Cardiovascular Pathology in Hutchinson-Gilford Progeria: Correlation With the Vascular Pathology of Aging


Objective—Children with Hutchinson-Gilford progeria syndrome (HGPS) exhibit dramatically accelerated cardiovascular disease (CVD), causing death from myocardial infarction or stroke between the ages of 7 and 20 years. We undertook the first histological comparative evaluation between genetically confirmed HGPS and the CVD of aging.

Methods and Results—We present structural and immunohistological analysis of cardiovascular tissues from 2 children with HGPS who died of myocardial infarction. Both had features classically associated with the atherosclerosis of aging, as well as arteriolosclerosis of small vessels. In addition, vessels exhibited prominent adventitial fibrosis, a previously undescribed feature of HGPS. Importantly, although progerin was detected at higher rates in the HGPS coronary arteries, it was also present in non-HGPS individuals. Between the ages of 1 month and 97 years, progerin staining increased an average of 3.34% per year (P<0.0001) in coronary arteries.

Conclusion—We find concordance among many aspects of cardiovascular pathology in both HGPS and geriatric patients. HGPS generates a more prominent adventitial fibrosis than typical CVD. Vascular progerin generation in young non-HGPS individuals, which significantly increases throughout life, strongly suggests that progerin has a role in cardiovascular aging of the general population. (Arterioscler Thromb Vasc Biol. 2010;30:2301-2309.)

Key Words: aging ■ atherosclerosis ■ pathology ■ peripheral arterial disease ■ progeria

Hutchinson-Gilford progeria syndrome (HGPS) is a rare, autosomal-dominant, fatal, progressive premature aging syndrome. Symptoms usually begin with failure to thrive or sclerodermatous skin changes, heralding generalized loss of subcutaneous fat, alopecia, osteopenia and acroosteolysis, and joint contracture. Death occurs at a mean age of 13 years because of myocardial infarction or stroke.¹ The majority of HGPS cases are caused by a single de novo nucleotide substitution at position 1824 (C→T) in the LMNA gene.²³ The normal LMNA protein product, lamin A, is a key component of the inner nuclear lamina, which functions in nuclear structure, chromatin organization, and gene transcription.⁴ The silent mutation in HGPS leads to alternative splicing at the 3' end of the LMNA mRNA and a 150-nucleotide deletion from the prelamin A transcript resulting in a mutant lamin A protein called progerin, which lacks 50 amino acids near its C-terminal end.⁵ In non-HGPS individuals, there is convincing evidence that the HGPS splice site is functional and can lead to progerin accumulation over time, although to a lesser degree than in children with HGPS.⁶ In HGPS, the cryptic donor splice site shares 6 of 7 bases with the consensus splice sequence, while non-HGPS individuals share 5 of 7 bases with the consensus splice sequence. Thus, non-HGPS individuals utilize the splice site less often. Progerin is not apparent in early passage non-HGPS cultured fibroblasts and skin biopsies, but it accumulates with increasing cell passage and donor age.⁷⁸ Thus, progerin is likely a previously unexplored contributor to human vascular disease and vascular aging. Pathological similarities and differences between validated HGPS and vasculature of the general population have not been previously studied. Although published case reports have included some pathology,⁹¹⁰ none were confirmed by mutation analysis. It is unknown whether these studies represent HGPS or other progeroid syndromes.

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because a number of publications describing HGPS are likely misdiagnoses. In the current study, we describe the histopathology and progerin distribution in 2 patients with 1824 (C>T), classic HGPS, along with a cohort of non-HGPS subjects with and without cardiovascular disease (CVD). Similarities and differences between CVD in HGPS and in normal aging are demonstrated.

Methods

An expanded Methods section is provided in the Supplemental Data, available online at http://atvb.ahajournals.org.

The study was approved by the institutional review boards of Rhode Island Hospital and Brown University. Informed consent was obtained from the parents of HG001 and HG120.

Clinical Information

Medical information for HG001 and HG120 was obtained from the Progeria Research Foundation Medical and Research Database (www.progeriaresearch.org/medical_database.html) at the Brown University Center for Gerontology and Health Care Research (Providence, RI). Of particular interest to this study, both HG001 (female) and HG120 (male) died of myocardial infarction, at ages 9.9 and 14.0, respectively. Both were normotensive, with largely normal lipid profiles throughout life. HG001 developed strokes at end stage, whereas HG120 did not. HG120 developed mild insulin resistance at age 7 years, without frank diabetes (HG001 unmeasured). For detailed case histories, see Supplemental Data.

Autopsy Specimens

Autopsy tissue from HG001 and HG120 were obtained from the Progeria Research Foundation Cell and Tissue Bank (www.progeriaresearch.org/cell_tissue_bank.html) at Rhode Island Hospital (Providence, RI).

Non-HGPS tissues were obtained from the CVPath Institute, Inc (Gaithersburg, Md).

Mutation Analysis

Mutational analysis of the LMNA exon 11 for HG001 and HG120 was performed via the Progeria Research Foundation Diagnostics Program (www.progeriaresearch.org/diagnostic_testing.html). For HG001, fibroblasts DNA was amplified and sequenced by PreventionGenetics (Marshfield, Wis). For HG120, liver DNA was amplified and sequenced by the Laboratory for Molecular Medicine (Cambridge, Mass).

