Evidence for Accelerated Biological Aging in Young Adults with Prader–Willi Syndrome

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Objective: Adults with Prader–Willi syndrome (PWS) are at increased risk of developing age-associated diseases early in life and, like in premature aging syndromes, aging might be accelerated. We investigated leukocyte telomere length (LTL), a marker of biological age, in young adults with PWS and compared LTL to healthy young adults of similar age. As all young adults with PWS were treated with growth hormone (GH), we also compared LTL in PWS subjects to GH-treated young adults born short for gestational age (SGA).

Design: Cross-sectional study in age-matched young adults; 47 with PWS, 135 healthy, and 75 born SGA.

Measurements: LTL measured by quantitative polymerase chain reaction, expressed as telomere/single copy gene ratio.

Results: Median (interquartile range) LTL was 2.6 (2.4–2.8) at a median (interquartile range) age of 19.2 (17.7–21.3) years in PWS, 3.1 (2.9–3.5) in healthy young adults and 3.1 (2.8–3.4) in the SGA group. Median LTL in PWS was significantly lower compared to both control groups ($P < .01$). In PWS, a lower LTL tended to be associated with a lower total IQ ($r = 0.35$, $P = .08$). There was no association between LTL and duration of GH treatment, cumulative GH dose, or several risk factors for type 2 diabetes mellitus or cardiovascular disease.

Conclusions: Young adults with PWS have significantly shorter median LTL compared to age-matched healthy young adults and GH-treated young adults born SGA. The shorter telomeres might play a role in the premature aging in PWS, independent of GH. Longitudinal research is needed to determine the influence of LTL on aging in PWS. **(*J Clin Endocrinol Metab* 105: 2053–2059, 2020)**

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Prader–Willi syndrome (PWS) is a rare disorder resulting in a variable phenotype with muscular hypotonia and failure to thrive during infancy and short stature, mental retardation, hyperphagia, and obesity in childhood and adulthood (1,2). PWS is caused by a lack of expression of the PWS region (q11–q13) on the paternally derived chromosome 15, mostly caused

by a paternal deletion or maternal uniparental disomy (mUPD) and in some cases by an imprinting center defect (ICD) or paternal chromosomal translocation (1,3).

Growth hormone (GH) is an approved treatment for children with PWS improving body composition, linear growth, physical strength, cognition, and adaptive functioning (4–9). Studies have shown that GH treatment also benefits adults with PWS, with a sustained improvement in body composition when GH is continued after attainment of adult height (10,11). However, up to now GH treatment is not registered for adults with PWS. Studies in adults with PWS who were not treated with GH describe an increased risk of age-associated diseases early in life, eg, diabetes mellitus type 2 (T2DM), cardiovascular disease (CVD), and cognitive decline (12,13). The mortality rate of people with PWS was estimated to be 3% per year across all ages, rising to 7% in those aged over 30 (14). One explanation for the increased mortality rate and risk of age-associated diseases could be that, like in premature aging syndromes, the aging process is accelerated in PWS (15–18).

Ageing is characterized by a progressive time-dependent decline of normal tissue and organ function and recent studies have shown that telomere shortening is involved in this process (19–22). Telomeres are highly conserved TTAGGG tandem repeat deoxyribonucleic acid (DNA) sequences at the end of each chromosome arm. Their main function is to protect the end of the chromosomes from inappropriate DNA repair mechanisms, preventing the loss of crucial DNA. Telomeres shorten during proliferation, and telomere length declines as a function of chronological age. When telomere length becomes critically short, the cell enters either senescence (ie, irreversible cessation of division) or apoptosis (ie, programmed cell death). The accumulation of senescent cells might be driving the process of tissue and organismal aging (21–23).

