Atherosclerosis in Ancient Humans, Accelerated Aging Syndromes and Normal Aging☆
Is Lamin A Protein a Common Link?

Michael I. Miyamoto*, Karima Djabali[†], Leslie B. Gordon[‡,§]
Mission Viejo, CA, USA; Garching, Germany; Providence, RI, USA; and Boston, MA, USA

ABSTRACT

Imaging studies of ancient human mummies have demonstrated the presence of vascular calcification that is consistent with the presence of atherosclerosis. These findings have stimulated interest in the underlying biological processes that might impart to humans an inherent predisposition to the development of atherosclerosis. Clues to these processes may possibly be found in accelerated aging syndromes, such as Hutchinson-Gilford progeria syndrome (HGPS), an ultra-rare disorder characterized by premature aging phenotypes, including very aggressive forms of atherosclerosis, occurring in childhood. The genetic defect in HGPS eventuates in the production of a mutant form of the nuclear structural protein lamin A, called progerin, which is thought to interfere with normal nuclear functioning. Progerin appears to be expressed in vascular cells, resulting in vessel wall cell loss and replacement by fibrous tissue, reducing vessel compliance and promoting calcification, leading to the vascular dysfunction and atherosclerosis seen in HGPS. Interestingly, vascular progerin is detectable in lower levels, in an age-related manner, in the general population, providing the basis for further study of the potential role of abnormal forms of lamin A in the atherosclerotic process of normal aging.

During the early stages of the Horus Study [1,2], among the fundamental questions to be addressed was whether ancient humans, presumably lacking exposure to modern risk factors for the development of atherosclerosis, would show signs of the disease process by computed tomographic analysis. An intriguing display in the Egyptian Museum of Antiquities in Cairo, describing the Pharaoh Menephtah (ca. 1200 BCE) as having had evidence of atherosclerosis, raised questions of how atherosclerosis in this ancient royal mummy was documented, and if accurate, what implications such an observation might have on our understanding of the disease.

Indeed, the Horus Study demonstrated evidence of the presence of atherosclerosis in a variety of ancient populations, with a surprisingly high prevalence and in an age-related manner [1,2]. These findings run counter to the prevailing wisdom that vascular disease is largely a modern scourge, promoted by factors such as sedentary lifestyles, unhealthy habits, calorie-rich diets, and their resultant metabolic aberrations that proliferated as we humans moved farther away from our ancient, more basic living circumstances [3].

Given that the mummies examined in this study represent examples of humans derived from eras without long-term or intense exposure to modern-day disease-promoting risk factors, questions then arise as to what specific factors might impart to humans an intrinsic risk of atherosclerosis. Is the aging process itself, indeed, among the most potent risk factors? If aging is paramount, what specific age-related biological processes might contribute to the development of vascular disease?

Syndromes of accelerated aging, very rare diseases characterized by patients developing physiologic aging processes compressed into short time frames, may provide relevant clues into vascular disease of normal aging. In a specific accelerated aging syndrome, Hutchinson-Gilford progeria syndrome (HGPS), affected individuals demonstrate a characteristic aging phenotype in childhood, including an aggressive form of atherosclerosis, with death occurring at a mean age of 14 years, due to myocardial infarction or stroke, in the face of normal metabolic parameters [4] and lack of significant family or smoking history [5].

As HGPS patients and many of the ancient humans in the Horus Study demonstrate atherosclerosis without intense exposure to conventionally accepted vascular risk factors, could they together provide insight into the biological processes that more generally contribute to the development of age-related vascular disease?

THE HORUS STUDY—EVIDENCE OF ATHEROSCLEROSIS IN ANCIENT HUMANS

Using computed tomographic imaging techniques and the presence of focal vascular calcification as a pathognomonic sign of atherosclerotic plaque, and leveraging the sensitivity of computed tomographic imaging to detect calcium, the Horus Team found radiographic evidence of atherosclerosis in 4 separate populations of pre-industrial humans, including groups of hunter-gatherers and forager-farmers [2] (Fig. 1). Vascular calcification consistent

with probable or definite atherosclerosis was observed in 47 of 137 mummies (34%) studied. Furthermore, the disease was found in groups with varying lifestyles, diets, and genetics with great temporal and geographic diversity. Of note, estimated age at death was found to be a determinant of the presence and extent of atherosclerosis, with the odds of increasing atherosclerosis severity increased by nearly 70% per decade of life (Fig. 2).

These findings of vascular calcifications in pre-industrial humans raise fundamental questions about the nature of atherosclerosis, and whether humans as a species possess an underlying, age-related predisposition to the disease, independent of the effects of conventionally accepted modern vascular risk factors. Potential factors promoting the development of atherosclerosis in at least some populations of ancient humans include chronic infections and inflammation, as well as exposure to smoke from indoor cooking fires [2].

If ancient human mummies represent a model of age-related atherosclerosis occurring in the absence of intense exposure to "modern" vascular risk factors, then perhaps an examination of features of HGPS may provide yet more important insights into some of the relevant age-dependent biological mediators involved.

HUTCHINSON-GILFORD PROGERIA SYNDROME— GENETICS AND ACCELERATED VASCULAR AGING

Due in part to its extreme rarity (1 in 4 million live births), HGPS was not described until the late 1800s by Jonathan Hutchinson and Hastings Gilford [6–8]. Gilford recognized that many of the features of the disease involved normative aging processes that, strikingly, appeared in young children. The condition is characterized by phenotypic features developing in childhood that are generally

found in the elderly, including alopecia, subcutaneous fat loss, decreased bone density, and a dramatic form of premature accelerated atherosclerosis (Fig. 3).

