Progeria Syndrome

Mechanisms of Premature Vascular Aging in Children With Hutchinson-Gilford Progeria Syndrome

Marie Gerhard-Herman, Leslie B. Smoot, Nicole Wake, Mark W. Kieran, Monica E. Kleinman, David T. Miller, Armin Schwartzman, Anita Giobbie-Hurder, Donna Neuberg, Leslie B. Gordon

Abstract—Hutchinson-Gilford progeria syndrome is a rare, segmental premature aging syndrome of accelerated atherosclerosis and early death from myocardial infarction or stroke. This study sought to establish comprehensive characterization of the fatal vasculopathy in Hutchinson-Gilford progeria syndrome and its relevance to normal aging. We performed cardiovascular assessments at a single clinical site on the largest prospectively studied cohort to date. Carotid-femoral pulse wave velocity was dramatically elevated (mean: 13.00±3.83 m/s). Carotid duplex ultrasound echobrightness, assessed in predefined tissue sites as a measure of arterial wall density, was significantly greater than age- and sex-matched controls in the intima-media (P<0.02), near adventitia (P<0.003), and deep adventitia (P<0.01), as was internal carotid artery mean flow velocity (P<0.0001). Ankle-brachial indices were abnormal in 78% of patients. Effective disease treatments may be heralded by normalizing trends of these noninvasive cardiovascular measures. The data demonstrate that, along with peripheral vascular occlusive disease, accelerated vascular stiffening is an early and pervasive mechanism of vascular disease in Hutchinson-Gilford progeria syndrome. There is considerable overlap with cardiovascular changes of normal aging, which reinforces the view that defining mechanisms of cardiovascular disease in Hutchinson-Gilford progeria syndrome provides a unique opportunity to isolate a subset of factors influencing cardiovascular disease in the general aging population. (Hypertension. 2012;59:92-97.) ● Online Data Supplement

Key Words: Hutchinson-Gilford progeria syndrome ■ atherosclerosis ■ arteriosclerosis ■ arterial stiffness ■ lamin ■ aging

Hutchinson-Gilford progeria syndrome (HGPS) is an extremely rare (incidence: 1 in 4–8 million) sporadic genetic disorder involving aberrant splicing of the LMNA gene,1,2 resulting in the production of a disease-causing mutant lamin A protein called progerin.1 Lamin A3 and, thus, progerin,4 are inner nuclear membrane proteins expressed in all layers of the vasculature in both HGPS and, at a reduced rate, in normal aging.5 Select clinical features of this disorder mimic those of accelerated human aging, with premature cardiovascular disease (CVD) as the basis for significant morbidity and mortality.5 Individuals with HGPS die at an average age of 13 years because of myocardial infarction or stroke.6 The rapid progression of CVD in HGPS presents an unmet need for developing objective cardiovascular end points when assessing treatment efficacy, and for exploring the intersections between HGPS and the general aging population. To date these issues have been modestly characterized.7 Based on dense arterial proteoglycan and collagen deposition in HGPS human4,8 and mouse model9 autopsies, along with abnormally echobright vasculature on carotid ultrasound in our previous natural history study,5 we hypothesized that end-stage cardiovascular events in HGPS are related to progressive impairment in vascular compliance. We, therefore, conducted a single center clinical trial involving the largest cohort of subjects with HGPS to date, using measures known to be associated with vascular stiffening in addition to established cardiovascular risk factors that influence CVD through independent mechanisms. Here we examined variables with potential clinical use in HGPS as measures of both disease severity and treatment efficacy for this unique model of accelerated cardiovascular aging.

Methods

Study Population and Design
Twenty-six children with classic p.G608G HGPS (c.1824 C>T in LMNA) were enrolled into a clinical trial for the study of progeria (NCT00425607). Several children were unable to perform some tests, and in those cases, the number was <26. Sixty-two age- and
sex-matched healthy control children without history of blood pressure (BP) or cardiac abnormalities were enrolled to establish normative pediatric reference data for parameters not available in the literature. These were internal carotid artery (ICA) echodensity and flow velocity, both obtained from a single carotid ultrasound. Fifty-seven subjects provided useful data for analysis. The Children’s Hospital Boston Committee on Clinical Investigation approved the study protocol.