Immunohistochemistry

Lamin staining was previously described in detail. Antibodies used in this study were as follows: mouse monoclonal anti-lamin A/C non-diluted (MAB3211; Chemicon, picum); monoclonal anti-smooth muscle α-actin fluorescein isothiocyanate–conjugated (1:100; clone 1A4; Sigma-Aldrich), and progerin-specific rabbit polyclonal antibody 972 (1:500). Sections of non-HGPS individuals were sub-

Results

We present structural and immunohistological analysis of cardiovascular tissues from 2 genetically confirmed classic (1824 C>T) cases of HGPS (a 9-year-old girl [HG001] and a 14-year-old boy [HG120] who died of myocardial infarction) and comparative analyses with a non-HGPS cohort.

Similarities Between HGPS Vascular Pathology and Conventional Atherosclerosis of Aging

The hematoxylin/eosin (H&E) and Movat stains of the coronary arteries of HGPS patients HG001 and HG120 revealed advanced atherosclerotic lesions. The atherosclerotic lesions in patient HG001 were variably cellular (Figure 1A and 1B), with approximately 70% chronic stenoses with stable eccentric lesions but no calcification or cholesterol crystals in the sections that were sampled. Similarly, many of the atherosclerotic lesions in HG120 were largely fibrotic and acellular, with chronic subtotal occlusion in the LAD (Figure 1C). Notably, the right coronary artery (RCA) in HG120 was 98% occluded (Figure 1D), with a classic complex plaque morphology, including a necrotic core (Figure 1E) and foci of chronic inflammation. Needle-shaped crystal formations were observed infrequently (Figure 1F). The LAD and RCA of patient HG120 displayed extensive calcification (Figure 1C and 1G), with appearance similar to calcification seen in most plaques associated with CVD in aging individuals after their 5th decades.

The HGPS intimal lesions were densely fibrotic and appeared to reflect the spectrum of atheromatous lesions present in advanced aging. There was medial thinning subjacent to thick plaque (Figure 1C and 1D), typical of medial changes in other vascular pathological settings. The LAD and RCA lesions showed no acute plaque rupture or thrombus formation; however, healed plaque ruptures were observed, suggesting that the clinical complications of atherosclerosis may have arisen from flow limiting stenoses rather than acute plaque rupture leading to sudden thrombotic occlusion. Supporting clinical history and autopsy findings are presented in the Supplemental Data.

Differences Between HGPS Vascular Pathology and Conventional Atherosclerosis of Aging

Arteries and veins in both HG001 and HG120 showed marked adventitial fibrosis, with a dense rim of collagen as manifested by Movat staining (Figure 1A to 1D, yellow) and H&E (Figure 2, deep pink). The adventitial changes were evident in large vessels such as the aorta of patient HG001 (Figure 2A) and the midcoronary artery of patient HG120 (Figure 2B and 2C). There was no increased medial matrix deposition, and the adventitial perivascular fibrosis showed mild, nonspecific chronic inflammation. Similar dense, perivascular adventitial fibrosis was also present around noncardiac vessels, including arteries of the salivary glands (Supplemental Figure IA), spleen, lymph nodes, lymphatic vessels, and pulmonary arteries (data not shown). Veins such as the central veins of the liver and the portal triad, epicardial, and hilar lymph node veins also exhibited extensive perivascular tissue fibrosis (Supplemental Figure IB to IF). In the noncardiovascular circulation, the findings were abnormal.
but less pronouncedly so than those in the cardiovascular circulation. In contrast, no similar dense adventitial fibrotic sheath was observed in the aortas or coronary arteries of 16-year-old healthy and 97-year-old atherosclerotic, non-HGPS individuals (Figure 2D to 2F).

Characterization of the ECM in the Plaque of HGPS Patients

We assessed the coronary lesions from both HGPS and adult coronary artery disease (CAD) patients for the accumulation and organization of ECM molecules known to be associated with progressive stages of adult atherosclerotic lesions. Figure 3A to 3O shows examples of typical ECM staining patterns for each type of lesion identified in the HGPS vessels, with an adult non-HGPS lesion of similar stage for comparison (Figure 3P to 3T).

In contrast to the adult samples demonstrating primarily fibroatheromas, the majority of HGPS lesions could be categorized as fibrous lesions, rich in collagen and proteoglycans (PG). Picrosirius Red staining for collagen (Figure 3A, 3F, and 3K) revealed an abundance of densely packed fibers of type I collagen (orange/red) in the majority of the lesions, with regions of more loosely organized type III collagen (yellow/green) typically located at the luminal surface, extending out to the shoulder regions of the plaques. Staining for the collagen-associated PG decorin (Figure 3B, 3G, and 3L) revealed a pattern of deposition that mirrored that of type I collagen. The majority of HGPS lesions displayed large regions of calcification (Figure 3H) and could be described as fibrocalcific. Evidence of previous plaque rupture or erosion was found in some lesions at the luminal surfaces, which displayed a majority of type III collagen, minimal decorin deposition, and abundant colocalized versican and hyaluronan (Figure 3L, 3M, and 3N, arrowheads).15,16

Macrophages were present in most lesions (Figure 3E, 3J, and 3O), indicating some degree of inflammatory involvement in lesion progression. Supplemental Figure II clearly shows the association of lipid pools with macrophages identified by surface receptors CD68 and CD44, and foam cells were also detected with H&E (Figure 1H).