We hypothesized that accelerated biological aging could partly explain the increased mortality rate and increased risk of developing age-associated diseases early in life in adults with PWS. Since telomeres are suggested to play a role in biological aging and telomere length had not yet been studied in people with PWS, we investigated leukocyte telomere length (LTL) in young adults with PWS and compared LTL to healthy young adults of similar age. As all young adults who participated in this study were treated with GH, we also investigated LTL in young adults born short for gestational age (SGA) who were also treated with GH. We hypothesized that LTL would be shorter in young adults with PWS compared to both groups, independent of GH treatment. Finally, we assessed whether cognitive functioning and putative risk factors for T2DM and CVD correlated with LTL.

Subjects and Methods

Subjects

We included 47 young adults participating in the Dutch Young Adult PWS (YAP) study, whose primary objective was to evaluate the effects and safety of GH treatment in young adults with PWS who were treated with GH during childhood. Inclusion criteria were (i) genetically confirmed diagnosis of PWS by a positive methylation test, (ii) at least 2 years of GH treatment during childhood, and (iii) having attained adult height, defined as a height velocity less than 0.5 cm per 6 months and epiphyseal closure as demonstrated by a radiograph of the left hand and wrist. Exclusion criteria were (i) medication to reduce weight (fat) or (ii) uncooperative behavior.

We compared LTL in GH-treated young adults with PWS to healthy participants from the PROGRAM cohort (24) and to young adults born SGA of similar age treated with ≥7 years of GH (1 mg/m^2/day) because of their short stature (25). The healthy participants from the PROGRAM cohort were all (i) 17 to 24 years old, (ii) born singleton, (iii) caucasian, and (iv) had an uncomplicated neonatal period without severe asphyxia, sepsis, or long-term complications of respiratory ventilation and/or oxygen supply. The Medical Ethics Committee of the Erasmus University Medical Center approved the study protocols. Written informed consent was obtained from patients and/or their parents or legal representatives.

Anthropometric measurements, body composition, and cognitive functioning

All patients with PWS were followed at the Dutch PWS Reference Center and treated with biosynthetic GH (Pfizer Inc., New York, NY, US) during childhood in a dose of 1.0 mg/m^2/day (≈0.035 mg/kg/day) and, at the time of LTL measurement, in a dose of 0.33 mg/m^2/day (≈0.012 mg/kg/day). The GH dose was regularly adjusted based on calculated body surface area and serum insulin-like growth factor 1 levels. Standing height was measured using a Harpenden Stadiometer and weight on a calibrated electric scale (ServoBalance KA-20-150S). Height, weight, and body mass index (BMI) were expressed as standard deviation score (SDS) using GrowthAnalyser 4.0 (available at www.growthanalyser.org), adjusting for age and sex according to Dutch reference values (26,27).

Systolic and diastolic blood pressure was measured using an appropriately sized cuff while sitting and expressed as SDS, adjusting for height and sex (28). Body composition was assessed by dual energy X-ray absorptiometry (DXA; Lunar Prodigy, GE Healthcare, Chalfont St Giles, UK). Total fat mass (FM; kg) and

lean body mass (LBM; kg) were assessed. All scans were made on the same machine, with daily quality assurance. The intra-assay coefficient of variation for fat tissue was 0.41 to 0.88% and for LBM, 1.57 to 4.49% (29). FM was also expressed as percentage of total body weight (FM%). LBM was calculated as fat-free mass minus bone mineral content. FM% SDS was calculated according to age- and sex-matched Dutch reference values and LBM SDS according to height- and sex-matched Dutch reference values (30). The Wechsler Adult Intelligence Scale was used to assess total IQ in patients over 16 years of age (31) and the Wechsler Intelligence Scale in patients below 16 years of age (32).