In a major milestone in the understanding of the disease, the genetic abnormality that underlies HGPS was elucidated, in 2003 [9,10], as a sporadic, autosomal dominant, silent mutation in the LMNA gene. The LMNA gene codes for lamins A and C, major structural proteins of the nuclear lamina, a protein scaffolding network underlying the inner nuclear membrane [11]. In about 90% of HGPS patients, a C $\rightarrow$ T mutation occurs at position 1,824 of codon 608 of exon 11; the other 10% of patients possess mutations within the intron 11 splice donor sequence and produce the same aberrant disease-causing protein [9]. In all cases, activation of a cryptic splice donor site 150 base pairs upstream of the 3′ end of exon 11 favors alternative splicing of the LMNA messenger ribonucleic acid, leading to production of a truncated mutant prelamin A, called progerin, with a 50 amino acid deletion near its C-terminus. Progerin acts in a dominant negative pattern; it is the presence of progerin, which causes disease [12].

Both wild-type lamin A and mutant progerin undergo a series of post-translational modifications, including the addition of a farnesyl group to the C-terminal end, which is thought to aid in the targeting of these proteins to the nuclear envelope [13]. Mature lamin A is then derived from cleavage of the C-terminal 15 amino acids, including the farnesyl group, by the metalloproteinase FACE1/Zmpste24 [14] (Fig. 4A [15]). However, in HGPS, progerin is missing its FACE1/Zmpste24 cleavage site due to this 50 amino acid deletion (Fig. 4B); it therefore retains its farnesyl group, preventing the movement of the mutant protein from the nuclear envelope to the nucleoplasm for protein degradation [9]. It stays intercalated within the nuclear membrane via hydrophobic interaction with lipids and/or
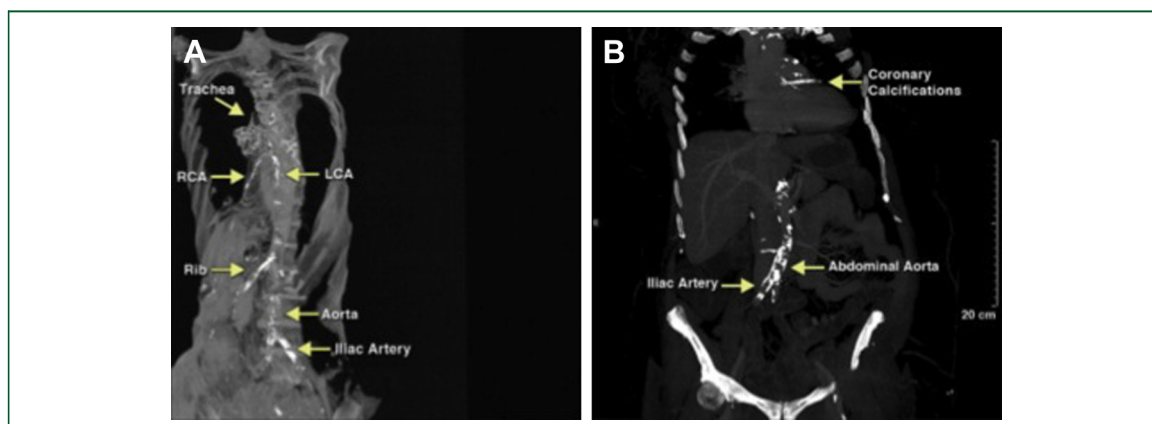

FIGURE 1. Atherosclerosis in an ancient Egyptian mummy and a modern Egyptian patient. (A) Computed tomographic image of the mummy of a princess who lived during the Second Intermediate Period shows calcifications in the coronary and iliac arteries and aorta, indicating this person had diffuse atherosclerosis. (B) Computed tomographic scan from a modern Egyptian patient showing similar calcifications in the coronary and iliac arteries. LCA, left coronary artery; RCA, right coronary artery. Reproduced with permission from: Thompson RC, Allam AH, Lombardi GP, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381:1211–22 [2].
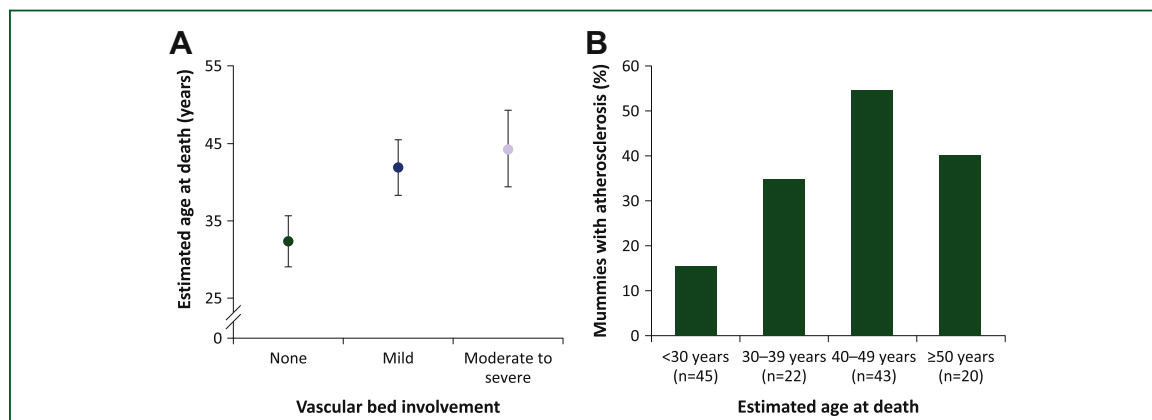
FIGURE 2. Age-related prevalence and extent of atherosclerosis in pre-industrial humans, in the Horus Study. (A) Mean age of death versus severity of atherosclerosis. (B) Frequency of atherosclerosis by age group. Reproduced with permission from: Thompson RC, Allam AH, Lombardi GP, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381:1211-22 [2].