Progerin Is Expressed in the Coronary Arteries and Plaques in HGPS

To evaluate whether progerin plays a direct role in HGPS-associated CVD, we evaluated whether progerin is physically present in cardiovascular pathological lesions. Progerin expression by immunohistochemistry (IHC) was assessed with a progerin-specific antibody that does not bind to normal lamins. Most medial vascular smooth muscle cells (VSMCs) in HGPS lesions stained positive for progerin (Figure 4A). Progerin and smooth muscle actin (SMA) colocalized in the VSMCs present in Figure 4B. In addition, progerin-positive cells were present within the intimal plaque (Figure 4C), the adventitial fibroblasts, the arteriolar VSMCs, and the arterio-
lar endothelial cells (EC) (Figure 4D). Although the atherosclerotic plaque in patient HG120 is mainly acellular, the few intimal smooth muscle cells (SMCs) present were strongly progerin positive (Figure 4E to 4H). In addition, we identified progerin-positive cells embedded in the highly fibrotic area of the adventitia (Figure 4I) and in the thinned media (Figure 4J). Because the archived specimens had been treated with HCl, which degrades DNA, we were not able to counterstain the nuclei for DNA. Quantification of the progerin-positive cells of the coronary in HG001 showed 68/1000 cells 6.5% progerin-positive cells in the plaque, 91/1000 cells 3.7% in the media and 77/1000 cells 6.4% in the adventitia (Figure 4K).

Progerin-positive EC were preserved on the surface of the plaque in both HGPS patients (Figure 4L and 4M). EC expressed lower levels of progerin compared with VSMCs (see Supplemental Figure III). These results show for the first time that progerin is well represented in all layers of the coronary vasculature in HGPS patients.

Progerin in the Arteries of Normal Aging Individuals
We assessed whether progerin was present in the coronary arteries of non-HGPS subjects by probing for progerin in 29 individuals ranging in age from 1 month to 97 years (Figure 5A). Tissues were derived from patients with and without risk factors. These samples represented a cross-sectional (and limited) sampling selected primarily for age distribution, and they were not intended to represent a statistical analysis of risk-related atherosclerosis. Nevertheless, there were no consistent differences among patients in various age stratifications with and without risk factors in the overall extent or patterns of atherosclerotic disease. At 1 month of age, the progerin staining rate was approximately 1.00 per 1000 cells in the adventitia, which was significantly higher than that in either media (0.01 per 1000 cells, P<0.0001) or plaque (0.06 per 1000 cells, P=0.0006). At the age of 97 years, the mean progerin-staining rate was 19.66 per 1000 cells in the adventitia, which was again significantly higher than both media (0.90 per 1000 cells, P<0.0001) and plaque (1.04 per 1000 cells, P=0.0001). The rate of progerin staining increased an average of 3.34% per year (P<0.0001), with no statistically significant difference in rate of increase between the 3 arterial wall layers (P=0.5288). Progerin was detected as punctate staining in the cell cytoplasm in non-HGPS individual (Figure 5C). When localized to the media, the progerin-positive cells were negative for SMA (Figure 5C, bottom row). Taken from...
together, our results show that progerin-positive cells reside in non-HGPS arteries and that vascular progerin accumulates in vivo with age.

As a control, we explored lamin A/C expression in a subset of young and elderly subjects by IHC. As expected, all cells from the media, adventitia, and intima were positive for lamin A/C across all age groups. Representative IHC with the lamin A/C antibody in a 3-year-old normal control and a 84-year-old with CAD is presented in Supplemental Figure IV.

HGPS Displays Severe Atherosclerosis of the Aorta

We observed thickened intima and adventitia in the ascending aorta from HG001 (Supplemental Figure VB and VD). The media was degenerated, with approximately 50% loss of medial SMCs predominantly on the luminal side. The aortic media exhibited foci of SMC loss (Figure 5F and 5H, arrow), and Movat staining suggested increased PG accumulation with modest elastic tissue fragmentation (Supplemental Figure VF). Progerin was highly expressed in the intima (data not shown), media, and adventitia (Supplemental Figure VH and VI). As previously observed in the coronaries, Picrosirius Red staining of HGPS aortas showed abundant adventitial type I collagen, with large, well-organized fibrils (Supplemental Figure VL). Taken together, our data show a severe adventitial thickening, which likely results in a stiffer, less compliant aorta.

Pathology of the Valves in HGPS

Consistent with previous reports showing thickened aortic and mitral valves by echocardiography in HGPS children, the mitral valve in patient HG001 showed extensive degenerative changes, including foci of calcification and expansion of fibrosa and ventricularis (Figure 6A). The spongiosa was markedly expanded (Figure 6B), and myofibroblasts were surrounded by large deposition of ECM. These findings correspond to generalized degeneration of the valvular tissue that are highly unusual in a young child but that occur frequently in geriatric mitral valves. High amounts of progerin were also present in the mesenchymal cells populating the valve (Figure 6C).
Figure 5. Progerin in coronary arteries of non-HGPS subjects with increasing age. A, Progerin-positive cells per 1000 total cells plotted as a function of age in years and the 3 arterial layers. Lines and bands represent the best fit lines and their 95% confidence intervals as determined by negative binomial general estimating equation, in the plaque, media, and adventitia. Samples from the adventitia had significantly higher rates of progerin-positive cells over the entire age range than media (P<0.001) and plaque (P<0.001). The 3 arterial layers showed significant increases in rate across ages (P<0.0001). B, Representative IHC in a 3-year-old (yo) normal control, a 43-year-old CAD patient, and a 93-year-old with advanced-complex plaque. Left to right: progerin (red), SMA (green), 4',6-diamidino-2-phenylindole (DAPI) (blue), and merged images. Bottom: example of progerin-positive cell that is not SMA-positive in the media of a 93-year-old with CAD. The arrows indicate the progerin-positive cells. ad indicates adventitia; m, media; i, intima. (Scale bars: 10 μm.)

