Title

Influence of molecular classes and growth hormone treatment on growth and dysmorphology in Prader-Willi syndrome: A multicenter study

Journal

Clinical Genetics, 100(1)

ISSN

0009-9163

Authors

Mahmoud, Ranim
Leonenko, Anna
Butler, Merlin G
et al.

Publication Date

2021-07-01

DOI

10.1111/cge.13947

Received: 14 November 2020 | Revised: 18 February 2021 | Accepted: 19 February 2021

DOI: 10.1111/cge.13947

ORIGINAL ARTICLE

CLINICAL GENETICS WILEY

Influence of molecular classes and growth hormone treatment on growth and dysmorphology in Prader-Willi syndrome: A multicenter study

Ranim Mahmoud[1,2] | Anna Leonenko[1] | Merlin G. Butler[3] | Pamela Flodman[1] | June-Anne Gold[1,4,5] | Jennifer L. Miller[6] | Elizabeth Roof[7] | Elisabeth Dykens[7] | Daniel J. Driscoll[6] | Virginia Kimonis[1,5]

[1]Department of Pediatrics, University of California, Irvine, CA, Children's Hospital of Orange County, Orange, California

[2]Department of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura, Egypt

[3]Departments of Psychiatry & Behavioral Sciences and Pediatrics, University of Kansas Medical Center, Kansas City, Kansas, USA

[4]Department of Pediatrics, Loma Linda University Medical School, Loma Linda, California

[5]Childrens Hospital of Orange County, Orange, California

[6]Department of Pediatrics, University of Florida, College of Medicine, Gainesville, Florida

[7]Vanderbilt Kennedy Center for Research on Human Development, Vanderbilt University, Nashville, Tennessee

Correspondence
Virginia Kimonis, M.D., Division of Genetics and Genomic Medicine, Department of Pediatrics, University of California Irvine, 101 The City Drive South, ZC4482, Orange CA 92868, USA.
Email: vkimonis@uci.edu

Funding information
The Prader-Willi Syndrome Association (USA) and the Angelman, Rett and Prader-Willi Syndromes Consortium (U54 HD06122), which was part of the National Institutes of Health (NIH) Rare Disease Clinical Research Network (RDCRN) was supported through collaboration between the NIH Office of Advancing Translational Science (NCATS) and the National Institute of Child Health and Human Development (NICHD)., Grant/Award Number: U54 HD06122 and RR019478. UC Irvine's Institute for Clinical and Translational Science was funded by a grant from the NIH Clinical and Translational Sciences Award program (UL1 TR000153).

Abstract

Prader-Willi syndrome (PWS) is a complex genetic disorder with three molecular classes but clinical ascertainment is based on distinctive features. The prevalence of dysmorphic features was studied in 355 PWS participants (61% deletion, 36% maternal disomy [UPD], and 3% imprinting defects) from the National Institute of Health PWS Rare Diseases Clinical Research Network. The effect of growth hormone (GH) treatment on growth and dysmorphic features was compared. Among participants, upslanting palpebral fissures were seen in 23%; strabismus in 42%; abnormal dentition in 32%; small hands in 63% and small feet in 70%; hypopigmentation in 30%; striae in 32% and skin picking in 26%. Compared to those with UPD, participants with deletions were found to be heavier ($p = 0.002$), had smaller head circumference (HC) ($p = 0.009$), higher incidence of a flat occiput ($p = 0.005$); low-anterior hairline ($p = 0.04$); abnormal dentition ($p = 0.009$); abdominal striae ($p = 0.045$), nail abnormalities ($p = 0.050$), and fair-haired ($p < 0.001$). Participants in both genetic groups receiving GH were taller ($p = 0.005$), had larger HCs ($p = 0.005$), and longer hands ($p = 0.049$). This study suggested that PWS genetic subtypes and GH treatment can influence growth and dysmorphic features that may impact clinical diagnosis of PWS, such as stature, head shape and appearance of the eyes, nose, and genitalia.

KEYWORDS
dysmorphology, genetic subtypes, genotype–phenotype, growth, growth hormone treatment, Prader-Willi syndrome

Ranim Mahmoud and Anna Leonenko are considered as co-first authors.

1 | INTRODUCTION

Prader-Willi syndrome (PWS) affects 1/15000–1/30000 live births and is genetically characterized by absence of expression of paternally inherited genes from the 15q11-q13 region, due to a paternal deletion, uniparental maternal disomy (UPD) 15, or imprinting defects (ID). Affected individuals have severe hypotonia, a poor suck with feeding difficulties and developmental delay during infancy and learning problems, hyperphagia with obesity in childhood, short stature, behavioral problems including frequent temper tantrums, skin picking, and possibly psychosis, schizophrenia, manic-depression and autism spectrum disorder (e.g., References 1-8). Individuals with PWS have abnormal function of the endocrine system, which includes growth hormone (GH)/insulin-like growth factor 1 axis dysfunction, hypogonadism, hypothyroidism, premature adrenarche, and adrenal insufficiency.[4-10] They also develop distinctive physical and dysmorphic facial features including small hands and feet, excessive body fat that often concentrates on the trunk and thighs, a narrow forehead, and deep-set almond-shaped eyes. Often these physical features and distinctive characteristics alert the clinician to the possible diagnosis of PWS but requires testing to identify the three known molecular genetic classes (i.e., paternally derived chromosome 15q11-q13 deletion, UPD 15 or imprinting center defects).[11]