FIGURE 3. Photograph of a 17-year-old girl with Hutchinson-Gilford progeria syndrome. Courtesy of The Progeria Research Foundation.

stronger association to integral membrane proteins, disrupting the overall nuclear laminar organization and structure. Nuclei from progerin-positive cells demonstrate a characteristic abnormal, blebbed appearance as well as other consistent signaling abnormalities (Figs. 5 [16] and 6). Because the nuclear lamina plays important roles in gene transcription and regulation, deoxyribonucleic acid (DNA) repair, and heterochromatin organization [17], progerin-induced effects on the nuclear lamina appear to underlie the cellular abnormalities observed in HGPS, including premature cellular senescence.

As part of the HGPS disease process, affected individuals universally demonstrate an aggressive vasculopathy, resulting in death at a young age due to myocardial infarction or stroke. The vasculopathy is characterized by loss of medial smooth muscle cells, adventitial thickening, intimal calcification, and atherosclerotic plaquing in the great vessels, small arteries, and arterioles [18]. Many, but not all, of the observed features of these vascular abnormalities share similarities with findings seen in senile atherosclerosis. Some features, such as inflammation and intimal lipid deposition, were not observed in an early description of HGPS vessels, although the subjects of these early reports were not genetically confirmed [18].

More recently, coronary arterial findings in 2 HGPS patients (ages 9.9 and 14 years) who died of myocardial infarction included dense fibrosis and medial thinning, with some lesions demonstrating a necrotic core and foci of chronic inflammation [19]. Coronary lesions were rich in collagen and proteoglycans and displayed large regions of calcification, similar to fibrocalcific atheroma (Fig. 7). Acute plaque rupture and thrombosis was not observed, but there was evidence of healed plaque rupture, suggesting a contribution of this process to the development of progressive, flow-limiting stenoses. In addition, thickening and calcification of the cardiac valves were observed, with high progerin expression present in valvular mesenchymal cells [19]. There
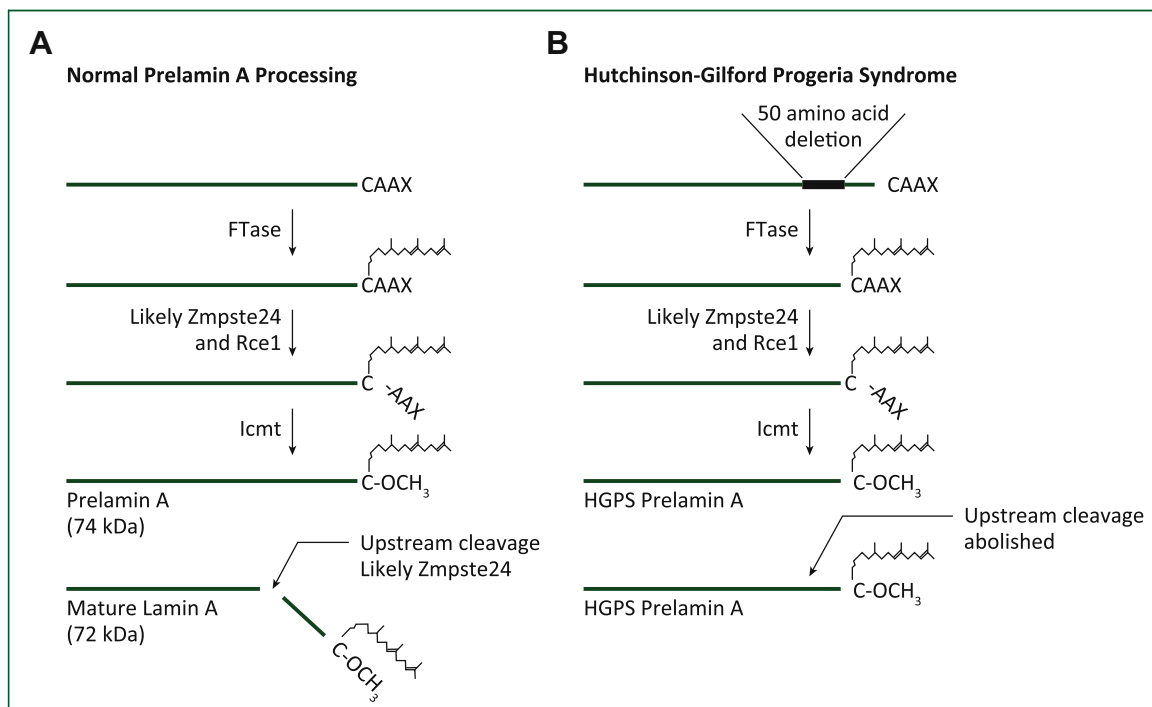
FIGURE 4. Prelamin A processing. (A) Normal prelamin A processing, with mature lamin A derived from the cleavage of the C-terminal 15 amino acids, including the farnesyl group. (B) Production of mutant lamin A (progerin), due to loss of the cleavage site, resulting in retention of the farnesyl group. Reproduced with permission from: Gordon LB, Brown WT, Rothman FG. LMNA and the Hutchinson-Gilford progeria syndrome and associated laminopathies. In: Epstein CJ, Erickson RP, Wynshaw-Boris A, editors. Inborn Errors of Development: The Molecular Basis of Clinical Disorders of Morphogenesis. 3rd edition. New York, NY: Oxford University Press [15].

was thickening of the left ventricular endocardium, with progerin found at increasing levels in endocardial fibroblasts.