Figure 6. Valve and endocardium pathology in HGPS. Valves: A, The mitral valve of patient HG001 is thick and degenerated, with visible calcification. Both the fibrosa (F) and the atrialis (A) are expanded. B, The spongiosa (S in panel A) is myxomatous and markedly expanded. The boxed area is shown at higher magnification on the right. The arrow points to high ECM content. C, IHC showing expression of progerin (red) in the valves of HG001 (4',6-diamidino-2-phenylindole [DAPI], blue). D, Endocardium of the left ventricle is thickened compared with another region of the left ventricle (E) with normal endocardium (H&E). Higher magnification is shown on the left corner. IHC: F, the endocardium contains abundant progerin-positive cells (red). DAPI (blue). The arrows in D and F indicate the enlarged endocardium. (Scale bars: A, B, D, and E, 500 μm; C and F, 50 μm.)
Cardiac (Endocardium) Fibrosis in HGPS

We looked for structural alteration within the cardiac muscle of HGPS patients. We observed a remarkable left endocardial thickening in HG001 (Figure 6D) characterized by a high PG content (data not shown), suggesting that the stromal cells had adopted a synthetic phenotype relative to nonaffected areas of the left ventricle (Figure 6E). It is not clear whether this was due to a primary progerin-induced lesion or is secondary to ischemia-induced ventricular luminal dilation resulting in endocardial fibrosis. Interestingly, high amounts of progerin were present in the endocardial fibroblasts (Figure 6F). Similar observations were made in the left ventricle of HG120.

Discussion

Relatively little is known regarding the cardiovascular pathology of HGPS. Although there are cardiac and vascular commonalities between HGPS and aging, such as severe vessel blockage, there is also a lack of classic risk factors in HGPS, such as hypercholesterolemia and increased serum high-sensitivity C-reactive protein18 early stage hypertension, and smoking. Isolated from these risk factors, the study of HGPS may provide an opportunity to discover new elements that influence the vascular disease of aging. Reports to date have not examined genetically confirmed HGPS and therefore are difficult to interpret. Here we describe the cardiovascular pathology in 2 children with the de novo heterozygous mutation 1824C>T in LMNA and typical HGPS disease course, who lack CVD risk factors established for the general population. In the face of this, we found global atherosclerosis and a pathological profile that overlaps significantly with classic atherosclerosis of aging.

Similar to geriatric CVD, we found a spectrum of early to late-stage plaques in the HGPS patient samples. Arterial lesions in both typical atherosclerosis and HGPS exhibit calcification, inflammation, and evidence of plaque erosion or rupture. Although HGPS lesions tended to have smaller atheromatous cores relative to more typical atherosclerosis, this may be attributable to the lack of hypercholesterolemia and dyslipidemia in the HGPS patients. In our study, the composition of the HGPS lesions indicates that the ECM is similar to adult CVD consistent with progressive atherosclerotic lesion development and an in situ inflammatory process.19 Most likely, multiple cell types are involved in the HGPS vascular pathology. Macrophages may have a role, as well as VSMCs which have potentially limited capacity for cell renewal.

In contrast to typical adult CVD, however, we identified markedly thickened adventitia in large, medium, and small arteries and in veins. This is a new finding, not noted in previous reports of progeria cases. It is anticipated that such profound fibrosis would lead to diminished vascular compliance, increased vessel stiffness, and potential predisposition to formation of intimal plaque. In HGPS, progerin accumulation may be a major factor that underlies the development of these premature vascular lesions.

The adventitia is rapidly gaining recognition as an active participant in the development of atherosclerosis and vascular response to injuries. Aortic stiffness can contribute to increased afterload and development of left ventricular hypertrophy, such as that observed in patient HG001. Progressive vascular stiffness occurs in geriatric patients and is considered a major predictor of adverse coronary events,20 although it is typically accompanied by a much milder degree of adventitial fibrosis.

What underlies increased adventitial fibrosis observed in HGPS? Changes in collagen deposition and organization in response to mechanical stress or inflammation can result in adventitial fibrosis and luminal narrowing.21 In vitro, HGPS fibroblasts have decreased viability and are susceptible to oxidative stress, and the nuclear lamina has a significantly reduced ability to rearrange under mechanical stress.22,23,24 Chronic ischemia can also induce adventitial fibrosis.25 These same factors play a role in the evolution of atherosclerosis of aging.26

Clinically, scleroderma-like skin findings and joint contractures in HGPS strongly imply that ECM abnormality is responsible for some disease sequellae. Further elucidation of the mechanisms that result in systemic vascular fibrosis in HGPS will aid more specifically targeted therapeutic interventions for this aspect of the disease. Given the abundance of dense collagen in the adventitia of the large and small arteries, it would be interesting to evaluate treatments that influence matrix architecture or tissue fibrosis, such as alagebrum27 or statins,28,29 respectively.