Written informed consent was obtained from parents and study assent from children aged ≥7 years. Participants with HGPS from 16 countries were flown to Children’s Hospital Boston for evaluations. Consent was provided in written and oral form in the language of the parents; interpreters were provided during testing periods for non-English–speaking participants. Outside medical charts and clinical information were obtained either from the Progeria Research Foundation Medical and Research Database (principal investigator, L.B.G.), with parental consent (Brown University Center for Gerontology and Healthcare Research, Providence, RI), or directly from parents and referring physicians.

Clinical Measures
A complete history and physical, venous blood collection, oral glucose tolerance testing, 12-lead ECG, and automated BP measures were performed. Either a pediatric 12- to 19-cm or infant size 8- to 13-cm BP cuff was selected for each child, based on the size that would allow the bladder to cover 80% of the upper arm. Insulin resistance was determined using the following equation: homeostasis model assessment-insulin resistance = (fasting glucose (mg/dl)) × (fasting insulin (μU/mL))/405. Height-age was determined by calculating the median age in the general population of a child with the height of each patient with HGPS, using Centers for Disease Control and Prevention sex-specific pediatric growth curves (http://www.cdc.gov/growthcharts/). All of the other studies were performed by a single cardiologist (M.G.-H.) in the morning, with the subjects fasting and resting supine in a quiet darkened, temperature-controlled (22°C) room.

Diagnostic carotid artery ultrasonography was performed in an Intersocietal Commission for the Accreditation of Vascular Laboratories–accredited laboratory using established protocols. A Philips iU22 ultrasound machine (Philips, Eindhoven, the Netherlands) equipped with a 17- to 5-MHz broadband linear-array transducer was used.

Velocity was obtained using pulsed wave Doppler performed with appropriate angle correction. Because the left-sided vessels were routinely performed later in the examination when the children might have been more tired or agitated, we assessed right and left sides separately to assure that any minor changes in vessel physiology have been more detected. Mean distal ICA velocity was derived using one third of the peak systolic velocity plus two thirds of the end diastolic velocity, as described previously.10 Gray map 5 was used uniformly after adjusting overall gain so that intraluminal blood appeared black. Digital gain compensation was kept perpendicular.

Distal common carotid artery far-wall intima-media thickness (IMT) was measured from the intima-lumen border to the media-adventitia border over a 2-cm segment according to standard protocol using edge-detection software (Medical Imaging Applications, Coralville, IA).11 Carotid-femoral pulse wave velocity (PWV(c-f)) and ankle-brachial index (ABI) measurements were determined as described previously.12,13 For detailed protocols, please see the online Data Supplement at http://hyper.ahajournals.org.

Echodensity values were quantified using ImageJ software (National Institutes of Health, Bethesda, MD) on a grayscale ranging from 0 (black) to 256 (white), where 0 was calibrated to equal the density of intraluminal blood (preset to appear as black).14 Prespecified rectangular regions were 1 cm in length, starting 1 cm proximal to the carotid bifurcation within the far wall of the right distal common carotid artery. Depths for prespecified sampling areas were the intima-media (from the intima-lumen interface to the media-adventitia border), the near adventitia (from the media-adventitia border to 0.4 mm depth), and the deep adventitia (from 1.6 to 2.0 mm depth beneath the media/adventitia interface). Histograms and percentiles were calculated using MATLAB 7.9 (The Mathworks, Inc, Natick, MA).

Statistical Analyses
Statistical comparisons between sex, age groups, or cases/controls were conducted using Wilcoxon rank-sum tests to account for asymmetry of distribution and nonhomogeneity of variance. Spearman correlation coefficients were used to quantify relationships between continuous variables. Statistical significance was defined as P≤0.05; there were no corrections for multiple comparisons.