ABNORMAL VASCULAR FUNCTION IN HGPS

It has been postulated that loss of medial vascular smooth muscle cells and replacement by fibrous elements results in a loss of vessel compliance, increased arterial stiffness, and vessel wall injury. Consistent with this, abnormalities in indices of vascular function have been demonstrated in HGPS patients [20]. Patients as young as 3 years of age (mean age 7.4 ± 3.4 years) were found to have measurable vascular abnormalities, including elevated carotid-femoral pulse wave
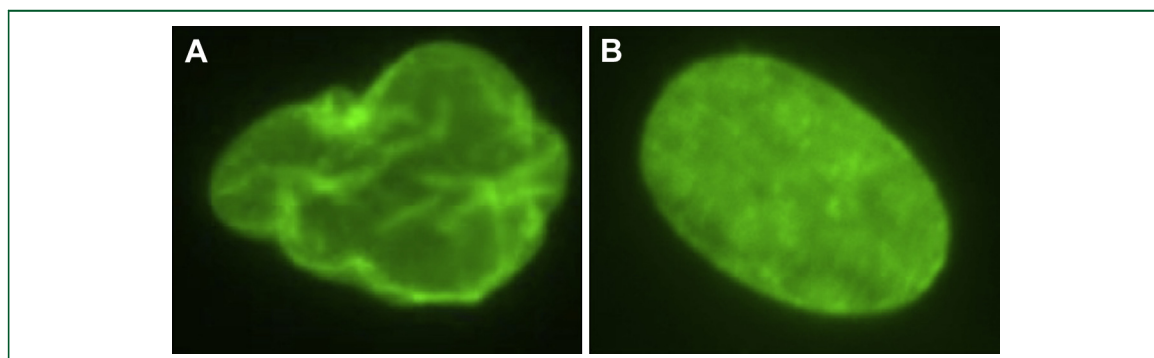

FIGURE 5. Nuclear blebbing and presence of progerin in Hutchinson-Gilford progeria syndrome and aging. Fibroblast nuclei stained with anti-lamin A antibody: (A) Hutchinson-Gilford progeria syndrome fibroblast, (B) normal aged fibroblast. The Hutchinson-Gilford progeria syndrome fibroblast demonstrates marked morphological abnormalities, with an irregular, blebbed appearance. Reproduced with permission from: Gordon LB. The premature aging syndrome Hutchinson-Gilford progeria syndrome: insights into normal aging. In: Fillet HM, Rockwood K, Woodhouse K, editors. Brocklehurst's Textbook of Geriatric Medicine and Gerontology. 7th edition. New York, NY: Saunders Elsevier; 2009. p. 66—72 [16].
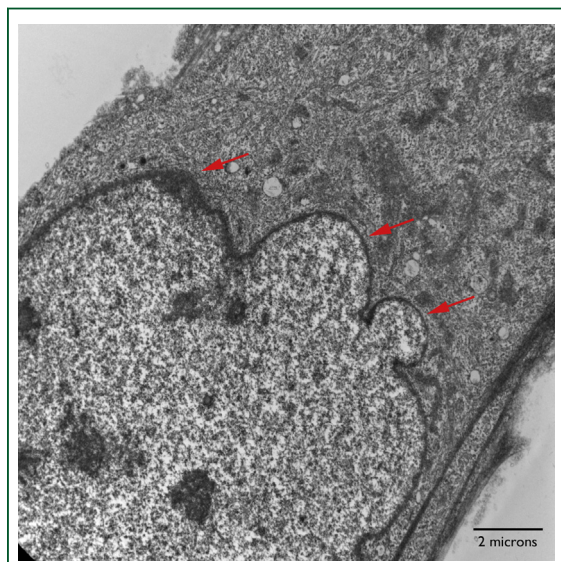
FIGURE 6. Nuclear abnormalities in Hutchinson-Gilford progeria syndrome. Low magnification transmission electron microscopic image of an HGPS nucleus showing several herniations (arrows). The altered nuclear structure is due to progerin-induced abnormalities of the nuclear envelope and lamina, the protein scaffolding network underlying the inner layer of the nuclear envelope. Note that heterochromatin is barely visible on the section. Loss of heterochromatin is one of the hallmarks of Hutchinson-Gilford progeria syndrome.

velocity, increased intima-media and adventitia echodensity (increased vascular stiffening), abnormal ankle-brachial indices, and elevated mean distal internal carotid artery flow velocity (signs of arterial occlusive disease). The equivalent pulse wave velocity for the group mean in HGPS patients was equal to that of a normal 60-year-old [21].

Notably, many of these vascular findings are commonly found in normal human aging. But in the case of HGPS, they occur in children without prolonged exposure to conventional vascular risk factors, with normal cholesterol and high-sensitivity C-reactive protein levels [4], lack of significant family history, and no smoking history, regardless of sex or ethnicity [5].

These observations have stimulated great interest in HGPS as a model for vascular aging and a possible, important role for altered lamin A expression in the development of arterial pathology in non-HGPS settings.