For the first time, we show that progerin is widely present in the arterial walls and intimal plaques of HGPS patients, involving coronary arteries, aortas, arterioles, and veins. VSMCs and adventitia showed dramatic accumulation of progerin localized into a thick, rim-like structure at the nuclear envelope. Ubiquitous progerin presence within the vasculature implies a direct role for this protein in progressive CVD, as well as possible indirect influence.

We also identify a new component in the typical aging process by demonstrating that progerin is present in the coronary arteries of non-HGPS aging individuals and increases with advancing age. Thus, resident vascular cells infrequently use the cryptic splice site in exon 11 of LMNA in vivo. Interestingly, in normal fibroblast lines, progerin-positive cells exhibit mitotic defects that increase with passage number.7,8 This observation supports a correlation between progerin-induced mitotic abnormalities and normal aging. The highest number of progerin-positive cells in non-HGPS arteries was in the adventitia, introducing the possibility that some vessel insult is initiated in this deep vessel layer and subsequently damages the intima, heralding plaque formation.

In our aging cohort, progerin-positive vascular cells were largely SMA negative. Although we did not attempt to further analyze their specific identity, their general shape was fibroblastoid. Some cells may be adventitial fibroblasts, or perhaps immune cells, such as macrophages or other cell types that accumulate in response to resident cell death. Cells within the media could potentially be inflammatory cells as well, or SMA-negative dedifferentiated VSMCs commonly found in atherosclerotic lesions.30 Future study to identify the progerin-positive cell types in aging vessels would help to
elucidate what roles they play in the development of atherosclerosis.

Although the rodent model shows prominent SMC dropout from the media of older HGPS arteries,12 medial SMC dropout was not a prominent feature in our human study. In our study, we could not distinguish the mild medial cell dropout in HGPS from the typical secondary effects of atherosclerosis. The reasons for the murine and human differences are unclear, but it should be noted that even in the mouse model, SMC dropout is highly variable within the vascular tree, and some areas did not display loss (F. Collins, personal communication). Thus, the available sampling from the HGPS human cases may not have encompassed the same areas of the aorta that showed severe dropout in mice. Of note, a prior human autopsy (though not definitively HGPS because of lack of genetic analysis) noted unusual aortic medial SMC depletion, the extent of which varied from site to site.10 Alternatively, medial cell death may not influence human vascular pathogenesis as strongly in the human as in the HGPS mouse model.

Additional work, beyond the scope of the current study, would be valuable in further elucidating a pathological association between progerin expression and the development of atherosclerosis in both HGPS and the general population. For example, does the comparatively small—but steadily increasing—level of progerin influence age-related atherosclerosis by inducing a low-level, smoldering, chronic injury? This might explain the differences in adventitial pathology between HGPS, in which progerin is extensive, and aging, in which progerin is low but persistently increasing. The question could be addressed by study of progerin expression in a larger cohort of non-HGPS individuals with a well-defined cardiovascular medical history (low versus high CVD risk).

We speculate that progerin accumulation in vascular cells causes nuclear defects and increased susceptibility to mechanical strain that in turn triggers some combination of cell death and inflammatory response, resulting in atherosclerosis. Because oxidative stress-induced free radicals have been implicated in vitro in the pathology of HGPS,24,31 a systematic quantitative comparison of lipid peroxidation products in HGPS and geriatric samples is warranted. Finally, because overexpression of farnesylated prelamin A has been implicated in progeroid damage,32,33 a systematic pathological examination of prelamin A expression in HGPS and in aging vessels could further identify key roles for altered lamin A proteins in these populations.

Atherosclerosis is a consequence of arterial wall healing in response to injury. In most individuals, this is a multifactorial process with contributions from a host of known risk factors (hypertension, hypercholesterolemia, etc.) but with a significant component of unidentified contributing factors. This study supports the possibility that progerin is a contributor to the risk of atherosclerosis in the general population. The current observations arise from a small-scale survey; however, the presence of progerin in aging vasculature merits examination as a potential new element influencing vascular health with aging.

Acknowledgments

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Disclosures

None.

References


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Supplemental Methods:

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Clinical Information: Medical information for HG001 and HG120 was obtained from The Progeria Research Foundation (PRF) Medical and Research Database (www.progeriaresearch.org/medical_database.html) at the Brown University Center for Gerontology and Health Care Research (Providence, RI).

Autopsy Specimens: Autopsy tissue from HG001 and HG120 were obtained from the PRF Cell and Tissue Bank (www.progeriaresearch.org/cell_tissue_bank.html) at Rhode Island Hospital (Providence, RI). Non-HGPS tissues were obtained from the CVPath Institute, Inc (Gaithersburg, MD).

Mutation Analysis: Mutational Analysis for HG001 and HG120 was performed via the PRF Diagnostics Program (www.progeriaresearch.org/diagnostic_testing.html). For HG001, fibroblasts (cell bank reference: HGADFN001) were cultured and DNA was isolated. Amplification and sequencing of the LMNA exon 11 was performed by PreventionGenetics (Marshfield, WI). For patient HG120, DNA was isolated from paraffin sections of the liver, submitted to two rounds of PCR followed by dideoxy sequencing. Amplification and sequencing of the LMNA exon 11 was performed by the Laboratory for Molecular Medicine (LMM) Cambridge, MA.