1.1 | Distinct facial and physical features

The facial and physical features seen in individuals with PWS were first described by Prader et al. in 1956.[12] These facial features include a small narrow bifrontal diameter, almond-shaped palpebral fissures, narrow nasal bridge, and thin upper lip with downturned corners of the mouth and decreased salivary secretions. Other physical features include short stature, small hands with flattened ulnar border, small feet, hypoplastic genitalia [1-8] and hypopigmentation in relationship to first-degree relatives in those with the 15q11-q13 deletion.[13]

GH deficiency has been documented in PWS with GH treatment considered the standard of care.[4,6,7,14] Overall, GH therapy affects linear growth by increasing adult height in addition to improvement of physical activity, strength and muscle mass that can impact craniofacial features, body habitus and quality of life with improved cognition and possibly behavior in PWS.[6,8,15,16]

The aim of this study was to compare the physical and dysmorphic features in individuals with PWS in a large cohort and in relationship to genetic subtypes and GH treatment to determine if GH treatment effects are different for individuals with either deletion or UPD.

2 | SUBJECTS AND METHODS

Data from 355 individuals with genetically confirmed PWS were collected at the University of California, Irvine, California; University of Florida Health Science Center, Gainesville, Florida; University of Kansas Medical Center, Kansas City, Kansas; and Vanderbilt University Medical Center, Nashville, Tennessee and entered into the National Institute of Health (NIH) funded Rare Disease Clinical Research Network (RDCRN) PWS registry.[17] Written informed consent was obtained from all participants or their guardians prior to enrollment using approved human subjects research consent forms at the four sites. Clinical and genetic data were obtained over an 8-year period from 2006 to 2014 using standardized measurements of physical and growth variables including craniofacial features noted by PWS specialists with over 100 combined years of experience and training as dysmorphologists at the sites.

2.1 | Dysmorphology evaluation

Physical and facial features, including continuous and categorical variables, were assessed. Continuous variables included physical measurements of height, weight, body mass index (BMI), head circumference (HC), craniofacial, arm span, hand, foot, and penile length. Data were collected for analysis at the initial enrollment visit per participant. For statistical purposes, the data were converted into age and gender-adjusted centiles using the WHO (World Health Organization) and CDC (Center for Disease Control) reference tables.[18,19] Categorical variables included esotropia, exotropia, head shape, narrow nasal bridge, flat philtrum, downturned corners of mouth, dental, genitalia, skin picking, hair texture, and pigment. The data were summarized using mean and SD for continuous variables. Participant groups were subdivided by PWS molecular genetic classes and GH use, duration, and onset, and then compared using two-group *t*-tests for continuous variables and chi-square tests for categorical variables. The statistical analyses were accomplished using SPSS 20 Statistics software (Armonk, NY). Statistical significance was considered at $p < 0.05$. This project was the focus of the Master's thesis by one of our co-authors (A.L.).[20]

3 | RESULTS

3.1 | Dysmorphic and clinical features in PWS

A total of 355 PWS study participants were analyzed and comprised of 160 males (45.1%) and 195 females (59.2%). Ninety-three percent of the PWS participants were Caucasian. The average age (±SD) for the 355 PWS participants was 13 (±1) years with a range of 2 months to 62 years. The mean age at diagnosis was 3.1 ± 6.7 years with a range from birth to 48 years. Sixty-two percent of the PWS participants were diagnosed at less than 1 year of age and 26% were diagnosed greater than 3 years of age. The PWS molecular genetic classes included 217 with 15q11-q13 deletions (61%), 127 with UPD (36%) and 11 with imprinting center defects (3%). Overall, 289 participants (81.4%) had a history of GH treatment with an average age of onset of 2 (±1) years, including 137 of 160 males (85.6%) and 152 of

TABLE 1 Phenotypic characteristics according to molecular class for all participants with Prader-Willi syndrome with or without growth hormone treatment

Variables	Deletion		UPD		
	N = 217 (M = 99, F = 118)	Mean (SD) or % frequency	N = 127 (M = 53, F = 74)	Mean (SD) or % frequency	p-value
Growth parameters:					
Height percentile for age and gender	160/217	42 (34)	109/127	45 (35)	0.510
Weight percentile for age and gender	207/217	75 (31)	123/127	62 (35)	**0.002**
Head circumference (HC) percentile for age and gender	192/217	51 (34)	115/127	61 (31)	**0.009**
BMI percentile for age and gender	209/217	86 (36)	116/127	84 (38)	0.137
Head:					
Microcephaly HC (<3rd percentile)	25/192	13%	3/127	2%	0.056
Prominent occiput	38	18%	26	20%	0.452
Flat occiput	59	27%	18	14%	**0.005**
Round face	74	34%	34	27%	0.157
Bitemporal narrowing	150	69%	82	64%	0.233
Craniosynostosis	2	0.9%	1	0.8%	0.550
Hair:					
Low-anterior hair line	56	26%	19	15%	**0.04**
Fair colored for family members	87	40%	19	15%	**<0.001**
Hypopigmented	86	40%	19	15%	**<0.001**
Eyes:					
Esotropia	81	37%	49	39%	0.529
Exotropia	7	3%	10	8%	0.091
Strabismus	84	39%	67	53%	**0.03**
Upslanting palpebral fissures	57	26%	23	18%	0.156
Downslanting palpebral fissures	13	6%	17	13%	**0.015**
Almond shaped	147	68%	73	58%	**0.045**
Inter-canthal distance percentile for age and gender	71	58 (33)	49	61 (32)	0.582
Inter-pupillary distance percentile for age and gender	70	65 (35)	47	67 (35)	0.685
Outer-canthal distance percentile for age and gender	71	50 (39)	49	48 (41)	0.749
Nose:					
Narrow	36	17%	18	14%	0.638
Mouth:					
Philtrum flat	50	23%	31	24%	0.957
Upper lip downturned	49	23%	35	28%	0.400
Abnormal dentition	91	42%	31	25%	**0.009**
Dental caries	41	19%	20	16%	**0.045**
Dental grinding	87	40%	45	36%	0.196
Ears:					
Posterior angulated	13	6%	17	14%	**0.019**
Ear length percentile for age and gender	71	44 (30)	47	53 (29)	0.075
Chest:					
Pectus excavatum	32	15%	24	19%	0.314
Pectus carinatum	6	3%	5	4%	0.551

