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Coronary Artery Disease in a Werner Syndrome-Like Form of Progeria Characterized by Low Levels of Progerin, a Splice Variant of Lamin A

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Abstract

Classical Hutchinson–Gilford progeria syndrome (HGPS) is caused by LMNA mutations that generate an alternatively spliced form of lamin A, termed progerin. HGPS patients present in early childhood with atherosclerosis and striking features of accelerated aging. We report on two pedigrees of adult-onset coronary artery disease with progeroid features, who were referred to our International Registry of Werner Syndrome (WS) because of clinical features consistent with the diagnosis. No mutations were identified in the WRN gene that is responsible for WS, among these patients. Instead, we found two novel heterozygous mutations at the junction of exon 10 and intron 11 of the LMNA gene. These mutations resulted in the production of progerin at a level substantially lower than that of HGPS. Our findings indicate that LMNA mutations may result in coronary artery disease presenting in the fourth to sixth decades along with short stature and a progeroid appearance resembling WS. The absence of early-onset cataracts in this setting should suggest the diagnosis of progeroid laminopathy. This study illustrates the evolving genotype–phenotype relationship between the amount of progerin produced and the age of onset among the spectrum of restrictive dermopathy, HGPS, and atypical forms of WS.

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Keywords

Hutchinson–Gilford progeria syndrome; Werner syndrome; lamin A; progeroid syndrome; genetic disorder; human

INTRODUCTION

Classical Hutchinson–Gilford progeria syndrome (HGPS) is a childhood onset genetic disorder characterized by a general appearance of accelerated aging with arteriosclerosis [Gordon et al., 2007; Merideth et al., 2008]. HGPS patients appear normal at birth and begin to exhibit growth retardation during the first year followed by accelerated degenerative changes and aged appearance due to alopecia, loss of subcutaneous fat, and skeletal changes. The most common cause of death is myocardial infarction at a median age of 13 [Olive et al., 2010]. HGPS is caused by a point mutation in exon 11 of *LMNA*, which generates a cryptic splice site and causes a 50-amino acid in-frame deletion [De Sandre-Giovannoli et al., 2003; Eriksson et al., 2003]. The accumulation of this deletion-mutant lamin A, termed progerin, is thought to be responsible for HGPS [Goldman et al., 2004]. Progerin is found in senescent cells in culture or in cells derived from normal elderly individuals, suggesting a role for progerin in the normal aging process [Scaffidi and Misteli, 2006; McClintock et al., 2007; Rodriguez et al., 2009; Olive et al., 2010].

Another well-known progeroid syndrome is the adult-onset condition, Werner syndrome (WS) [Leistriz et al., 2007]. WS is caused by mutations of the *WRN* gene which encodes a nuclear helicase [Yu et al., 1996; Friedrich et al., 2010]. WS patients develop normally until their teens, when they fail to experience the usual pubertal growth spurt. They then begin to suffer from skin atrophy, loss and graying of hair, bilateral ocular cataracts, type 2 diabetes mellitus, osteoporosis, and gonadal atrophy. Premature and severe forms of atherosclerosis and multiple cancers have been observed by middle age. The most common cause of death is myocardial infarction at a median age of 54 [Huang et al., 2006].

The International Registry of Werner Syndrome (University of Washington, Seattle, WA; <http://www.wernersyndrome.org>) recruits patients based on established clinical criteria of WS [Leistriz et al., 2007]. Approximately 20% of the patients clinically diagnosed as possible WS do not carry *WRN* mutations and are operationally categorized as “atypical WS.” A small subset of atypical WS subjects carries heterozygous mutations in the *LMNA* gene, mostly at the N-terminal regions of lamin A/C [Chen et al., 2003; Saha et al., 2010]. Unlike HGPS, these mutations do not generate progerin and coronary atherosclerosis is not a cardinal clinical feature in these patients.

We recently identified novel *LMNA* mutations that result in the expression of progerin in two pedigrees presenting with prominent cardiovascular disease along with an aged appearance. In both cases, arteriosclerosis was noted in the fourth to fifth decades of life, correlating with significantly lower expression of progerin compared to HGPS. Our findings indicate that laminopathies should be considered in adult cases with premature arteriosclerosis accompanied by the appearance of accelerated aging.