Results
Demographic and Anthropometric Profiles
Anthropometric and historical cardiovascular characteristics are presented in Table S1, available in the online Data Supplement. Mean age was 7.4±3.4 years (range: 3.1–16.2). All of the patients fell well below the third percentile for height (94.96±11.6 cm), weight (10.46±2.7 kg), and body mass index (11.5±1.2 kg/m²). Mean height-age was 3.4±1.5 years, which is 4.1 years younger than chronologic age.

Metabolic Profile
Metabolic profile data are presented in Table S2. With oral glucose tolerance testing, only a small fraction of patients had abnormal fasting (16%) or 2-hour glucose levels (8%). In contrast, 52% of patients had hyperinsulinemia, and 36% were insulin resistant. One patient with insulin resistance had accompanying glucose intolerance, and 1 patient, age 16 years, had diabetes mellitus.

Serum lipid profiles agreed with previous studies (Table S2).14 High-density lipoprotein was significantly low in 8 (33%) of 24 patients. Mean cholesterol, low-density lipoprotein, and triglycerides were in the normal range. Leptin was undetectable or below normal in 92% of patents. All of the patients had normal liver and kidney function, assessed with serum aspartate aminotransferase, alanine aminotransferase, bilirubin (total and direct), serum urea nitrogen, and creatinine.

ECG Findings
Eighty-five percent of patients (22 of 26) had normal 12-lead ECGs. Four patients (15%; ages 9–16 years) had significant ECG abnormalities: 2 exhibited borderline left ventricular hypertrophy (1 with atrial enlargement), and 2 exhibited left ventricular hypertrophy with strain.

Blood Pressure
We evaluated BP in relationship to both chronologic age and height-age. When assessed by chronologic age, systolic and diastolic pressures were ≥95th percentile in 7 (27%) of 26 and 9 (35%) of 26 patients, respectively. Using height-age norms to account for patient size, systolic and diastolic pressures were elevated in 12 (46%) of 26 and 14 (54%) of 26 patients, respectively. Four of 26 children were taking antihypertensive medications (3 taking β-blockers and 1 taking an angiotensin-converting enzyme inhibitor). Elevations in both systolic and diastolic BPs resulted in overall pulse pressure within the normal range for both chronologic age and height-age (n=25; median: 41 mm Hg; range: 24–65 mm Hg).
children in the control cohort. With right and left ICA flow velocities averaged, 76.0 cm/s represented the 95th percentile in the control group; values above this were defined as elevated. In the HGPS population, 15 children (63%) had bilateral flow velocity elevation; 4 children (17%) had unilateral flow velocity elevation; and 5 children (21%) were within normal limits bilaterally.

### Intima-Media Thickness
Similar to our previous study, all of the 26 children with HGPS had IMT measures using right, left, and combined ICA values (each 0.43±0.03 mm) within the normal range and similar to controls for right (n=55; 0.40±0.04 mm), left (n=54; 0.39±0.04 mm), and combined values (0.40±0.03 mm).19

### Common Carotid Artery Echodensity
Vascular echobrightness on ultrasound increases with tissue density.20,21 Carotid artery wall echodensity was assessed within predefined areas of the intima-media, near adventitia, and deep adventitia in 22 HGPS subjects and compared with 52 age- and sex-matched controls (Figure 3A through 3D). Predefined areas were transformed into histograms representing the spectrum of echobrightness for the entire area captured (Figure 3E through 3G). Most control histograms (Figure 3E) and some HGPS histograms (Figure 3F) were fully captured on the grayscale. However, in some cases, the vessel wall was uniformly white beyond the limit of echodetection, resulting in a histogram compressed against the upper limit wall (Figure 3G). To better define the differences with these data, we compared density at both the 10th and 50th percentiles for each subject. At both the 10th and 50th percentiles, mean wall echodensities of the intima (10th percentile P=0.0001; 50th percentile P=0.02), near adventitia (10th percentile P=0.02; 50th percentile P=0.003), and deep adventitia (10th percentile P=0.03; 50th percentile P=0.01) were significantly greater in the HGPS cohort when compared with controls (Figure 3H). There was no significant association between PWVcf and echodensity at the 10th and 50th percentiles within each of the echodensity sites (n=19).