Laboratory measurements

Blood samples were collected after overnight fasting. Genomic DNA was isolated from peripheral leukocytes using standard procedures. DNA samples were kept frozen at –20°C until assayed. All LTL measurements were performed in the same laboratory at the University of Leicester (Leicester, UK). LTL was measured by the quantitative polymerase chain reaction–based technique as previously described (33–35). Telomere sequence copy number (T) and single copy gene number in genome 36B4 (S) were measured in separate reactions and calculated relative to a calibrator sample (genomic DNA from K562 cell line) included on each run. Leukocyte telomere length was subsequently expressed as T/S ratio. For quality control, all samples were checked for concordance between duplicate values and samples with >0.2 cycle difference in take-off value were excluded and rerun. Samples which amplified outside of the linear range of the assay were also excluded and rerun. Reproducibility of the assay was tested by rerunning 47 samples of the age-matched healthy participants and the SGA group together with the PWS samples (36). The correlation between the original and new LTL results was 0.87, and the mean coefficient of variation, 6.4%.

Blood levels of glucose, insulin, total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol, and triglyceride were determined in the Biochemical and Endocrine laboratories of the Erasmus Medical Center (Rotterdam, The Netherlands).

Statistical analysis

Statistical analyses were performed with SPSS 24.0 (SPSS Inc., Chicago, IL, US). LTL was expressed as T/S ratio and data as median (interquartile range [IQR]). Continuous variables of young adults with PWS, healthy young adults, and young adults born SGA were compared using Kruskal–Wallis and Mann–Whitney U tests, and categorical variables were compared using Chi-square tests. Analysis of covariance was used to correct for age, gender, gestational age, birth length, and birth weight SDS.

In young adults with PWS, gender and genotypic differences in clinical characteristics were calculated by independent samples *t* tests in case of a Gaussian distribution and by Mann–Whitney U tests in case of a non-Gaussian distribution. Correlations between LTL and anthropometric measurements, body composition, cognitive functioning, and metabolic health parameters were calculated by Pearson correlation analysis in case of a normal distribution and by Spearman correlation analysis in case of a non-Gaussian distribution. *P* values less than .05 were considered statistically significant.

Results

Clinical characteristics

Forty-seven young adults with PWS (24 females) with a median (IQR) age of 19.2 (17.7–21.3) years participated in the current evaluation of LTL (Table 1). We compared LTL to 135 age-matched healthy participants (71 females; median age 20 years) and to 75 young adults born SGA (33 females; median age 20 years) who were treated with GH during childhood because of their short stature. The distribution of males and females was similar between the 3 groups. Gestational age, BMI, and FM% in the PWS group were significantly higher and LBM significantly lower compared to the healthy participants and the SGA group ($P < .02$). Age, birth weight, and birth length SDS and adult height were lower in young adults with PWS compared to healthy participants ($P < .04$). Compared to the SGA group, birth weight, birth length, adult height SDS, and duration of GH treatment were higher in young adults with PWS ($P < .02$), and age was similar ($P = .47$).

Telomere length in PWS and control groups

Median (IQR) LTL was 2.6 (2.4–2.8) in the PWS group (Fig. 1). Forty-four young adults with PWS (94%) had an LTL below the 50th percentile of healthy young adults and 20 (43%) below the 10th percentile. The healthy age-matched young adults and the young adults born SGA had a similar median (IQR) LTL, which were 3.1 (2.9–3.5) and 3.1 (2.8–3.4), respectively (Fig. 1). Median LTL was significantly lower in young adults with PWS compared to both control groups ($P < .01$), also after correction for age, gender, gestational age, birth weight, and birth length SDS.

Table 1. Clinical characteristics of young adults with PWS, healthy young adults, and young adults born SGA

