Syndrome Association (USA) 40-year mortality survey. Genetics in Medicine, 19(6), 635–642.27854358

Butler MG, McGuire A, & Manzardo AM (2015). Clinically relevant known and candidate genes for obesity and their overlap with human infertility and reproduction. Journal of Assisted Reproduction and Genetics, 32(4), 495–508.25631154

Butler MG, Nelson TA, Driscoll DJ, & Manzardo AM (2015a). Evaluation of plasma substance P and beta-endorphin levels in children with Prader-Willi syndrome. Journal of Rare Disorders, 3(2), 1–14.26191532

Butler MG, Nelson TA, Driscoll DJ, & Manzardo AM (2015b). High plasma neurotensin levels in children with Prader-Willi syndrome. American Journal of Medical Genetics Part A, 167A(8), 1773–1778.25847417

Butler MG, Sturich J, Lee J, Myers SE, Whitman BY, Gold JA, … Driscoll DJ (2011). Growth standards of infants with Prader-Willi syndrome. Pediatrics, 127(4), 687–695.21402637

Butler MG, Sturich J, Myers SE, Gold JA, Kimonis V, & Driscoll DJ (2009). Is gestation in Prader-Willi syndrome affected by the genetic subtype? Journal of Assisted Reproduction and Genetics, 26(8), 461–466.19760168

Cassidy SB, Schwartz S, Miller JL, & Driscoll DJ (2012). Prader-Willi syndrome. Genetics in Medicine, 14(1), 10–26.22237428

Copet P, Jauregi J, Laurier V, Ehlinger V, Arnaud C, Cobo AM, … Thuilleaux D (2010). Cognitive profile in a large French cohort of adults with Prader-Willi syndrome: Differences between genotypes. Journal of Intellectual Disability Research, 54(3), 204–215.20136683

Craig ME, Cowell CT, Larsson P, Zipf WB, Reiter EO, … Albertsson Wikland K (2006). Growth hormone treatment and adverse events in Prader-Willi syndrome: Data from KIGS (the Pfizer International Growth Database). Clinical Endocrinology, 65(2), 178–185.16886957

Dang V, Surampalli A, Manzardo AM, Youn S, Butler MG, Gold JA, & Kimonis VE (2016). Prader-Willi syndrome due to an unbalanced de novo translocation t(15;19)(q12;p13.3). Cytogenetic and Genome Research, 150(1), 29–34.27894106

Driscoll DJ, Waters MF, Williams CA, Zori RT, Glenn CC, Avidano KM, & Nicholls RD (1992). A DNA methylation imprint, determined by the sex of the parent, distinguishes the Angelman and Prader-Willi syndromes. Genomics, 13(4), 917–924.1505981

Gold JA, Ruth C, Osann K, Flodman P, McManus B, Lee HS, … Kimonis VE (2014). Frequency of Prader-Willi syndrome in births conceived via assisted reproductive technology. Genetics in Medicine, 16(2), 164–169.23928912

Hassan M, & Butler MG (2016). Prader-Willi syndrome and atypical submicroscopic 15q11-q13 deletions with or without imprinting defects. European Journal of Medical Genetics, 59(11), 584–589.27659713

Henkhaus RS, Kim SJ, Kimonis VE, Gold JA, Dykens EM, Driscoll DJ, & Butler MG (2012). Methylation-specific multiplex ligation-dependent probe amplification and identification of deletion genetic subtypes in Prader-Willi syndrome. Genetic Testing and Molecular Biomarkers, 16(3), 178–186.21977908

Holland A, Whittington J, Cohen O, Curfs L, Delahaye F, Dudley O, … Vogels A (2009). The European Prader-Willi Syndrome Clinical Research Database: An aid in the investigation of a rare genetically determined neurodevelopmental disorder. Journal of Intellectual Disability Research, 53(6), 538–547.19457156

Johnson L, Manzardo AM, Miller JL, Driscoll DJ, & Butler MG (2016). Elevated plasma oxytocin levels in children with Prader-Willi syndrome compared with healthy unrelated siblings. American Journal of Medical Genetics Part A, 170(3), 594–601.26615966