### Ankle-Brachial Indices
Patients exhibited both elevated and depressed ABI (Table). Seventy-eight percent of children had ABI abnormality in one or both limbs; only 5 children (22%) were within normal limits bilaterally, with ABI values between 1.0 and 1.2.

### Discussion
Accelerated CVD leads to debilitating morbidity in HGPS and culminates in mortality from myocardial infarction or stroke at an average age of 13 years.6 For the first time, we have identified elevated PWVcf, increased intima-media and adventitia echodensity, abnormal ABI, and increased ICA mean flow velocity as pervasive disease features in HGPS. Evidence of vascular dysfunction is apparent in 100% of our cohort, in children as young as 3 years of age. Elevated PWVcf and wall echodensity identify vascular stiffening as an early and important contributor to cardiovascular decline; abnormalities in ABI and distal ICA mean flow velocity

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**Figure 1.** Carotid-femoral pulse wave velocity (PWVcf) is elevated in Hutchinson-Gilford progeria syndrome (HGPS); PWVcf for each of 21 children with HGPS. Solid horizontal bar represents published pediatric normal range (mean±SD).16

**Figure 2.** Internal carotid artery (ICA) flow velocity is elevated in Hutchinson-Gilford progeria syndrome (HGPS). Mean (±SE) distal ICA flow velocity in meters per second for left and right sides in control cohort (n=53), white bars; and HGPS cohort (n=24), black bars. **P<0.0001 between control and HGPS groups on both left and right sides.

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**Carotid-Femoral Pulse Wave Velocity**

Mean PWVcf was 13.00±3.83 m/s (n=21), which is 228% of the highest published mean pediatric normal values of 5.7±0.9 m/s.16 (Figure 1), and mean values were comparable to those of typical adults >60 years of age.17,18 The Spearman correlation coefficient between PWVcf and homeostasis model assessment-insulin resistance was 0.456 (P=0.04). PWV was significantly increased (P=0.03) in the children who were insulin resistant (n=8; PWVcf median: 16.1; range: 10.1–18.8) versus those who were not (n=12; PWVcf median: 12.6; range: 7.2–17.5).

**Diagnostic Carotid Ultrasonography**

Atherosclerotic plaque limited to the ICA was evident in 2 (8%) of 26 patients (Figure S1). One subject had total occlusion of the ICA. No other significant stenoses were evident by systolic velocity ratios.

Mean distal ICA flow velocities10 were assessed as a measure of distal small vessel function. We observed increased flow velocities bilaterally in >80% of the HGPS cohort compared with controls (P<0.0001 for both right- and left-sided comparisons; Figure 2). To assess flow abnormality by patient, we next calculated the fraction of children with HGPS whose mean velocities were ≥95th percentile of

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**Figure 3.**

- **A** and **D**: Control histograms (Figure 3A through 3D). Most control histograms were fully captured on the grayscale. However, in some cases, the vessel wall was uniformly white beyond the limit of echodetection, resulting in a histogram compressed against the upper limit wall (Figure 3G). To better define the differences with these data, we compared density at both the 10th and 50th percentiles for each subject. At both the 10th and 50th percentiles, mean wall echodensities of the intima (10th percentile P=0.0001; 50th percentile P=0.02), near adventitia (10th percentile P=0.02; 50th percentile P=0.003), and deep adventitia (10th percentile P=0.03; 50th percentile P=0.01) were significantly greater in the HGPS cohort when compared with controls (Figure 3H). There was no significant association between PWVcf and echodensity at the 10th and 50th percentiles within each of the echodensity sites (n=19).