(Continues)

TABLE 1 (Continued)

Variables	Deletion N = 217 (M = 99, F = 118)	Deletion Mean (SD) or % frequency	UPD N = 127 (M = 53, F = 74)	UPD Mean (SD) or % frequency	p-value
Abdomen:					
Abdominal striae	83	38%	32	25%	**0.024**
Abdominal pale striae	62	29%	21	17%	**0.045**
Extremities:					
Hand length percentile for age and gender	190	38 (31)	114	36 (33)	0.773
Foot length percentile for age and gender	185	24 (25)	106	23 (26)	0.841
Shorter fifth finger	86	40%	35	28%	**0.024**
Nail abnormalities	56	26%	16	13%	0.050
Large thighs	112	52%	50	39%	**0.025**
Spine:					
Scoliosis	96	44%	50	39%	0.050
Genitalia:					
Bilateral cryptorchidism	74/99	75%	28/53	53%	0.263
Micropenis (<5th percentile)	26/99	26%	14/53	26%	0.173
Scrotum rugation poor	32/99	32%	18/53	34%	0.920
Scrotum hypoplastic	46/99	46%	25/53	47%	0.570
Labia minora hypoplastic	53/118	45%	37/74	50%	0.526
Clitoris hypoplastic	48/118	41%	27/74	36%	0.531
Skin:					
Face skin picking	14	6.5%	6	4.7%	**0.010**

Note: Eleven participants with imprinting defects were not included in the analysis. Bold numbers represent statistically significant values.
Abbreviations: F, female; M, male; UPD, uniparental maternal disomy.

TABLE 2 Growth hormone (GH) intake based on the age of onset of GH treatment

Age groups		Growth hormone treated cohort	
	Years	Frequency	%
1	0–1	121	41.7
2	1–4	84	29.2
3	4–12	52	18.1
4	12–21	19	6.6
5	21–70	13	4.5
Total		289	100

195 females (77.9%). In addition, 179 of 217 (82.4%) had deletions, 103 of 127 (81%) with UPD, and 7 of 11 (63.6%) individuals with imprinting center defects received GH treatment (Table 1).

In the entire PWS cohort (N = 355 participants), microcephaly (HC less than third percentile) was found in 8% of participants, flat occiput in 22%, upslanting palpebral fissures in 23%, craniosynostosis in 0.8%; strabismus in 42%; abnormal dentition in 32%; small hands in 63% and small feet in 70%, hypopigmented hair in 30%; striae in 32% and skin picking in 26%. Bilateral cryptorchidism was present in 63% and hypoplastic scrotum in 44% of males. Hypoplastic clitoris and labia minora were found in 38% and 46% of females, respectively during the initial baseline clinic visit (Table 1). Forty of the 160 males had recorded penile stretched length and 15 (38%) were considered to have a micropenis (<5th percentile).

3.2 | Comparison between the 15q11-q13 deletion and UPD 15 in the entire PWS cohort

When comparing deletion with UPD, participants with deletions were found to be heavier (mean weight percentile of 75 ± 31 vs. 62 ± 35, p = 0.002), but BMI percentile was not significantly different (mean BMI percentile of 86 ± 36 vs. 84 ± 38, p = 0.137). Those with deletions had a smaller HC with mean HC percentile of 51 ± 34 vs. 61 ± 31 (p = 0.009). However, height was not different between the two PWS molecular classes (p = 0.510). Almond-shaped eyes (68% vs. 58%; p = 0.045), a flat occiput (27 vs. 14%; p = 0.005), low-anterior hairline (26% vs. 15%; p = 0.04), and abnormal dentition (42% vs. 25%; p = 0.009) were seen at a higher incidence in the deletion group compared with UPD. Individuals with the deletion had a higher incidence of shorter fifth fingers (40% vs. 28%; p = 0.024) and nail

TABLE 3 Phenotypic characteristics of the study PWS participants according to growth hormone treatment status