Kubota T, Sutcliffe JS, Aradhya S, Gillessen-Kaesbach G, Christian SL, Horsthemke B, … Ledbetter DH (1996). Validation studies of SNRPN methylation as a diagnostic test for Prader-Willi syndrome. American Journal of Medical Genetics, 66(1), 77–80.8957518

Manzardo AM, Johnson L, Miller JL, Driscoll DJ, & Butler MG (2016). Higher plasma orexin A levels in children with Prader-Willi syndrome compared with healthy unrelated sibling controls. American Journal of Medical Genetics Part A, 170(8), 2097–2102.27214028

Manzardo AM, Loker J, Heinemann J, Loker C, & Butler MG (2017). Survival trends from the Prader-Willi Syndrome Association (USA) 40-year mortality survey. Genetics in Medicine, 10.1038/gim.2017.92. Epub ahead of print.

Miller JL, Lynn CH, Driscoll DC, Goldstone AP, Gold JA, Kimonis V, … Driscoll DJ (2011). Nutritional phases in Prader-Willi syndrome. Am Journal of Medical Genetics Part A, 155A(5), 1040–1049.

Milner KM, Craig EE, Thompson RJ, Veltman MW, Thomas NS, Roberts S, … Bolton PF (2005). Prader-Willi syndrome: Intellectual abilities and behavioural features by genetic subtype. Journal of Child Psychology and Psychiatry, 46(10), 1089–1096.16178933

Papenhausen P, Schwartz S, Risheg H, Keitges E, Gadi I, Burnside RD, … Tepperberg J (2011). UPD detection using homozygosity profiling with SNP genotyping microarray. American Journal of Medical Genetics, 155(4), 757–768.

Roof E, Stone W, MacLean W, Feurer ID, Thompson T, & Butler MG (2000). Intellectual characteristics of Prader-Willi syndrome: Comparison of genetic subtypes? Journal of Intellectual Disability Research, 44(Pt 1), 25–30.10711647

Scheimann AO, Butler MG, Miller JL, Lee PD, Stevenson DA, Heinemann J, & Driscoll DJ (2012). Letter to the editor: Long-term experience with duodenal switch in adolescents. Obesity Surgery, 22(3), 517–518.21874367

Scheimann AO, Nadler EE, Driscoll DJ, Butler MG, Miller JL, Markovic TP, & Goldstone AP (2015). Laparoscopic sleeve gastrectomy in 108 obese children and adolescents ages 5 to 21 years by Alqahtani AR, Antonisamy B, Alamri H, Elahmedi M, Zimmerman VA. Annals of Surgery, 261(4), e118.24045441

Shoffstall AJ, Gaebler JA, Kreher NC, Niecko T, Douglas D, Strong TV, … Butler MG (2016). The high direct medical costs of Prader-Willi syndrome. The Journal of Pediatrics, 175, 137–143.27283463

Zarcone J, Napolitano D, Peterson C, Breidbord J, Ferraioli S, Caruso-Anderson M, … Thompson T (2007). The relationship between compulsive behaviour and academic achievement across the three genetic subtypes of Prader-Willi syndrome. Journal of Intellectual Disability Research, 51(Pt. 6), 478–487.17493030

**Table 1.**

The study timeline and important dates regarding protocol approval, subject enrollment and study amendments

| Item | Calendar date | Comment |
|---|---|---|
| Protocol approval | January 2006 | |
| First PWS patient enrolled | September 2006 | |
| First EMO patient enrolled | October 2006 | |
| Amendment 1 | August 2007 | |
| Amendment 2 | October 2008 | Additional site at Vanderbilt University was added. Follow-up visits changed to every 2 years per subject at age 3 years of age |
| Amendment 3 | May 2009 | |
| Amendment 4 | July 2010 | MS-MLPA added as an alternative genetic test |
| Amendment 5 | July 2012 | Sample size increased from 300 to 400. RNCA collection added. EMO criteria relaxed to recruit subjects with single gene mutation as cause for obesity. |
| Final patient visit | January 2014 | |

Baseline subject characteristics for both Prader-Willi syndrome (PWS) and early-onset morbid obesity (EMO) are shown separately in Table 2. One subject with PWS was considered “enrolled” in the study but no information was provided.