- **B** and **C**: Typical histograms (Figure 3B through 3C) captured the spectrum of echobrightness for the entire area. Most control histograms (Figure 3B) were fully captured; however, in some cases, the vessel wall was uniformly white beyond the limit of echodetection, resulting in a histogram compressed against the upper limit wall (Figure 3C). To better define the differences with these data, we compared density at both the 10th and 50th percentiles for each subject. At both the 10th and 50th percentiles, mean wall echodensities of the intima (10th percentile P=0.0001; 50th percentile P=0.02), near adventitia (10th percentile P=0.02; 50th percentile P=0.003), and deep adventitia (10th percentile P=0.03; 50th percentile P=0.01) were significantly greater in the HGPS cohort when compared with controls (Figure 3H). There was no significant association between PWVcf and echodensity at the 10th and 50th percentiles within each of the echodensity sites (n=19).

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**Discussion**

Accelerated CVD leads to debilitating morbidity in HGPS and culminates in mortality from myocardial infarction or stroke at an average age of 13 years.6 For the first time, we have identified elevated PWVcf, increased intima-media and adventitia echodensity, abnormal ABI, and increased ICA mean flow velocity as pervasive disease features in HGPS. Evidence of vascular dysfunction is apparent in 100% of our cohort, in children as young as 3 years of age. Elevated PWVcf and wall echodensity identify vascular stiffening as an early and important contributor to cardiovascular decline; abnormalities in ABI and distal ICA mean flow velocity
under-score the presence of arterial occlusive disease. Taken together, these abnormalities promote dynamic increases in ventricular afterload, impaired vascular autoregulation, and cardiac and cerebrovascular decompensation.

We identified 3 main contributors to vascular stiffening. First, PWVcf was dramatically elevated overall. This finding is consistent with the elevated augmentation index detected previously in HGPS. PWV is inversely related to arterial wall distensibility and serves as the gold standard for measures of arterial stiffness. Moreover, increased PWV is predictive of subsequent cardiovascular events. Second, we discovered abnormally echodense vascular walls in the HGPS cohort. We hypothesize that this corresponds with the dramatically thickened, fibrotic matrix detected previously on histopathologic analyses of both children with HGPS and a transgenic mouse model of HGPS. These autopsy studies have described a diffuse arteriopathy marked by striking depletion of vascular smooth muscle cells from the media, along with deposition of replacement proteoglycans and collagen in both media and adventitia. Such maladaptive vascular remodeling would predict lower arterial compliance and impaired flow reserve. Third, in agreement with our previous study, we found that a significant fraction of patients had systolic and diastolic BPs above the 95th percentile. This fraction increased after adjusting for height-age, because the HGPS cohort is extremely small for age, and somatic size and chronologic age contribute to BP. These elevated BP trends would be expected in the setting of significant vascular stiffness, because prehypertensive adults display both impaired arterial functions and elevated PWV. The observed elevation in PWVcf without elevation of pulse pressure in this cohort may be related to overestimation of diastolic pressure by the automated cuff, altered volume status and heart rate in fasting patients, or disproportionate changes in the aorta compared with the peripheral arteries. Characteristics of large versus small vessel disease and their relative contribution to pulse pressure and systemic vascular.