PROGERIN EXPRESSION IN NORMAL HUMAN AGING

The altered form of lamin A is detectable in cells and tissues from healthy, non-HGPS individuals. This important observation has further intensified interest in the possible role of progerin in age-related vascular disease.

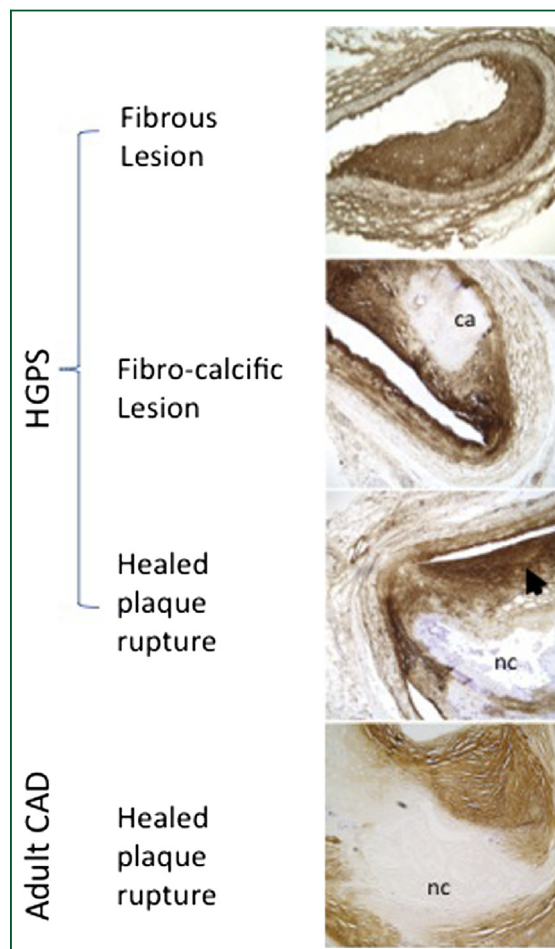The production of progerin in normal cells is thought to be due to sporadic use of the unmutated cryptic HGPS

FIGURE 7. Histological similarity of coronary artery atherosclerotic plaque in Hutchinson-Gilford progeria syndrome and adult coronary artery disease. ca, calcification; nc, necrotic core. Reproduced with permission from: Olive M, Harten I, Mitchell R, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arterioscler Thromb Vasc Biol 2010;30:2301—9 [19].

splice site. Progerin has been detected in skin fibroblast cell lines from healthy, elderly individuals, at levels approximately 50-fold less than those observed in HGPS cells, but nevertheless accompanied by observable nuclear defects and altered nuclear localization of wild-type lamin A, similar to that seen in HGPS cells [22]. The in vivo correlate shows that progerin is present in an age-dependent manner in normal aging human skin biopsies [23]. Furthermore, progerin expression has been detected in coronary arteries in the general population, in an age-related manner, using immunohistochemical techniques [19]. In an examination of coronary arteries from 29 patients ranging in age from 1 month to 97 years, progerin staining with an antiprogerin antibody was observed in the adventitia, as well as the media and atherosclerotic plaque (although at lower levels than

those in the adventitia) (Fig. 8). The progerin staining rate increased from 1 per 1,000 cells at 1 month of age to 19.66 per 1,000 cells at age 97 years. The progerin-positive cell types were thought to be adventitial fibroblasts or, less likely, immune cells that accumulate in response to cell death.

POSSIBLE MECHANISMS OF PROGERIN-INDUCED VASCULAR DISEASE

Although the mechanisms by which progerin ultimately leads to the accelerated degree of vascular disease in HGPS, and perhaps to age-related atherosclerosis, remain unknown, several hypotheses, based on both pre-clinical and clinical data, can be proposed. In one hypothesis, the nuclear abnormalities and dysregulation of the function of the nuclear lamina affect gene expression [24] and DNA repair [25]; interfere with mitosis, causing abnormal chromosome segregation and binucleation [26]; and interact with upstream telomere dysfunction [27] to induce cellular senescence, resulting in the loss of vascular smooth muscle cells and/or adventitial fibroblast dysfunction. Cell death promotes extracellular matrix accumulation as seen in the

thick, dense vascular walls in HGPS. Collectively these factors would result in adverse effects on vessel wall compliance. Progerin-related DNA damage may also promote vascular calcification by inducing a senescence-associated secretory phenotype, leading to osteogenic differentiation of vascular smooth muscle cells, as well as a reduction in the production and accumulation of extracellular pyrophosphate, which is normally a potent inhibitor of vascular calcification [28,29].

Atherosclerotic changes associated with laminopathies other than HGPS further support a potential pivotal role for abnormal lamin A in vascular disease. Altogether, there are >180 known mutations in nuclear lamin genes, associated with 13 known diseases, some of which are associated with premature vascular disease [11,30,31]. Besides HGPS, for example, premature atherosclerosis is observed in familial partial lipodystrophy, Dunnigan type (*LMNA* missense mutations in exons 8 and 11), and atypical Werner syndrome (3 autosomal-dominant *LMNA* mutations). These diseases are caused not by progerin, but by other abnormal forms of prelamin A. These observations point to the potential role of such abnormal forms of lamin A in atherosclerosis.