Histochemistry: Tissues were fixed in 2% paraformaldehyde and embedded in paraffin or frozen in OCT medium. Cross-sections (6 µm) were stained with hematoxylin/eosin and Movat.
Images were captured using a Nikon Eclipse 6600 Microscope equipped with QImaging Retiga 1300 digital camera.

**Immunohistochemistry (IHC):** Lamin staining was previously described in detail. Briefly, antibodies used in this study were: mouse monoclonal anti-lamin A/C non-diluted (MAB3211; Chemicon, pure); monoclonal anti-smooth muscle α-actin FITC-conjugated (1:100; clone 1A4; Sigma-Aldrich); Alexa Fluor 594 or 555-conjugated were used as secondary antibodies (1:500; Molecular Probes). Lamin A/C expression was explored using the MAB3211 antibody in 9 control individuals (3 individuals/age group: 0 to 20-years old, 80-years-old and above.)

Progerin antibody is a rabbit polyclonal 972 and used at 1/500 dilution. Anti-progerin antibody stains progerin specifically, and does not cross-react with lamin. Sections of non-HGPS individuals were subjected to a 2 min EDTA antigen retrieval treatment in a pressure cooker and further stained with the anti-progerin antibody. Slides were mounted in DAPI-containing medium (Vector Laboratories). Fluorescence emission images were obtained with a confocal microscope system (LMS 510; Zeiss) using 40x or 65x oil lenses. Progerin-positive cells and progerin negative cells were quantified on sections of LAD of non-HGPS individuals and a negative binomial generalized estimating equation used to model percent progerin staining as a function of age, allowing for within-subject correlation. Rate was calculated by modeling progerin-stained cell counts, offset by the logarithm of the total cell count (proc genmod, SAS version 9.2, SAS Institute, Cary, NC). There were no differences in inferences after Holm adjustment for multiplicity and so unadjusted p-values were reported.

**Histological Evaluation of Extra Cellular Matrix:** Decorin, biglycan and versican were detected with a one hour pre-incubation with chondroitinase ABC (Sigma) followed by either rabbit polyclonal antiserum for decorin (LF-122 diluted 1:500, from Larry Fisher, National...
Institute of Dental Research, Bethesda, MD), biglycan (LF-51 diluted 1:2000) or a mouse monoclonal antibody against human versican (2B1 (Calbiochem) diluted 1:1000). Macrophages were detected by heating sections in sodium citrate buffer (10 mM sodium citrate, 0.05% Tween 20, pH 6.0) for 30 minutes followed by a monoclonal mouse anti-human CD68 (KP1 (Dako) diluted 1:100). Hyaluronan was detected with a biotinylated hyaluronan binding protein preparation (b-HABP, 3 µg/ml) made as described. CD44 was detected with a mouse monoclonal anti-human CD44 (A3D8 (Abcam) diluted 1:50). Biotin-conjugated secondary antibodies were used and detected with Vectastain ABC kit (Vector) followed by peroxidase substrates NovaRed (Vector) or DAB (Vector). IgG isotype controls were carried out for each antibody (data not shown). Collagen was visualized with Picrosirius Red and viewed under polarized light. Lipid was detected in frozen sections of HG001 using Oil Red O. All images of ECM were obtained using a Leica (Deerfield, IL) DM 2500 scope equipped with a Diagnostic Instruments (Sterling Heights, MI) Insight 4 megapixel color CCD camera with SPOT software.

Clinical Information:

Case history HG001

Early Course and Diagnosis: HG001 was a white female who died at age 9.9 years, after a clinical course typical for HGPS. She was born at 39 weeks gestation. Birth weight was 3-5%ile (2.5 kg) and hovered between 5-10%ile until age 5.7 months when she dropped below the 3%ile indefinitely. Birth height was 25%ile (47.6 cm) and gradually decreased to below the 3% ile at age 18 months. Despite nutritional intervention, she reached a maximum weight and standing height of 11.8 kg and 102 cm, respectively. Developmental milestones and intellect were normal throughout life. At age 2.5 months, the skin on the lower trunk and legs became mottled and
sclerotic. Over the next year, she developed typical signs of HGPS including prominent veins, receding mandible, high-arched palate, and delayed and crowded dentition; alopecia began at age 1.6 years. Skeletal findings included hypoplastic clavicles, coxa valga, and eventual bilateral avascular necrosis of the hips, without history of fracture. She was diagnosed clinically with HGPS at age 2.9 years. Karyotype analysis at age 6 months was normal 46XX. Genetic diagnosis was accomplished post-mortem (see Methods).

**Family History:** Parents were in good health and without history of hypertension or hyperlipidemia. Maternal grandparents developed hypertension and hypercholesterolemia in their sixth decade. Paternal grandparents had history of cancer as adults, with paternal grandfather deceased at 36 (pneumonia, cancer involving low back). There was no family history of lipodystrophy.

**Blood Lipids:** Lipid profiles were obtained at ages 7 and 22 months (Supplemental Table 2), with normal findings at 7 months and slightly elevated serum triglycerides at age 22 months. No lipid profiles were available at older ages.