	PWS	Healthy	SGA
Men/women (n)	23/24	64/71	42/33
Genetic subtype			
Deletion/mUPD/ICD[a]	21/22/3/1	NA	NA
Gestational age (weeks)	40.6[b] (38.9; 41.7)	37.0 (33.0; 40.0)	37.4 (33.0; 40.0)
Birth weight (SDS)	–1.2[b] (–2.1; –0.1)	0.4 (–0.4; 1.0)	–2.7 (–3.3; –1.6)
Birth length (SDS)	–1.1[b] (–2.2; –0.2)	0.1 (–0.4; 0.7)	–3.1 (–4.5; –2.3)
Age (yrs)	19.2[c] (17.7; 21.3)	20.0 (19.0; 22.0)	20.0 (18.0; 21.7)
Height (SDS)	–1.0[c,d] (–1.8; –0.3)	0.00 (–0.4; 0.7)	–1.5 (–2.0; –0.8)
BMI (kg/m^2)	25.0[b] (21.8; 27.5)	21.8 (20.5; 23.4)	20.2 (18.8; 22.1)
BMI for age (SDS)	1.2[b] (0.0; 1.8)	–0.1 (–0.6; 0.6)	–0.9 (–1.4; 0.1)
Duration of GH treatment (years)	13.0[d] (11.5; 14.0)	NA	9.2 (7.1; 11.0)
Fat percentage (%)	39.8[b] (36.0; 43.6)	22.4 (14.3; 31.4)	18.9 (11.6; 27.8)
Fat percentage (SDS)	2.2[b] (1.8; 2.5)	0.6 (–0.1; 1.1)	0.5 (–0.1; 1.1)
Lean mass (SDS)	–1.3[c,d] (–2.1; –0.5)	–0.6 (–1.3; 0.1)	–0.7 (–1.6; 0.1)
Telomere length (T/S ratio)	2.6[b] (2.4; 2.8)	3.1 (2.9; 3.5)	3.1 (2.8; 3.4)

Data expressed as median (interquartile range).
Abbreviations: BMI, body mass index; mUPD = maternal uniparental disomy; ICD = imprinting center defect; PWS, Prader–Willi syndrome; SDS, standard deviation score; SGA, short for gestational age; T/S ratio, telomere/single copy gene ratio.
[a]genotype unknown.
[b]$P < 0.01$ compared to healthy young adults and SGA group.
[c]$P < 0.04$ compared to healthy young adults.
[d]$P < 0.02$ compared to SGA group.

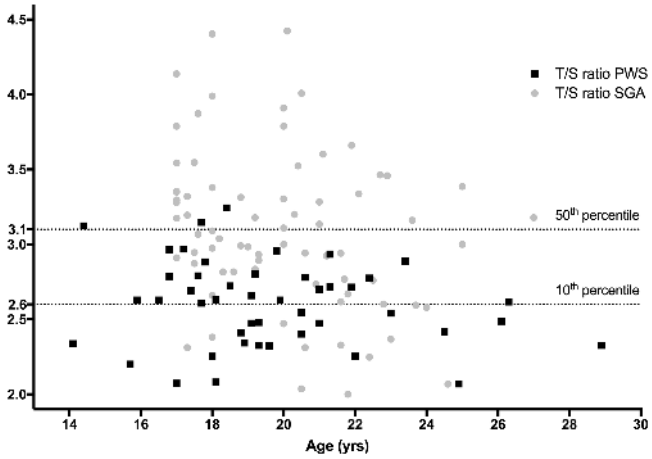

Figure 1. Leukocyte telomere length for age in 47 GH-treated young adults with PWS and 75 young adults born SGA who were also treated with GH. The dotted lines represent the 10th and 50th percentile of the healthy young adults. Forty-four young adults with PWS (94%) have a LTL below the 50th percentile and 20 (43%) below the 10th percentile of the healthy young adults.

Correlation analyses in PWS

Age was not significantly associated with LTL ($r = –0.18$, $P = .22$), which could be related to the fact that subjects in our study population had approximately the same age. There was no significant difference in LTL between males and females; median (IQR) LTL was 2.5 (2.3–2.8) in males and 2.7 (2.4–2.8) in females with PWS ($P = .35$). Median (IQR) LTL was similar in young adults with a deletion ($n = 21$) and a mUPD/ICD ($n = 25$): 2.6 (2.4–2.8) in both groups ($P = .97$).