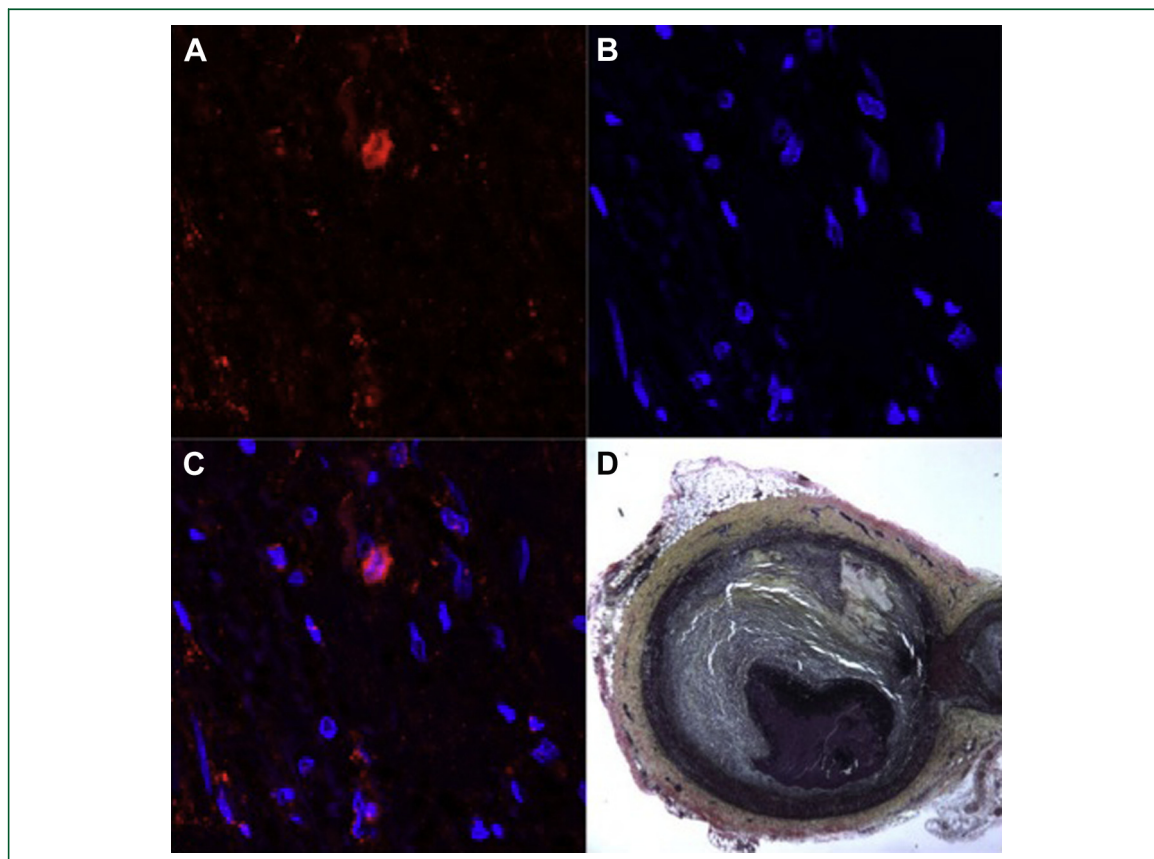FIGURE 8. Progerin expression in adult coronary artery disease. (A) Red: antiprogerin antibody staining in vessel media. (B) Blue: 4′,6-diamidino-2-phenylindole fluorescent stain of deoxyribonucleic acid. (C) Merged signal of antiprogerin and 4′,6-diamidino-2-phenylindole, demonstrating progerin-positive nuclei. (D) Photomicrograph of coronary atherosclerotic plaque. Staining performed in a coronary artery from a 31-year-old.

A repeating cycle of vascular cell damage, perhaps promoted by oxidative and mechanical stress, leading to a cascade of events resulting in increased vascular stiffness and plaque formation, placing the vasculature at risk for further oxidative and mechanical damage, may underlie the accelerated vasculopathy observed in HGPS, a subset of non-progerin-producing laminopathies, and possibly, more generally, some proportion of age-related vascular disease. Further elucidation of the specific events involved in such a process may improve the understanding of the fundamental underpinnings of atherosclerosis.

IMPLICATIONS, A POSSIBLE PARADIGM OF ATHEROSCLEROSIS, AND FUTURE DIRECTIONS

The observations of severe vascular disease occurring at a very young age in HGPS patients and the detection of progerin in non-HGPS coronary artery disease together suggest that at least a part of an inherent human predisposition to atherosclerosis may be attributable to the vascular effects of progerin and/or other abnormal lamin A forms. That such a basic human propensity to the disease exists should not be surprising, perhaps. Clinically, the occurrence of serious disease states related to atherosclerosis, such as acute coronary and stroke syndromes, is not uncommonly observed in modern patients lacking obvious vascular risk factors. In explaining these patients' situations, medical providers often vaguely cite complex genetic risk, "bad luck," or other, unrecognized factors. It is tempting to speculate that age-related vascular progerin production may be a specific, possibly even major, example of such factors.

A central role for abnormal lamin A in atherosclerosis associated with certain laminopathies, and possibly normal aging, has stimulated interest in novel therapeutic targets. For example, protein farnesyltransferase inhibitors, which inhibit farnesylation of prelamin A, thereby reducing the toxicity of mutant lamin A, are being studied as potential treatments to ameliorate some of the devastating manifestations of HGPS. One of these inhibitors, lonafarnib, has demonstrated decreases in arterial pulse wave velocity and carotid artery echodensity, indices of vascular stiffness, in children with HGPS [32]. Whether these therapies may also be effective in preventing or reducing the burden of age-associated atherosclerosis remains the subject of very early, but intriguing, speculation.