**Medications:** The only medication taken routinely was low dose aspirin, which was taken daily between ages 4 and 7 years, and then resumed at 9.8 years of age. Ibuprofen and acetaminophen were taken intermittently for pain (hip pain, headache).

**Glucose Tolerance:** Fasting insulin and glucose levels were not measured.

**Neurovascular:** At age 7.5 years, HG001 began experiencing recurrent morning headaches. Over the next two years, the headaches increased in frequency, and were accompanied by nausea.

Between ages 8 and 9.5 years, prolonged headaches preceded three transient ischemic attacks. At age 9.8 yrs, two weeks prior to death, she had a cerebrovascular accident characterized by syncope and fatigue, as well as severe headache with vomiting and seizure-like activities - body
stiffening followed by whole body limpness, incontinence of urine, and right-sided weakness. EEG demonstrated posterior slowing; MRI/MRA showed bilateral occipital-parietal edema, left greater than right.

**Cardiac:** Blood pressures were within normal limits for age throughout life; detailed blood pressure history is shown in Supplementary Table I. Annual electrocardiograms (EKG) and cardiac echocardiograms were normal from age 3 to 8.8 years, with a systolic vibratory Still's murmur noted. At age 8.8 yrs, she developed symptoms of occasional chest discomfort, a new murmur, and an echocardiogram demonstrating mild mitral stenosis, reserved ventricular function, and normal-appearing proximal coronary arteries. Chest pain frequency and duration increased over the next year, without additional findings on exam or EKG, until two weeks prior to death when she exhibited cardiomegaly on chest X-ray (CXR). EKG revealed LVH, and a new diastolic murmur was heard. On the day of death the patient presented with substernal chest pain, vomiting, fever, sinus tachycardia, a gallop rhythm, ST segment changes on EKG, and interstitial edema on CXR. Troponin-1 values were 4.0 and 6.0 (normal 0-0.8), consistent with acute myocardial infarct. Despite oxygen, morphine, nitroglycerine drip and propranolol, she developed bradycardia, followed by ventricular tachycardia, ventricular fibrillation, and asystole.

**Case History HG120**

HG120 was a white male who died at age 14.0 years, after a clinical course typical for HGPS. Early Course and Diagnosis: HG0120 was a white male born at 36 weeks gestation, weighing 2.98 kg (10%ile). Despite nutritional intervention, his weight was below the 3rd %ile at 11 months and remained so for his lifetime. Developmental milestones and intellect were normal throughout life. By one year of age, external features (alopecia, micrognathia, dystrophic nails,
absence of subcutaneous fat, and dental crowding) suggested HGPS. Clinical diagnosis of HGPS was made at age 3; karyotype analysis performed at age 7.5 was normal.

**Family History:** Parents and sibling reported good health, with no history hypertension or hyperlipidemia. Paternal extended family members reported to have late onset diabetes; paternal grandmother developed hypertension and myocardial infarction in later years. There was no family history of lipodystrophy.

**Blood Lipids:** Lipid profiles were obtained at ages 4.2, 7.0, 12.9 and 13.9 years (Supplemental Table II). The only abnormal findings were at age 12.9 years, when cholesterol and triglycerides were slightly elevated.

**Medications:** Aspirin was started at 12 years at dose of 240 mg twice per day, (reportedly for myalgias). At age 13, he was prescribed Theophyllin 100 mg TID. At age 13.6, he began daily Digoxin and diuretics (spironolactone and chlorothiazide), using nitroglycerin for chest pain. At age 13.8 years, after presenting with pulmonary edema he was treated acutely with furosemide and continued on above regimen. At age 14 years, daily oral furosemide was added, along with home oxygen and bed elevation for symptoms of dyspnea at rest.

**Glucose Tolerance:** There was mild insulin resistance by age 7, without frank diabetes. Insulin sensitivity was tested twice via intravenous stimulation tests. First, at age 4.2 - Tolbutamide and glucagon stimulation tests were normal, indicating normal insulin sensitivity. Second, at age 7.0, an intravenous carbohydrate tolerance test (insulin and glucagon stimulation test), revealed normal fasting blood sugar with normal response to glucagon, although blood sugar response to insulin was muted, suggesting mild insulin resistance.

**Cardiac:** HG120 was normotensive, and without clinical evidence of transient ischemic attacks or strokes throughout his life; detailed blood pressure history is shown in Supplementary Table I.
Cardiac exam, EKGs, CXR, and echocardiograms were initially within normal limits; at age 8, a grade I/VI systolic ejection murmur was detected, and at age 9, there was intermittent left lower chest pain. At age 12, he developed increased interstitial markings on CXR without overt pulmonary infection, and within the next 8 months experienced increasing episodes of angina, two-flight dyspnea on exertion, grade II/VI systolic murmur, prominent interstitial markings, peribronchial thickening, and small areas of atelectasis on CXR. At age 12.9, there was a grade III/VI systolic murmur and bilateral carotid bruits, as well as anginal episodes at rest 1-2 times each week. EKG showed LVH, and echocardiogram showed normal ejection fraction, but with septal wall motion abnormalities. Pulmonary function testing revealed obstructive lung disease with diffuse increased interstitial markings. Over the subsequent year, he developed increasing episodes of angina and dyspnea; physical exam revealed a prominent S3, hyperkinetic carotids, pulsatile jugular venous distension to 4 cm in the upright position, and the EKG showed inferior and lateral ischemia. Despite digoxin, diuretics, nitrates, and salicylate, he developed progressive congestive heart failure and angina; he died of cardiac arrest at home.