Variables	Growth hormone treatment		No growth hormone treatment		
	N = 289 (M = 137, F = 152)	Mean (SD) or % frequency	N = 66 (M = 23, F = 43)	Mean (SD) or % frequency	p-value
Growth parameters:					
Height percentile for age and gender	238/289	47 (33)	36/66	17 (31)	**0.005**
Weight percentile for age and gender	277/289	70 (32)	60/66	74 (33)	0.432
Head circumference (HC) percentile for age and gender	260/289	58 (32)	55/66	52 (29)	**0.005**
BMI percentile for age and gender	276/289	83 (37)	57/66	85 (44)	0.789
Head:					
Prominent occiput	37	13%	16	24%	0.004
Flat occiput	63	22%	17	26%	0.976
Round face	72	25%	27	41%	0.001
Bitemporal narrowing	192	66%	28	42%	0.906
Craniosynostosis	2	0.6%	1	2%	0.158
Hair:					
Low-anterior hair line	48	17%	17	26%	0.142
Hypopigmented	99	34%	13	20%	0.057
Eyes:					
Esotropia	122	42%	15	23%	**0.012**
Exotropia	18	6%	5	8%	0.553
Strabismus	135	47%	24	36%	0.357
Upslanting palpebral fissures	59	20%	13	20%	0.793
Downslanting palpebral fissures	25	9%	5	8%	0.955
Almond shaped	156	54%	43	65%	**0.005**
Inter-canthal distance percentile for age and gender	99	54 (32)	24	46 (25)	0.246
Inter-pupillary distance percentile for age and gender	98	56 (36)	22	37 (32)	**0.031**
Outer-canthal distance percentile for age and gender	99	54 (38)	24	41 (34)	0.116
Palpebral fissure percentile for age and gender	97	58 (37)	24	45 (38)	0.145
Nose:					
Narrow	79	27%	25	38%	**0.020**
Mouth:					
Philtrum flat	112	39%	27	41%	0.317
Upper lip downturned	65	23%	21	32%	0.683
Normal dentition	172	60%	41	62%	0.265
Dental caries	52	18%	10	15%	0.294
Ears:					
Posterior angulated	23	8%	6	9%	0.583
Ear length percentile for age and gender	97	53 (31)	24	48 (25)	0.462
Chest:					
Pectus excavatum	52	18%	0	0%	**0.003**
Pectus carinatum	6	2%	3	6%	0.187
Abdomen:					
Abdominal pale striae	54	19%	17	26%	**0.030**

(Continues)

TABLE 3 (Continued)

Variables	Growth hormone treatment N = 289 (M = 137, F = 152)	Mean (SD) or % frequency	No growth hormone treatment N = 66 (M = 23, F = 43)	Mean (SD) or % frequency	p-value
Extremities:					
Hand length percentile for age and gender	165	38 (33)	55	34 (27)	**0.049**
Foot length percentile for age and gender	167	23 (25)	53	25 (26)	0.649
Nail abnormalities	52	18%	10	15%	0.274
Mid-thigh circumference percentile	115	42 (14)	43	35 (8)	**0.003**
Large thighs	137	47%	31	46%	0.509
Spine:					
Scoliosis	152	53%	28	42%	0.422
Genitalia:					
Bilateral cryptorchidism	82/137	60%	20/23	87%	0.648
Micropenis (<5th percentile)	8/137	6%	7/23	30%	0.063
Scrotum rugation poor	46/137	34%	9/23	39%	0.613
Scrotum hypoplastic	66/137	48%	9/23	39%	0.390
Labia minora hypoplastic	79/152	52%	11/43	26%	**0.005**
Clitoris hypoplastic	64/152	42%	11/43	26%	**0.044**
Skin:					
Face skin picking	14	5%	6	9%	**0.010**

Note: These measurements are based on normative data. Bold numbers represent statistically significant values.

abnormalities (26% vs. 13%; p = 0.050). The participants with the deletion were more fair-haired (40% vs. 15%; p < 0.001) than their family members which may reflect the loss of a single biallelically expressed *OCA2* gene allele found in the distal 15q11-q13 region and deleted in the deletion process leading to hypopigmentation.[13,21] Interestingly, the UPD group had a higher incidence of strabismus (53% vs. 39%; p = 0.03), downward slanting of the fissures (13% vs. 6%; p = 0.015) and posterior angulated ears (14% vs. 6%; p = 0.019) than in the deletion group.

3.3 | Comparison between GH treated and non-GH treated participants with PWS

The study participants were categorized according to their history of GH treatment (treated vs. not treated) regardless of their PWS molecular class. Table 2 tabulates the frequency of individuals taking GH in different age groups. We found that 41.7% of individuals began GH by 1 year of age and 70% were started by 4 years of age. Not surprisingly, individuals who received GH were taller (p = 0.005), had longer hands (p = 0.049), and had larger HCs (p = 0.005); however, weight was not statistically different. Mean BMI percentile was 83 ± 37 in the GH treated group vs. 85 ± 44 in the non-GH treated group, (p = 0.789). The data were standardized for gender and age, and each GH group had the same molecular class distribution (e.g., 62% deletion). We found that individuals on GH were taller (p = 0.005), had larger head size (p = 0.005), had a lower incidence of almond-shaped eyes (p = 0.005), a narrow nose (p = 0.020), abdominal pale striae (p = 0.030), skin picking of the face (p = 0.010), larger thigh circumferences (p = 0.003), longer hand length (p = 0.049), increased incidence of esotropia (p = 0.012), hypoplastic labia minora (p = 0.005) and hypoplastic clitoris (p = 0.044) (see Table 3).