From these considerations, a proposed paradigm of human atherosclerosis may be offered (Fig. 9): 1) The development of atherosclerosis in humans is an inexorable process, perhaps in part related to progressive, age-related progerin and/or other abnormal lamin A expression and accumulation. 2) Atherosclerosis remains clinically silent, until it reaches a threshold burden of disease (flow-limiting stenosis) and/or disease activity (plaque rupture and thrombosis). 3) The background rate of the development of atherosclerosis may be accelerated to varying degrees by multiple factors, many of which may be interrelated,
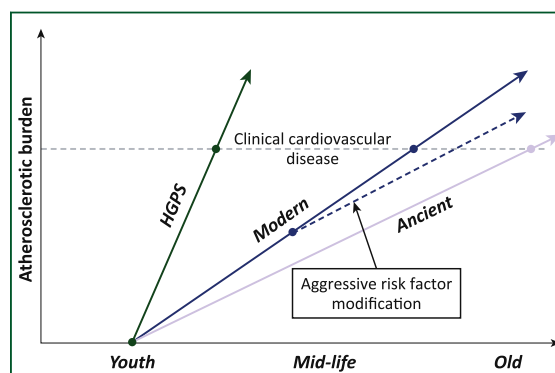

FIGURE 9. A proposed paradigm of atherosclerosis in antiquity, accelerated aging syndromes, and normative aging. The progressive, age-related nature of atherosclerosis may be in part related to expression of abnormal lamin A. Other factors such as biological regulators, infections, modern vascular risk factors, and genetic influences may modulate the rate of progression of atherosclerosis, determining the point at which clinical disease becomes manifest. Treatments that mitigate these factors may retard the rate of atherosclerosis development, preventing or deferring the onset of symptomatic disease stages. HGPS, Hutchinson-Gilford progeria syndrome.

including modern vascular risk factors (e.g., sedentary lifestyles, obesity, tobacco use, hypertension, metabolic derangements), chronic infections/inflammation (perhaps particularly relevant to ancient peoples), and genetic predispositions that have been found to be associated with higher risk of clinical vascular disease. 4) Effective risk factor management and medical therapies may retard the progression of atherosclerosis, reducing or delaying the risk of reaching a threshold level of disease or disease activity associated with clinical disease manifestations. It should be noted that the evidence of atherosclerosis in ancient humans seen in the Horus Study was not proof of the presence of clinical, symptomatic disease. However, widespread vascular calcification in multiple beds was observed in some mummies. In addition, some ancient Egyptian hieroglyphic papyri describe symptoms consistent with angina, acute myocardial infarction, and congestive heart failure [33,34].

Future study of the regulators of progerin expression in the general population will be important to examine possible environmental, infectious, and genetic influences, which may help to unify certain seemingly disparate components of the disease process. In non-HGPS skin fibroblasts, for example, progerin expression has recently been found to be stimulated by exposure to long wave ultraviolet light [35]. It is possible that other cellular stressors such as oxidative damage and mechanical stress may have similar effects on vascular cells. It is interesting to note that HGPS children with the exact same mutation may express disease progression at very different rates, with

some dying of vascular disease at age 7 years and others at age 17, suggesting that modifying biological factors involving specific regulatory proteins influence the amount of progerin production or its damaging effects, and, hence, the rate of progression of vasculopathy. Further study of these modifying factors may shed further light on the atherosclerotic process in normal aging and provide potential therapeutic targets.

The Horus Team plans to probe for the presence of progerin in ancient human mummy tissue samples, and, if detectable, to evaluate possible correlations of age and protein expression, as well as progerin detection and radiographic signs of atherosclerosis.

SUMMARY

It is perhaps ironic that humans living thousands of years ago, as well as modern children suffering an extremely rare genetic disease may together help to shed light on some of the fundamental processes that underlie one of the most common diseases of modern humans. If, from such investigations, our understanding of vascular disease is significantly advanced, perhaps forming the basis for novel preventive treatments, we will owe a debt of gratitude to both the ancients and those with HGPS.

REFERENCES

1. Allam AH, Thompson RC, Wann LS, et al. Atherosclerosis in ancient Egyptian mummies: the Horus Study. JACC Cardiovasc Imaging 2011; 4:315–27.
2. Thompson RC, Allam AH, Lombardi GP, et al. Atherosclerosis across 4000 years of human history: the Horus study of four ancient populations. Lancet 2013;381:1211–22.
3. O'Keefe JH Jr., Cordain L. Cardiovascular disease resulting from a diet and lifestyle at odds with Our Paleolithic genome: how to become a 21st-century hunter-gatherer. Mayo Clin Proc 2004;79:101–8.
4. Gordon LB, Harten IA, Patti ME, Lichtenstein AH. Reduced adiponectin and HDL cholesterol without elevated C-reactive protein: clues to the biology of premature atherosclerosis in Hutchinson-Gilford Progeria Syndrome. J Pediatr 2005;146:336–41.
5. Meredith MA, Gordon LB, Clauss S, et al. Phenotype and course of Hutchinson-Gilford progeria syndrome. N Engl J Med 2008;358: 592–604.
6. Hutchinson J. Congenital absence of hair and mammary glands with atrophic condition of the skin and its appendages, in a boy whose mother had been almost wholly bald from alopecia areata from the age of six. Med Chir Trans 1886;69:473–7.
7. Hutchinson J, Gilford H. On a condition of mixed premature and immature development. Med Chir Trans 1897;80:2–42.
8. Gilford H. Progeria: a form of senilism. Practitioner 1904:188–217.
9. Eriksson M, Brown WT, Gordon LB, et al. Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 2003;423:293–8.
10. De Sandre-Giovannoli A, Bernard R, Cau P, et al. Lamin A truncation in Hutchinson-Gilford progeria. Science 2003;300:2055.
11. Capell BC, Collins FS, Nabel EG. Mechanisms of cardiovascular disease in accelerated aging syndromes. Circ Res 2007;101:13–26.
12. Fong LG, Ng JK, Lammerding J, et al. Prelamin A and lamin A appear to be dispensable in the nuclear lamina. J Clin Invest 2006;116:743–52.
13. Hennekes H, Nigg EA. The role of isoprenylation in membrane attachment of nuclear lamins: a single point mutation prevents proteolytic cleavage of the lamin A precursor and confers membrane binding properties. J Cell Sci 1994;107:1019–29.