There was no significant association between LTL and gestational age, birth weight, birth height, adult height, BMI, or FM% ($P > .28$). A lower LTL tended to be associated with a lower LBM SDS and a lower total IQ ($r = 0.29$, $P = .06$ and $r = 0.36$, $P = .08$, respectively), showing that LBM SDS and cognitive functioning in young adults with PWS tend to be lower in those with a shorter LTL. Since GH treatment could potentially cause increased replicative stress by inducing catch-up growth, we analyzed whether LTL was associated with the duration of GH treatment or the cumulative GH

Table 2. Risk factors CVD and T2DM

Risk factor	
Systolic blood pressure (SDS)	0.8 (0; 1.6)
Diastolic blood pressure (SDS)	0.6 (0.3; 1.2)
Total Cholesterol (mmol/L)	4.3 (3.9; 4.8)
HDL (mmol/L)	1.3 (1.1; 1.5)
LDL (mmol/L)	2.8 (2.3; 3.2)
Triglycerides (mmol/L)	0.8 (0.7; 1.2)
HOMA-IR	1.7 (1.2; 2.9)

Data expressed as median (interquartile range).
Abbreviations: HDL, high-density lipoprotein cholesterol. HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; LDL, low-density lipoprotein cholesterol; SDS, standard deviation score.

dose, but we found no significant association between either. Table 2 shows metabolic health parameters in young adults with PWS. Neither blood pressure SDS and serum cholesterol levels, nor Homeostatic Model Assessment for Insulin Resistance were associated with LTL ($P > .21$).

Discussion

This cross-sectional study in 47 GH-treated young adults with PWS shows that median leukocyte telomere length (LTL) is shorter in young adults with PWS compared to age-matched healthy young adults and young adults born SGA who were also treated with GH. We found no significant association between LTL and the duration of GH treatment or the cumulative GH dose. This is the first study to show that a shorter LTL might play a role in the reported accelerated aging process in adults with PWS, independent of GH treatment (15–17). Sinnema et al described excess functional impairment, morbidity and mortality, and evidence of premature aging in 12 adults with PWS above the age of 50 years (17). Aging is characterized by a progressive time-dependent functional decline of tissues and telomere shortening is considered to be involved in this process (20,21). Individuals with shorter telomere length show increased mortality risk, providing support for an association between telomere length and lifespan (37).

Besides the finding that LTL is shorter in young adults with PWS, we found a tendency toward an association between a shorter LTL and a lower total IQ, which might imply a role of LTL in cognitive functioning in PWS. A cross-sectional MRI-study in 20 young adults with PWS showed that predicted brain age was, on average, 8.7 years higher than chronological age (38). Together with a brain structure resembling healthy older brains, indicative of premature neuronal loss and atrophy, this suggests premature brain aging in young adults with PWS (38). The same research group found a 2.5-year higher predicted brain age than chronological age in 46 adults with Down syndrome. A higher brain-predicted age was significantly associated with lower cognitive functioning (39), and shorter telomeres have been associated with cognitive decline and dementia status in patients with Down syndrome (40,41). These studies support that people with PWS and Down syndrome age prematurely. Our study suggests a possible role of shorter LTL in this accelerated aging process in adults with PWS. However, longitudinal studies on LTL, cognitive functioning and brain development in a larger cohort of children and (older) adults with PWS are needed to elucidate the natural course of brain development in relation to LTL and cognitive functioning.