Table. Blood Pressure and Ankle-Brachial Index

<table>
<thead>
<tr>
<th>Blood Pressure (n=26)</th>
<th>Systolic</th>
<th>Diastolic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (range), mm Hg</td>
<td>104 (80–134)</td>
<td>69.5 (40–96)</td>
</tr>
<tr>
<td>≥95 percentile for chronologic age, n (%)</td>
<td>7 (27)</td>
<td>9 (35)</td>
</tr>
<tr>
<td>≥95 percentile for height-age, n (%)</td>
<td>12 (46)</td>
<td>14 (54)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ABI Stratification (n=23)</th>
<th>No. of Subjects With 1 Limb in Category</th>
<th>No. of Subjects With 2 Limbs in Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>High abnormal, ≥1.20</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Normal range, 1.01–1.20</td>
<td>9</td>
<td>5</td>
</tr>
<tr>
<td>Borderline low, 0.91–1.00</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>Low abnormal, 0.80–0.90</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

ABI indicates ankle-brachial index.
resistance will be important in understanding the basis for the marked abnormalities observed in PWVcf. Future HGPS studies will benefit from rigorous longitudinal assessment of these parameters in both static and dynamic states.

We experienced several study limitations. In young children, vascular testing is limited by the child’s ability to withstand testing and by a paucity of standards for comparison. Where pediatric standards were unavailable to quantitatively evaluate vessel wall density and distal ICA mean flow velocity, we tested an age- and sex-matched healthy control cohort, with predictably larger body mass index. Where possible, we relied on comparison with published pediatric normal control studies (eg, PWVcf), which lends itself to confounding factors, such as interrater reliability. In addition, noninvasive carotid artery ultrasonography is subject to technical limitations. Although we used a pediatric ultrasound transducer with the highest available frequency, the relative lack of subcutaneous tissue6 precluded adequate image resolution along the carotid arterial near wall in many cases. Therefore, all of the measures were acquired from the far wall according to standard protocol11 and where image resolution was excellent for all of the subjects. Echodensity in the HGPS cohort often saturated the upper limit of detection; technical expansion of the 256 grayscale would allow additional density capture. Finally, longitudinal analyses may further clarify the relationship of age to the vasculopathy of HGPS in future studies.

Understanding the intersections and distinctions between HGPS and normal aging can inform both fields of study and help us to interpret the clinical influence that progerin may have after the end-stage manifestation of atherosclerotic disease, the vascular pathology seen in HGPS.4,8 Although arterial intima-media density has been correlated with pathologic4,25 support for key roles of altered lamin A and progerin in human aging. In addition, noninvasive measures, including PWVcf, carotid wall echodensity and ICA flow velocity, offer quantitative insights into accelerated vasculopathy in HGPS and may provide sensitive indicators of disease progression or remission with adjuvant therapies.

The end-stage manifestation of atherosclerotic disease, the presence of carotid plaque, was found in only 2 of our older HGPS subjects. As with aging, this implies that visible plaque occurs later in disease. In contrast, elevated mean ICA flow velocity was seen across all ages in HGPS. In aging, increased ICA flow velocity directly correlates with degree of stenosis.31 This abnormality may be an early indicator of an ongoing process that culminates in plaque formation, as a consequence of narrowing of the distal ICA. Alternatively, elevated ICA flow velocity may be attributed to increased flow within the brain because of collateral formation around occlusive disease.

ABI is a useful tool to detect peripheral vascular disease in the general aging population, where it indicates calcifications or vessel stiffness when high, as well as lower extremity occlusive disease when low.12 Both high and low ABI are predictors for cardiovascular mortality. We detected both elevated and depressed ABI in HGPS, which likely reflects a composite picture of peripheral vascular stiffening and occlusive disease that could actually yield misleading normal ABI values in this mixed disease setting.