14. Sinensky M, Fantle K, Trujillo M, McLain T, Kupfer A, Dalton M. The processing pathway of prelamin A. J Cell Sci 1994;107:61–7.
15. Gordon LB, Brown WT, Rothman FG. *LMNA* and the Hutchinson-Gilford progeria syndrome and associated laminopathies. In: Epstein CJ, Erickson RP, Wynshaw-Boris A, editors. Inborn Errors of Development: The Molecular Basis of Clinical Disorders of Morphogenesis. 3rd edition. New York, NY: Oxford University Press. In Press.
16. Gordon LB. The premature aging syndrome Hutchinson-Gilford progeria syndrome: insights into normal aging. In: Fillet HM, Rockwood K, Woodhouse K, editors. Brocklehurst's Textbook of Geriatric Medicine and Gerontology. 7th edition. New York, NY: Saunders Elsevier; 2009. p. 66–72.
17. Zuela N, Bar DZ, Gruenbaum Y. Lamins in development, tissue maintenance and stress. EMBO Rep 2012;13:1070–8.
18. Stehbens WE, Wakefield SJ, Gilbert-Barness E, Olson RE, Ackerman J. Histological and ultrastructural features of atherosclerosis in progeria. Cardiovasc Pathol 1999;8:29–39.
19. Olive M, Harten I, Mitchell R, et al. Cardiovascular pathology in Hutchinson-Gilford progeria: correlation with the vascular pathology of aging. Arterioscler Thromb Vasc Biol 2010;30:2301–9.
20. Gerhard-Herman M, Smoot LB, Wake N, et al. Mechanisms of premature vascular aging in children with Hutchinson-Gilford progeria syndrome. Hypertension 2012;59:92–7.
21. Redheuil A, Yu WC, Wu CO, et al. Reduced ascending aortic strain and distensibility: earliest manifestations of vascular aging in humans. Hypertension 2010;55:319–26.
22. Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. Science 2006;312:1059–63.
23. McClintock D, Ratner D, Lokuge M, et al. The mutant form of lamin A that causes Hutchinson-Gilford progeria is a biomarker of cellular aging in human skin. PLoS One 2007;2:e1269.
24. Marji J, O'Donoghue SI, McClintock D, et al. Defective lamin A-Rb signaling in Hutchinson-Gilford progeria syndrome and reversal by farnesyltransferase inhibition. PLoS One 2010;5:e11132.
25. Musich PR, Zou Y. DNA-damage accumulation and replicative arrest in Hutchinson-Gilford progeria syndrome. Biochem Soc Trans 2011;39: 1764–9.
26. Cao K, Capell BC, Erdos MR, Djabali K, Collins FS. A lamin A protein isoform overexpressed in Hutchinson-Gilford progeria syndrome interferes with mitosis in progeria and normal cells. Proc Natl Acad Sci U S A 2007;104:4949–54.
27. Cao K, Blair CD, Faddah DA, et al. Progerin and telomere dysfunction collaborate to trigger cellular senescence in normal human fibroblasts. J Clin Invest 2011;121:2833–44.
28. Liu Y, Drozdov I, Shroff R, Beltran LE, Shanahan CM. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. Circ Res 2013;112:e99–109.
29. Villa-Bellosta R, Rivera-Torres J, Osorio FG, et al. Defective extracellular pyrophosphate metabolism promotes vascular calcification in a mouse model of Hutchinson-Gilford progeria syndrome that is ameliorated on pyrophosphate treatment. Circulation 2013;127:2442–51.
30. Capell BC, Collins FS. Human laminopathies: nuclei gone genetically awry. Nat Rev Genet 2006;7:940–52.
31. Al-Shali KZ, Hegele RA. Laminopathies and atherosclerosis. Arterioscler Thromb Vasc Biol 2004;24:1591–5.
32. Gordon LB, Kleinman ME, Miller DT, et al. Clinical trial of a farnesyltransferase inhibitor in children with Hutchinson-Gilford progeria syndrome. Proc Natl Acad Sci U S A 2012;109:16666–71.
33. Wreszinsk W. [Der frosse medizinische Papyrus des Berliner Museums.] Microfilm. Washington, DC: Library of Congress Preservation Microfilming Program, available from Library of Congress Photoduplication Service. Available at: http://openlibrary.org/b/OL1050325 M/grosse-medizinische-Papyrus-des-Berliner-Museums-%28Pap.-Berl.-3038%29; 1995. Accessed September 1, 2013.
34. Ebbel B. The Papyrus Ebers. Copenhagen, Denmark: Levinn and Munksgaard; 1937. p. 48.
35. Takeuchi H, Rünger TM. Longwave UV light induces the aging-associated progerin. J Invest Dermatol 2013;133:1857–62.