**Table 2.**

Baseline characteristics, BMI, age, IQ, and molecular class for individuals with Prader–Willi syndrome and early-onset morbid obesity

| Characteristic | Prader–Willi syndrome, *N* = 355 (%) | Early-onset morbid obesity, *N* = 36 (%) |
|---|---|---|
| Gender | | |
| Male | 158 (44.5%) | 18 (50%) |
| Female | 197 (55.5%) | 18 (50%) |
| Race[a] | | |
| Caucasian | 330 | 28 |
| African American | 17 | 6 |
| Asian | 16 | 1 |
| Other/unknown | 11 | 1 |
| BMI mean (SD) *n*[b] | 25.6 (10.2) 317 | 36.8 (13.1) 32 |
| Age (years) mean (SD) *n* | 13.4 (12.0) 355 | 10.0 (4.8) 36 |
| Age category (years) | | |
| 0 < 2 years | 39 (11.0%) | 0 (0%) |
| 2 < 5 years | 56 (15.8%) | 5 (13.9%) |
| 5 < 12 years | 105 (29.6%) | 20 (55.6%) |
| 12 < 21 years | 77 (22.0%) | 11 (30.6%) |
| ≥21 years | 78 (22.0%) | 0 (0%) |
| IQ mean (SD) n | 67 (16) 256 | 84 (21) 25 |
| Molecular class | | |
| Deletion | 217 (61.1%) | |
| Type I deletion | 79 (36.4%) | |
| Type II deletion | 120 (55.3%) | |
| Atypical deletion | 18 (8.3%) | |
| Uniparental disomy | 127 (35.8%) | |
| Imprinting defect | 11 (3.0%) | |

[a]Subjects could report more than one race or refuse to answer entirely.

[b]The average BMI was low for an obesity syndrome as 56% of our PWS participants were less than 12 years of age.

[c]The full-scale IQ was reported for those 6 years and older from historical educational records and/or KBIT-2 testing.

**Table 3.**

Mortality and study exposure information

| Study exposure variable | Prader–Willi syndrome (*n* = 355) | Early-onset morbid obese (*n* = 36) |
|---|---|---|
| Cumulative amount of time on study (years) | 598.3 | 42.5 |
| Average amount of time on study (years) ± SD (range) | 1.7 ± 1.8 [0–6.5] | 1.2 ± 1.7 [0–4.9] |
| Total study visits in age category | | |
| 0–2 years | 50 | 0 |
| 2–5 years | 113 | 5 |
| 5–12 years | 226 | 34 |
| 12–21 years | 136 | 18 |
| Above 21 | 134 | 0 |
| Deaths | 6 | 1 |
| Mortality incidence rate per 100 years exposure (95%CI) | 1.0 (0.4, 2.2) | 2.4 (0.0, 13.1) |

**Table 4.**

Deaths reported in study participants and related information during the rare disease study

| Age at death (years) | Group | Molecular class | Gender | Autopsy performed | Manner of death—description | Cause of death |
|---|---|---|---|---|---|---|
| 51.72 | PWS | Uniparental disomy | Female | Unknown | | Natural causes |
| 32.44 | PWS | Uniparental disomy | Male | Unknown | | Natural causes |
| 32.60 | PWS | Deletion | Male | Unknown | | Natural causes |
| 1.93 | PWS | Uniparental disomy | Female | Unknown | Pneumonia and septicemia | Pneumonia and septicemia |
| 50.79 | PWS | Uniparental disomy | Female | No | Gastric necrosis | Gastric necrosis |
| 44.43 | PWS | Imprinting defect | Female | Yes | Died in sleep, autopsy results pending | Unknown |
| 14.79 | EMO | | Female | No | Diabetic ketoacidosis and resultant brain herniation | Brain herniation |