Echodensity is an emerging area of study in cardiology and aging. Echobrightness on ultrasound increases as tissue density increases; each pixel represents one returning echo at a particular depth where the ultrasound encounters tissue. The brightness of an image is determined by the number of returning echoes, which is proportional to the density of the tissue being evaluated.21 We found that the intima-media complex in HGPS is of normal thickness but abnormal echodensity. This relationship between echogenicity and tissue pathology can occur in the face of normal IMT. Lind et al14 performed a study of human aging common carotid artery vasculature in randomly chosen people over age 70 years. Those with normal IMT had the same increased echogenicity as those with widened IMT within both the intima-media and within plaques. In diseased cardiovascular tissue and plaque, both animal and human studies have supported duplex ultrasound as a useful tool for detecting tissue pathology with dysregulated extracellular matrix through increased echogenicity. When correlating echogenicity with tissue pathology in rats, Tabel et al20 demonstrated hyperchoic tissue only within older infarcts where thick collagen fibers were present. New infarcts with thinner, normal-looking collagen fibrils were not hyperchoic. This dense fibrous matrix is reminiscent of the vascular pathology seen in HGPS.4,8 Although arterial intima-media density has been correlated with cardiovascular risk factors and carotid plaque echogenicity in elderly adults,14 the striking degree of adventitial abnormalities observed in this study has not yet been evaluated in aging and should be the subject of future studies.

**Perspectives**

The HGPS vascular phenotype is associated with significant premature cardiovascular morbidity and mortality occurring in the absence of the common comorbidities that are prevalent in adult populations, such as smoking, hypercholesterolemia, and obesity. We characterized this single gene disorder as a disease of vascular stiffening in the setting of gradual vascular stenosis, much like that seen over a lifetime in normative aging. This study’s human clinical investigations help us to translate the in vitro,7,9,26,27,32 mouse model,9,33 and human pathological4,25 support for key roles of altered lamin A and progerin in human aging. In addition, noninvasive measurements, including PWVcf, carotid wall echodensity and ICA flow velocity, offer quantitative insights into accelerated vasculopathy in HGPS and may provide sensitive indicators of disease progression or remission with adjuvant therapies.

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Acknowledgments

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Disclosures

L.B.G. is the parent of a child with HGPS who participated in this study.

References

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Short Title: Vascular Aging in Progeria

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Online-only Methods:

Carotid-femoral pulse-wave velocity (PWVcf) measurements were determined by using a duplex ultrasound with simultaneous ECG acquisition to measure the propagation time of the pressure pulse from the carotid to femoral arteries. The onset of the arterial pulse waveform was identified by using the intersecting tangent method.\(^1\) Propagation time (\(\Delta t_{cf}\)) was calculated by measuring the time lag between the R-wave of the simultaneous ECG and the arrival of the arterial pulse at both the carotid (\(\Delta t_c\)) and (\(\Delta t_f\)) femoral arteries. The distance between the carotid and femoral arteries (\(l_{cf}\)) was measured and recorded. PWVcf was calculated using the formula \(PWV_{cf} = \frac{l_{cf}}{\Delta t_{cf}}\). Published normal ranges used for comparison were performed using this method.\(^2,3\)

Ankle brachial indices (ABIs) were performed using appropriate sized pediatric sphygmomanometers with subjects in the supine position after resting for ten minutes. Systolic pressures were obtained in each arm and ankle. A Doppler probe and photoplethysmograph were used to monitor the pulse while the sphygmomanometer was inflated to suprasystolic pressure above each artery. The sphygmomanometer was then deflated and the pressure at which the pulse returned was recorded. ABIs were calculated by dividing the systolic ankle pressure by the highest systolic arm pressure.

References:


Online-only Results:

Table S1. HGPS Cohort Anthropometrics and CV History (n=26*)

<table>
<thead>
<tr>
<th>Anthropometrics at Study Entry†</th>
<th>Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronologic Age</td>
<td>7.4 ± 3.4</td>
</tr>
<tr>
<td>Height Age</td>
<td>3.4 ± 1.5</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.46 ± 2.7</td>
</tr>
<tr>
<td>Standing Height (cm)</td>
<td>94.96 ± 11.6</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>11.5 ± 1.2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Historical CV Characteristics</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of CV events</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cerebrovascular accident</td>
<td>4</td>
<td>15%</td>
</tr>
<tr>
<td>Supraventricular tachycardia</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>Congenital heart disease</td>
<td>1</td>
<td>4%</td>
</tr>
<tr>
<td>ROSS Classification</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-asymptomatic</td>
<td>23</td>
<td>88%</td>
</tr>
<tr>
<td>II-exertional symptoms</td>
<td>3</td>
<td>12%</td>
</tr>
<tr>
<td>History of Hypertension</td>
<td>5</td>
<td>19%</td>
</tr>
<tr>
<td>History of Treatment for Hypertension</td>
<td>4</td>
<td>22%</td>
</tr>
<tr>
<td>ACE-I or β-blocker</td>
<td>2</td>
<td>8%</td>
</tr>
<tr>
<td>Diuretic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*11 males (42%) and 15 females (58%); all tanner stage 1
† anthropometrics are also included in Gordon et al.²
Table S2. Glucose Metabolism and Lipid Values in HGPS Cohort