**Autopsy Findings:** Both the original review at the institutions where autopsies were performed and re-review by the study group are concordant.

**HG001:**

The heart displayed normally sized RV and RV wall thickness, and no abnormalities noted grossly or microscopically in the pulmonary circulation. There was gross and histologic evidence of myocardial infarction (MI) involving the posterior intraventricular (IV) septum as well as smaller infarctions involving the circumference of the left ventricle and papillary muscles. The MIs were of a variety of ages, ranging from remote (months) to subacute (2-3
weeks), with multifocal acute (2-3 day old) infarction. The coronary arteries all showed severe multifocal stenoses microscopically, with the right coronary focally exhibiting 95% chronic occlusion, the left circumflex artery up to 90% chronic occlusion distally, and the left coronary artery up to 50% chronic stenosis. There was no acute plaque hemorrhage, rupture, or thrombosis identified in any of the sampled coronary artery segments; additional wet tissue was not available for analysis. The findings point to both acute and chronic MI in the setting of severe three-vessel coronary artery disease, likely with a terminal arrhythmia as the cause of death.

**HG 120:**
The right ventricle showed papillary muscle atrophy and RV dilation without hypertrophy. The main pulmonary artery was normal in size as were its branches. There is no mention of pulmonary artery atherosclerotic plaque in the original autopsy report (as a surrogate to assess for chronic pulmonary hypertension), nor was it visualized histologically. There was gross and histologic evidence of MI in the interventricular septum, extending into the anterior and posterior left ventricle in a focally transmural fashion. The MIs were of a variety of ages, ranging from remote (months) to subacute (2-3 weeks), with multifocal acute (2-3 day old) infarction. Microscopically, the coronary arteries all showed severe multifocal chronic stenoses (>95%) many of which were calcified. There was recent plaque hemorrhage in the left main coronary artery, but no areas of acute vessel thrombosis were sampled at the time of autopsy; additional wet tissue was not available for analysis. The findings point to both acute and chronic MI in the setting of severe three-vessel coronary artery disease, likely with a terminal arrhythmia as the cause of death.
## Supplemental Tables and Figure Legends

### Supplemental Table 1

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*When a series of pressures was obtained over several days, one representative set is shown

**Age after which medications that could affect blood pressure were routinely administered

### Supplemental Table II

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<td><strong>Age 7.0 years</strong></td>
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<td>Phospholipids (mg%)</td>
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* When a series of pressures was obtained over several days, one representative set is shown.

** Age after which medications that could affect blood pressure were routinely administered.
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<td>Triglycerides (mg%)</td>
<td>118</td>
<td>&lt;140</td>
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*Abnormal Value

**Suppl. Figure I:** A, Artery of the salivary gland surrounded by fibrosis (arrow). B, View of a hepatic vein with increased fibrosis (arrow) and C, dense fibrotic matrix is present in the hepatic triad (arrow) in HG120 (H&E stain). Perivascular fibrosis is also present in epicardial vein of D, patient HG001 (Movat stain) and E, patient HG120 and in F, the hilar lymph node vein (H&E stain). (Scale bars: 50 µm).

**Suppl. Figure II:** RCA of case HG001 was analyzed for the presence of lipid (Oil Red O), macrophages (CD68), the hyaluronan receptor CD44, and hyaluronan (HABP). Positive staining indicated in red. Sections were counterstained with hematoxylin. (Scale bars: 100 µm).

**Suppl. Figure III:** Endothelial Cells in HGPS patient have low levels of progerin. Small capillaries of the coronary arteries of HG001 are stained with progerin (red), CD31 (green) and DAPI (blue). (Scale bars: 10 µm).

**Suppl. Figure IV:** Lamin A/C in aging arteries

Lamin A/C is present in the normal coronary arteries of a 3-years-old control and in a typical coronary artery with complex plaque from a 84 years-old individual. Media, intima and adventitia were imaged. Lamin A/C (red), SMA (green) and DAP (blue). (Scale bars: 10 µm).
Suppl. Figure V: Stenosis and VSMC loss in HGPS aorta

Comparison of a 16-year-old non-HGPS (left) and HGPS (right) aorta. A, Normal individual (16-year-old) and B, HGPS proximal aorta (H&E stain). HGPS proximal aorta has an enlarged intima (i) compared to the control. C, Control aorta, D, Dense and thickened adventitia of the distal aorta in HGPS (Movat stain). E, F, Higher magnification of the pictures of the media in C and D. White arrow indicates VSMC death. G, Control aorta stained with Lamin A/C (red) and SMA (green). H, Anti-progerin (red) and SMA antibodies (green) show VSMC loss in HGPS. I, Control aorta was stained with Lamin A/C (red) and SMA (green). J, Fibroblasts and small arterioles are progerin-positive in HGPS adventitia. K, Adventitia of a 16-years-old control aorta with less condensed collagen (yellow green) compared to L, highly condensed collagen fibers (red) present in HGPS adventitia (Picrosirius red stain). (Scale bars: A-D 500 µm, E-F 50 µm, G-J 10 µm, K-L 25 µm).

References


Lipid
Oil red O

Macrophages
CD68

Hyaluronan
Receptor
CD44

Hyaluronan
HABP