Because of the wide age range of our participants, we studied the frequencies of dysmorphic features by age of initiation of GH treatment in different age groups (birth to 1 year, 1 to 4 years of age, 4 to 12 years, or 12 to 21 years), versus GH treatment initiated during adulthood (i.e., 21 years or older) (Table 4). This analysis was done to test the hypothesis that if GH treatment was initiated at a younger age, then a stronger effect may be present by ameliorating the physical and dysmorphic features associated with PWS. Individuals who had GH treatment initiated at a younger age (from birth to 1 year) in contrast to an older age group showed lower incidences of micrognathia (p = 0.039), slit-like eyes (p = 0.025), a narrow nose (p = 0.013), abdominal or central distribution of fat (p = <0.05), kyphosis (p = <0.05), and short fifth fingers (p = 0.026). Participants who started GH treatment at a younger age had fewer dysmorphic features as they received treatment for a longer duration. However, no statistically significant difference was found when the duration of treatment was compared among these age groups (one-way ANOVA; p = 0.818), but both micrognathia and slit-like eyes were statistically more common in the older age groups.

TABLE 4 Physical features compared with age of growth hormone (GH) treatment initiation

	0–1 N = 121		1–4 N = 84		4–12 N = 52		12–21 N = 19		21–70 N = 13		
Age of initiation of GH (years)	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**Chi-square p-value**
Head and face:											
Prominent occiput	15	13.9	12	15.8	5	10.2	2	11.8	2	15.4	0.928
Flat occiput	34	31.5	19	25.0	15	30.6	2	11.8	3	23.1	0.471
Round face	25	23.1	23	30.3	18	36.7	5	29.4	1	7.7	0.198
Narrow nose	22	20.4	22	28.9	22	44.9	7	41.2	6	46.2	**0.013**
Bitemporal narrowing	81	75.0	54	71.1	32	65.3	13	76.5	12	92.3	0.355
Craniosynostosis	3	2.8	0	0.0	0	0.0	0	0.0	0	0.0	0.360
Hypopigmented hair	50	46.3	23	30.3	15	30.6	8	47.1	3	23.1	0.089
Hyperpigmented hair	2	1.9	1	1.3	2	4.1	0	0.0	1	7.7	0.521
Chin:											
Micrognathia	24	22.2	18	23.7	5	10.2	6	35.3	6	46.2	**0.039**
Prognathia	11	10.2	5	6.6	8	16.3	2	11.8	0	0.0	0.317
Retrognathia	7	6.5	6	7.9	3	6.1	0	0.0	0	0.0	0.661
Eyes:											
Almond shaped	61	56.5	46	60.5	31	63.3	10	58.8	8	61.5	0.945
Slit-like eyes	5	4.6	5	6.6	8	16.3	4	23.5	2	15.4	**0.025**
Strabismus	61	56.5	40	52.6	22	44.9	7	41.2	5	38.5	0.467
Esotropia	53	49.1	40	52.6	18	36.7	6	35.3	5	38.5	0.341
Exotropia	6	5.6	3	3.9	4	8.2	4	23.5	1	7.7	0.065
Ptosis	15	13.9	11	14.5	10	20.4	3	17.6	4	30.8	0.515
Epicanthal folds	38	35.2	22	28.9	11	22.4	5	29.4	3	23.1	0.549
Hypertelorism	8	7.4	7	9.2	3	6.1	1	5.9	0	0.0	0.809
Hypotelorism	9	8.3	11	14.5	7	14.3	2	11.8	0	0.0	0.424
Extremities:											
Short fifth fingers	23	21.3	24	31.6	23	46.9	4	23.5	4	30.8	**0.026**
Back:											
Kyphosis	5	4.6	11	14.5	17	34.7	4	23.5	7	53.8	**<0.05**
Abdomen:											
Abdominal (central) distribution of fat	44	40.7	53	69.7	32	65.3	11	64.7	12	92.3	**<0.05**

3.4 | Comparison of effect of GH treatment on specific PWS molecular genetic classes

Analysis of effects of GH treatment was also undertaken for each individual molecular genetic class separately (Table 5). The duration of GH treatment was also calculated based on the reported age at initiation of GH treatment, if at first visit the participant was on GH or whether they were currently on GH, as well as at their current age, or age when discontinued. The mean age of starting GH treatment was 4 ± 0.4 years (range from birth to 49 years) with an average duration of 13 ± 0.8 years (range from birth to 53 years) with no significant differences in the two groups. Similar trends were noted in the molecular classes; individuals with the deletion versus UPD on GH treatment or non-GH treatment had a higher incidence of hypopigmented hair, or fairer hair color than their family members (p = 0.029). There were no differences in the frequency of deletion or UPD participants on GH treatment or not on GH treatment or age difference found in the two molecular class groups. Other findings that showed differences when comparing effects of GH treatment on deletion versus UPD are included in Table 5. There was a greater weight percentile (p = 0.021); hypopigmentation (p = 0.030), a higher incidence of lower anterior hair line (p = 0.046); almond-shaped eyes (p = 0.023), dental caries (p = 0.007) and kyphosis (p = 0.001) in the deletion group without GH treatment; and a higher incidence of abdominal striae (p = 0.006), hypopigmentation (p < 0.001), scoliosis (p = 0.011) and interestingly flattened occiput (p = 0.002), in the deletion group on GH treatment. Interestingly the UPD group had a higher incidence of scoliosis (p = 0.039), and broad nasal bridge (p = 0.037) in those not on GH treatment; and more downslanting fissures (p = 0.006) and posteriorly angulated ears (p = 0.002) in the group on GH treatment.