There is a strong correlation between telomere length in different tissues in humans and in other mammals, which shows that telomere length in leukocytes reflects systemic telomere length in other tissues (42). Besides chronological aging, a wealth of genetic and environmental factors are reported to affect telomere length (22). Even though none of the genes in the PWS region on chromosome 15 are associated with telomere homeostasis, several clinical features of people with PWS are associated with an increased risk of shorter telomeres, including obesity, a reduced level of physical activity, and increased psychosocial stress. Furthermore, adults with PWS are prone to develop T2DM and CVD in early life (12,13), and several studies have shown that shortened telomeres are associated with an increased risk of T2DM and CVD (20,22,37). As none of the investigated young adults with PWS were diagnosed with T2DM and CVD, we investigated the interaction between LTL and putative risk factors for T2DM and CVD. We found no significant correlation between LTL and age, BMI, FM%, blood pressure, serum cholesterol levels, and Homeostatic Model Assessment for Insulin Resistance. This is in accordance with an earlier study demonstrating that the association between LTL and CVD is independent of risk factors for CVD (35). Probably our study group of young adults with PWS was too young to already have T2DM or CVD. Also, with the more recent trends of early diagnosis, GH treatment from a young age, and the enhanced prevention of potentially impairing health conditions, T2DM and CVD might occur later in life. It would be interesting to analyze the association between (risk factors for) T2DM and CVD and LTL at a later age, when age-associated diseases become more apparent.

All young adults with PWS who participated in the current study were treated with long-term GH improving body composition, linear growth, physical strength, cognition, and adaptive functioning (4–9). To evaluate whether GH treatment would cause increased

replicative stress and shorter LTL, we compared young adults with PWS to young adults born SGA who were also treated with GH. The fact that LTL in young adults with PWS was shorter compared to both groups and similar in healthy young adults and GH-treated young adults born SGA make adverse effects of long-term GH treatment on LTL very unlikely (36). Besides, the lack of an association between LTL and the duration of GH or the cumulative GH dose in young adults with PWS is reassuring with regard to possible negative effects of GH on LTL.

One could even argue that GH treatment might positively influence LTL by improving body composition. We know that long-term GH treatment during childhood counteracts the clinical course of increasing obesity in children with PWS and has substantially changed their phenotype (4). Combined with an early diagnosis and multidisciplinary support from a very young age, GH treatment has resulted in a new generation of children and young adults with PWS without severe obesity. Thus, it could be that if our group of young adults had not been treated with GH, they would have had a higher BMI and FM%, and their LTL could have been even shorter. The limited age range and Dutch origin of our PWS cohort is both a strength and a constraint of our study, since it eliminates the confounding effect of age and nationality, but restricts generalizations of the results to other age groups and nationalities. Also, we were not able to compare LTL in GH-treated young adults with PWS to (young) adults with PWS who were not treated with GH and more research is needed on LTL across the life course and in people of different origin to be able to determine its role in aging for both GH-treated and untreated people with PWS.

In contrast to previous studies, we found no significant difference in median LTL between males and females. Females have been reported to have longer LTL, which is thought to be due to higher levels of estrogen, conferring anti-inflammatory and antioxidant properties and promoting telomerase expression (22). Since serum estrogen levels are generally low in females with PWS, this could explain the lack of a difference between males and females in our study (43). We also found similar LTL in young adults with a deletion and those with a mUPD or ICD. A recent study by Whittington et al reported on 26 adults with PWS over the age of 40 years. In 3 participants (aged 41, 48, and 55), there was a significant deterioration in executive functioning with possible dementia. All were women, had UPD or a disomic region, and had had psychotic episodes (18). Psychiatric disorders are reported more commonly in people with a mUPD and a higher level of psychosocial stress is associated with shorter telomeres (22). Based on these studies, a difference in LTL between the deletion and mUPD subtypes was expected. However, since the study group of Whittington et al is considerably older than our group and none of the affected individuals were treated with GH, it is difficult to compare results. We suggest investigating possible differences in LTL between genetic subtypes in future studies.

In conclusion, we found a shorter LTL in 47 GH-treated young adults with PWS compared to untreated healthy young adults and young adults born SGA who were also treated with GH, which might suggest that a shorter LTL is involved in the reported accelerated aging process in adults with PWS. More research on LTL across the life course is needed to be able to determine its exact role in aging in people with PWS.

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Additional Information

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Data Availability: Restrictions apply to the availability of data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will, on request, detail the restrictions and any conditions under which access to some data may be provided.

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