<table>
<thead>
<tr>
<th>Serum Test</th>
<th>Mean ±SD</th>
<th>Fraction (%) of Patients in Abnormal Range*</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasting Glucose (mg/dl)</strong></td>
<td>88.32±13.73</td>
<td>2/25 (8%)</td>
<td>&lt;100</td>
</tr>
<tr>
<td><strong>OGTT 2 hr Glucose (mg/dl)</strong></td>
<td>106.1±33.9</td>
<td>3/25 IGT (12%)</td>
<td>Normal &lt;140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/25 Diabetic (4%)</td>
<td>Impaired glucose tolerance 140-200</td>
</tr>
<tr>
<td><strong>Fasting Insulin (uU/mL)</strong></td>
<td>22.98±42.41</td>
<td>13/25 (52%)</td>
<td>Diabetes &gt;200</td>
</tr>
<tr>
<td>†HOMA - IR</td>
<td>5.6+/10.3</td>
<td>9/25 (36%)</td>
<td>Normal &lt;2.5</td>
</tr>
<tr>
<td><strong>Leptin (µg/L)</strong></td>
<td>not assessable</td>
<td>22/26 Undetectable (84%)</td>
<td>Girls 1.23-5.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/26 Below Normal (8%)</td>
<td>Boys 0.65-3.04</td>
</tr>
<tr>
<td><strong>HbA1C (%)</strong></td>
<td>5.58±0.55</td>
<td>1/25 (4%)</td>
<td>&lt;6</td>
</tr>
<tr>
<td>‡Total Cholesterol (mg/dl)</td>
<td>149.28±30.03</td>
<td>1/25 Borderline (4%)</td>
<td>Desirable:&lt;170</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/25 High (8%)</td>
<td>Borderline 170-199; High≥&gt;200</td>
</tr>
<tr>
<td><strong>HDL cholesterol (mg/dl)</strong></td>
<td>43.67±14.38</td>
<td>8/24 (33%)</td>
<td>Desirable: &gt;35</td>
</tr>
<tr>
<td><strong>LDL cholesterol (mg/dl)</strong></td>
<td>85.52±31.83</td>
<td>2/23 Borderline (9%)</td>
<td>Desirable: &lt;110</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1/23 High (4%)</td>
<td>Borderline: 110-129; High≥≥130</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dl)</strong></td>
<td>91.19±93.35</td>
<td>1/25 Borderline (4%)</td>
<td>Desirable: &lt;150</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2/25 High (8%)</td>
<td>Borderline 150-199; High≥≥200</td>
</tr>
</tbody>
</table>

*In cases where patients were unable to fast, results are reported for fewer than 26 patients
†homeostasis model assessment-insulin resistance (HOMA-IR) = fasting (glucose)(insulin)/405
‡Five patients were taking cholesterol-lowering drugs (4 taking statins and one taking ezetimibe) at the time of study visit
Figure S1. Carotid occlusions. Carotid ultrasonography in two HGPS individuals demonstrating A, complete distal internal carotid stenosis. Note colored area of Doppler flow (Positive Flow) prior to total occlusion (No Flow). B, a non-occlusive atherosclerotic plaque (P) juts out within the vascular lumen (L).