TABLE 5 Effect of growth hormone (GH) treatment on PWS molecular classes

	GH treatment (N = 282)					No GH treatment (N = 62)				
	Deletion (M = 89, F = 92)		UPD (M = 41, F = 60)			Deletion (M = 10, F = 26)		UPD (M = 12, F = 14)		
Physical characteristics	N = 179	Mean (SD)	N = 103	Mean (SD)	p-value	N = 38	Mean (SD)	N = 24	Mean (SD)	p-value
Growth parameters:										
Height percentile for age and gender	139/282	35 (30)	78	42 (34)	0.228	38/62	40 (35)	24	21 (26)	0.124
Weight percentile for age and gender	139/282	62 (35)	78	58 (36)	0.471	38/62	61 (36)	24	30 (34)	**0.021**
Head circumference percentile for age and gender	130/282	44 (31)	75	51 (30)	0.197	38/62	44 (29)	24	42 (31)	0.827
BMI percentile for age and gender	139/282	83 (38)	78	82 (37)	0.895	38	86 (48)	24	83 (42)	0.748
Head:										
Prominent occiput	31	17%	19	18%	0.547	7	18%	7	29%	0.382
Round face	56	31%	22	21%	0.083	13	34%	10	41%	0.657
Flat occiput	49	27%	12	12%	**0.002**	10	26%	6	25%	0.837
Bitemporal narrowing	116	65%	61	59%	0.143	34	89%	21	87%	0.882
Craniosynostosis	2	1%	0		0.285	2	5%	1	4%	0.914
Hair:										
Hypopigmented	69	38%	16	16%	**<0.001**	17	45%	3	13%	**0.030**
Low-anterior hair line	41	23%	15	20%	0.233	16	42%	4	16%	**0.046**
Low-posterior hair line	64	36%	35	47%	0.638	18	47%	8	33%	0.250
Eyes:										
Inter-canthal distance percentile for age and gender	129	54 (33)	70	57 (31)	0.585	38	46 (26)	24	63 (30)	0.909
Inter-pupillary distance percentile for age and gender	139	57 (35)	78	56 (37)	0.923	38	40 (31)	24	32 (38)	0.626
Outer-canthal distance percentile for age and gender	139	58 (38)	77	50 (38)	0.345	38	40 (31)	24	43 (38)	0.864
Palpebral fissure length percentile for age and gender	139	55 (37)	78	63 (37)	0.339	38	40 (36)	24	59 (40)	0.279
Almond shaped	99	55%	53	51%	0.245	30	70%	12	50%	**0.023**
Strabismus	63	35%	54	52%	**0.011**	21	55%	13	54%	0.461
Esotropia	64	36%	41	40%	0.734	17	45%	8	33%	0.157
Exotropia	6	3%	8	7%	0.187	1	2%	2	8%	0.454
Ptosis	19	12%	12	10%	0.872	6	16%	5	21%	0.666
Hypotelorism	8	4%	12	16%	0.276	6	16%	3	13%	0.200
Hypertelorism	8	4%	2	3%	0.330	3	7%	2	8%	0.209
Telecanthus	9	5%	7	9%	0.968	4	10%	2	8%	0.656
Upslanting palpebral fissures	43	24%	18	18%	0.345	14	36%	5	21%	0.165
Downslanting palpebral fissures	9	5%	14	14%	**0.006**	4	10%	2	8%	0.383
Nose:										
Broad nasal bridge	15	8%	11	15%	0.733	0	0%	4	16%	**0.037**
Narrow nasal bridge	31	17%	13	17%	0.580	2	5%	5	21%	0.344
Mouth:										
Flat philtrum	43	24%	25	33%	0.974	7	18%	6	25%	0.823
Thin upper lip	91	51%	50	66%	0.866	28	73%	11	46%	0.098
Full upper lip	20	11%	13	17%	0.918	4	10%	1	4%	0.311

TABLE 5 (Continued)

	GH treatment (N = 282)					No GH treatment (N = 62)				
	Deletion (M = 89, F = 92)		UPD (M = 41, F = 60)			Deletion (M = 10, F = 26)		UPD (M = 12, F = 14)		
Physical characteristics	N = 179	Mean (SD)	N = 103	Mean (SD)	p-value	N = 38	Mean (SD)	N = 24	Mean (SD)	p-value
Wide-spaced dentition	57	32%	23	31%	0.259	14	37%	6	25%	0.061
Dental caries	34	19%	17	23%	0.639	7	18%	3	13%	**0.007**
Enamel hypoplasia	68	38%	36	48%	0.922	16	42%	6	25%	0.061
Ears:										
Ear length percentile for age and gender	139	48 (32)	78	61 (25)	0.074	38	51 (26)	24	41 (24)	0.376
Low-set ears	22	12%	18	19%	0.252	7	18%	1	4%	0.246
Posterior angulated ears	4	3%	12	14%	**0.002**	3	7%	1	4%	0.731
Chest:										
Pectus excavatum	30	17%	22	21%	0.521	0	0%	0	0%	0.471
Pectus carinatum	4	2%	2	2%	0.321	2	5%	3	12%	0.572
Abdomen:										
Abdominal striae	69	39%	22	21%	**0.006**	14	37%	10	41%	0.437
Spine:										
Scoliosis	65	47%	29	37%	**0.011**	31	82%	21	88%	**0.039**
Kyphosis	25	18%	13	17%	0.509	13	34%	4	16%	**0.001**
Genitalia: (N = 137 males; N = 152 females)										
Bilateral cryptorchidism	65	73%	17	41%	0.635	9	90%	11	92%	0.074
Hypoplastic scrotum	39	44%	22	53%	0.452	7	70%	3	25%	0.128
Poor scrotal rugae	27	30%	15	37%	0.747	5	50%	3	25%	0.749
Hypoplastic labia minora	45	49%	34	85%	0.513	8	31%	3	21%	0.416
Hypoplastic clitoris	39	42%	25	62%	0.721	9	35%	2	14%	0.218

4 | DISCUSSION

The aim of our study was to analyze differences in phenotypic features seen in PWS between the two main PWS molecular classes and the effect of GH treatment on physical characteristics or dysmorphism. This study was based on the largest dataset to date consisting of 355 PWS participants whose phenotypical features were collected using standard forms and measures at four USA sites by PWS experts and trained dysmorphologists. Our study found that individuals with the 15q11-q13 deletion were heavier, had a smaller HC with a flattened occiput, were hypopigmented, had less strabismus, lower anterior hair line, less downslanting palpebral fissures, but more almond-shaped eyes, more dental problems, less posteriorly angulated ears, more abdominal striae, and shorter fifth fingers. However, no statistical differences in height between the two molecular classes were found. In another study of 64 individuals with PWS, participants with deletions were also heavier, and had smaller HCs, but were taller.[22]

Our results are consistent with previous studies regarding a higher prevalence of characteristic facial features, including almond-shaped palpebral fissures, a narrow nasal bridge, and downturned mouth in individuals with the 15q11-q13 deletion when compared to UPD.[1,3,23,24] We also noted a higher incidence of abnormal dentition, low-anterior hairline, shorter fifth finger, nail abnormalities, larger thighs, abdominal striae, hypoplastic labia minora, hypoplastic clitoris, and more facial skin picking in those with UPD. Not surprisingly, individuals with the deletion were more likely to have fair skin and hair than their family members[13] compared to UPD, attributed to loss of a single copy of the *OCA2* albinism gene in the 15q11-q13 region due to the deletion process.[21] Individuals with UPD were noted to have an increased incidence of hypoplastic female genitalia, almond-shaped eyes, and more skin picking in the face region. More atypical presentations were also found in the UPD group. Involvement of abnormal maternal recessive gene alleles due to cross-over events in maternal meiosis with loss of heterozygosity and isodisomy of chromosome 15 regions may be present in those with UPD accounting for more variable presentation of clinical findings, behavior, and a later diagnosis.[11]

Surprisingly, we found that only 41% of individuals started GH treatment under the age of 1 year, 29% between the ages of 1–4 years, and 30% started GH over the age of 4 years. We studied the effects of GH on the incidence of dysmorphic features,

understandably, those individuals who received GH treatment were taller, had larger HCs, and had longer hand lengths compared to the untreated PWS cohort participants in this study. Also, individuals who had GH treatment initiated at a younger age had lower incidences of micrognathia, slit-like eyes, narrow nasal bridge, abdominal distribution of fat, kyphosis and short fifth fingers. However, the duration of GH treatment had no significant effect on the frequencies or types of dysmorphic features. As in prior orthopedic reports,[25-27] we did see differences in the overall incidence of more scoliosis in the deletion group in the GH treatment, more scoliosis in the UPD group without GH treatment and more kyphosis in the deletion group without GH treatment as analysis of effects of GH treatment was done for each individual molecular class separately for each clinical variable. GH treated individuals in the deletion group had a more flattened occiput, hypopigmented or fairer hair, abdominal striae, or scoliosis. In contrast, the GH treated individuals in the UPD group had a higher incidence of hypoplastic labia majora and clitoris, downslanting eyes and strabismus. We also found that the overall incidence of scoliosis in patients on GH was significantly higher in the deletion group compared to UPD in our study.

In summary, PWS is a relatively rare condition and the strength of our study lies in the large size of the cohort increasing the power to show statistically significant PWS genetic subtype-phenotype correlations. We also found that GH treatment had different influences among the molecular classes as described in our report, but the authors encourage further studies to examine the effects of GH treatment in PWS and whether GH treatment which improves stature and foot size may also impact on more subjective characteristics such as facial features, both positively or negatively, and possibly other PWS findings or dysmorphic changes.

ACKNOWLEDGMENTS

The authors acknowledge the gracious participation and provision of information provided by the families in the Prader-Willi Syndrome Rare Disease Natural History Study which was funded by NIH U54 grants HD061222 and RR019478, as well as the Prader-Willi Syndrome Association | USA. We thank UCI's Institute for Clinical and Translational Science, which was funded by a grant from the NIH Clinical and Translational Sciences Award program (UL1 TR000153). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.

CONFLICT OF INTEREST

There is no conflict of interest reported by the authors.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/cge.13947.

DATA AVAILABILITY STATEMENT

Data are available upon request

ORCID

Merlin G. Butler https://orcid.org/0000-0002-2911-0524
Virginia Kimonis https://orcid.org/0000-0003-1567-4449

REFERENCES

1. Butler MG, Meaney FJ, Palmer CG. Clinical and cytogenetic survey of 39 individuals with Prader-Labhart-Willi syndrome. *Am J Med Genet*. 1986;23(3):793-809.
2. Butler MG. Prader-Willi syndrome: current understanding of cause and diagnosis. *Am J Med Genet*. 1990;35(3):319-332.
3. Cassidy SB. Prader-Willi syndrome. *J Med Genet*. 1997;34(11):917-923.
4. Butler MG, Lee PDK, Whitman BY. *Management of Prader-Willi Syndrome*. 3rd ed. New York: Springer-Verlag; 2006.
5. Cassidy SB, Driscoll DJ. Prader-Willi syndrome. *Eur J Hum Genet*. 2009;17(1):3-13.
6. Angulo MA, Butler MG, Cataletto ME. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. *J Endocrinol Invest*. 2015;38(12):1249-1263.
7. Butler MG, Miller JL, Forster JL. Prader-Willi syndrome - clinical genetics, diagnosis and treatment approaches: an update. *Curr Ped Rev*. 2019;15(4):207-244.
8. Driscoll DJ, Miller JL, Schwartz S, et al. Prader-Willi Syndrome. 1998 Oct 6 [Updated 2017 Dec 14]. In: Adam MP, Ardinger HH, Pagon RA, et al., eds. *GeneReviews®* [Internet]. Seattle (WA): University of Washington; 1993-2018. https://www.ncbi.nlm.nih.gov/books/NBK1330.
9. Unanue N, Bazaes R, Iniguez G, Cortes F, Avila A, Mericq V. Adrenarche in Prader-Willi syndrome appears not related to insulin sensitivity and serum adiponectin. *Horm Res*. 2007;67(3):152-158.
10. Grugni G, Marzullo P. Diagnosis and treatment of GH deficiency in Prader-Willi syndrome. *Clin Endocrin Metab*. 2016;30(6):785-794.
11. Butler MG, Hartin SN, Hossain WA, et al. Molecular genetic classification in Prader-Willi syndrome: a multisite cohort study. *J Med Genet*. 2019;56(3):149-153.
12. Prader A, Labhart H. Willi Ein syndrom von adipositas, kryptorchismus und oligophrenie nach myatonieartigem zustand in neugeborenenamter. *Schweiz Med Wochenschr*. 1956;86:1260-1261.
13. Butler MG. Hypopigmentation: a common feature of Prader-Labhart-Willi syndrome. *Am J Hum Genet*. 1989;45(1):140-146.
14. Bakker NE, Lindberg A, Heissler J, et al. Growth hormone treatment in children with Prader-Willi syndrome: three years of longitudinal data in prepubertal children and adult height data from the KIGS database. *J Clin Endocrinol Metab*. 2017;102(5):1702-1711.
15. Butler MG, Smith BK, Lee J, et al. Effects of growth hormone treatment in adults with Prader-Willi syndrome. *Growth Horm IGF Res*. 2013;23(3):81-87.
16. Butler MG, Matthews NA, Patel N, et al. Impact of genetic subtypes of Prader-Willi syndrome with growth hormone therapy on intelligence and body mass index. *Am J Med Genet A*. 2019;179(9):1826-1835.
17. Butler MG, Kimonis V, Dykens E, et al. Prader-Willi syndrome and early-onset morbid obesity NIH rare disease consortium: a review of natural history study. *Am J Med Genet A*. 2018;176(2):368-375.
18. Grummer-Strawn LM, Reinold C, Krebs NF, Centers for Disease Control and Prevention (CDC). Use of World Health Organization and CDC growth charts for children aged 0-59 months in the United States. *MMWR Recomm Rep*. 2010;59(RR-9):1-15.
19. Growth Charts - 2000 CDC Growth Charts - United States. CDC.Gov, 2021, https://www.cdc.gov/growthcharts/cdc_charts.htm.
20. Leonenko A. Analysis of phenotypic features in a large cohort of individuals with Prader-Willi Syndrome: differences between gender, molecular type, growth hormone exposure in various age groups. eScholarship, University of California, 2018.
21. Spritz RA, Bailin T, Nicholls RD, et al. Hypopigmentation in the Prader-Willi syndrome correlates with P gene deletion but not with haplotype of the hemizygous P allele. *Am J Med Genet*. 1997;71(1):57-62.

22. Oldzej J, Manazir J, Gold JA, et al. Molecular subtype and growth hormone effects on dysmorphology in Prader-Willi syndrome. *Am J Med Genet A*. 2020;182(1):169-175.
23. Cassidy SB, Forsythe M, Heeger S, et al. Comparison of phenotype between patients with Prader-Willi syndrome due to deletion 15q and uniparental disomy 15. *Am J Med Genet*. 1997;68(4):433-440.
24. Gillessen-Kaesbach G, Robinson W, Lohmann D, Kaya-Westerloh S, Passarge E, Horsthemke B. Genotype-phenotype correlation in a series of 167 deletion and non-deletion patients with Prader-Willi syndrome. *Hum Genet*. 1995;96(6):638-643.
25. Nagai T, Obata K, Ogata T, et al. Growth hormone therapy and scoliosis in patients with Prader-Willi syndrome. *Am J Med Genet A*. 2006;140(15):1623-1627.
26. Murakami N, Obata K, Abe Y, et al. Scoliosis in Prader-Willi syndrome: effect of growth hormone therapy and value of paravertebral muscle volume by CT in predicting scoliosis progression. *Am J Med Genet A*. 2012;158A(7):1628-1632.
27. Nakamura Y, Murakami N, Iida T, et al. The characteristics of scoliosis in Prader-Willi syndrome (PWS): analysis of 58 scoliosis patients with PWS. *J Orthop Sci*. 2015;20(1):17-22.

How to cite this article: Mahmoud R, Leonenko A, Butler MG, et al. Influence of molecular classes and growth hormone treatment on growth and dysmorphology in Prader-Willi syndrome: A multicenter study. *Clinical Genetics*. 2021;100:29–39. https://doi.org/10.1111/cge.13947