CLINICAL REPORTS

The first patient (Werner Syndrome Registry BUCK1010, Fig. 1) is a 37-year-old man referred to us for possible WS. He first presented at age 11 for evaluation of short stature. His height was 124 cm (Z score -3.01). He did not undergo a pubertal growth spurt nor did he respond to treatment with growth hormone. At age 23 years, his lipid profile included a total cholesterol of 124 mg/dl, HDL 28 mg/dl, LDL 60 mg/dl, and VLDL 36 mg/dl. He was not taking statin medication. At age 31, he was diagnosed with diabetes mellitus, hypertension, and had chest pain; an echocardiogram showed aortic stenosis. He underwent valve replacement and triple coronary artery bypass surgery. He developed claudication and non-healing ulcers. By age 36, he developed extensive peripheral vascular disease, and underwent stenting of the left popliteal and superior mesenteric arteries with resolution of a foot ulcer and abdominal pain. Other clinical features included hyperlipidemia, osteopenia by DEXA scan, and severe periodontal disease with tooth loss. There was no history of delay in tooth eruption or shedding of primary teeth. Ocular cataracts (which are seen in virtually all WS subjects) were noticeably absent. Physical exam at age 36 showed a height of 153.2 cm (Z score -3.99), and blood pressure of 154/69. His appearance was progeroid, with alopecia, loss of subcutaneous fat, sparse axillary and pubic hair, loss of subcutaneous fat, and mild skin tightness over the distal extremities. There was pes planus and delayed capillary refill with an ulcer of the left hallux.

There are two siblings (Fig. 1G). His 40-year-old brother is healthy, and of normal stature and appearance. The 32-year-old sister (Fig. 1) has short stature and small size (135 cm, Z score -4.3 ; weight 28.2 kg, (Z score -7.9) with a progeroid appearance and hair loss. She had undergone plastic surgery for a beaked nose. She was diagnosed with hypercholesterolemia at age 24, but does not have peripheral vascular disease or diabetes. She had delayed shedding of primary teeth; only 4 or 5 were lost spontaneously. The rest were extracted because eruption of secondary teeth had begun. She has had significant gum disease and loss of teeth. Her periods are normal. Examination was notable for loss of subcutaneous tissue, thin facial appearance with beaked nose, sparse fine hair of the scalp, axillary and pubic areas, hypoplastic nails, and patchy, fine hypopigmentation over her entire body.

The parents were non-consanguineous and of German and Irish descent. Their father was 63, of normal stature and appearance, and had a myocardial infarction at age 47. Their mother was 61-year old and described as shortest in her family (147 cm, Z score -2.51). She suffered from severe chest tightness and dyspnea in her 30s that was originally diagnosed as anxiety or panic attacks. At age 54, she suffered her first myocardial infarction, and underwent multi-vessel bypass surgery. Her right coronary artery was totally occluded and calcified. After the initial bypass, she developed recurring occlusions, and so far has had a total of 10 stents placed. She was diagnosed with osteoporosis in her 40s and diabetes at 56. Musculoskeletal problems included non-congenital hip dysplasia, with bilateral hip replacement surgery at 53 and numerous back operations for ruptured discs. She underwent hysterectomy at age 36, sparing her ovaries; she experienced hot flashes in her late 30s. She had longstanding gum disease, multiple dental cavities, and root canal surgeries. She has never had cataracts. Her facial appearance is not particularly aged, however, by comparison,

her 62-year-old sister has a much more youthful appearance. She has five siblings. One brother died young in a motor vehicle accident; the other four siblings are well without coronary artery disease or diabetes in their 50s and 60s. The mother's mother was of normal stature (163 cm) and did not have diabetes or myocardial infarctions. The mother's father was 180 cm tall; he had diabetes, poorly healing wounds, some related to fractures that developed osteomyelitis, and had his first myocardial infarction at age 60. Photographs of him are notable only for a beaked nose.

The second patient (Werner Syndrome Registry LUS1010) was a 46-year-old woman of Polish and German ancestry referred to us for possible WS. Clinical features included short stature (138.5 cm, Z score -3.8), progeroid appearance, beaked nose, scleroderma-like skin, premature graying and alopecia, atrophic skin, and dystrophic nails. Secondary sexual characteristics were under-developed with absent breast tissue and sparse axillary and pubic hair. She had an unusually high-pitched voice. The distal interphalangeal crease was absent on the right fourth finger with limited movement at this joint. She did not have diabetes, but was treated for hyperlipidemia (prior to treatment with a statin drug, triglycerides were 443 mg/dl (normal <200 mg/dl) and total cholesterol was 276 mg/dl (normal <200 mg/dl) and hypertension. She had significant coronary artery disease, aortic calcification, a myxomatous mitral valve with prolapse with a III/VI systolic murmur, and hypertension. She did not have ocular cataracts, but had otosclerosis with a 3-year history of ringing in her left ear. She had symptomatic endometriosis.

Ten days prior to her death, she suffered abdominal pain and underwent emergent right hemicolectomy for mesenteric ischemia with bowel necrosis. She developed pulmonary edema and did not tolerate extubation. Coronary artery catheterization revealed 80% stenosis of her left main coronary artery and 80% stenosis of the ostium of the right coronary artery. Severe mitral regurgitation was also diagnosed. She underwent triple coronary artery bypass surgery and mitral valve replacement, never regained consciousness and life support was terminated 1 month after her 48th birthday. Family history was notable for four brothers and one sister, who were all of normal to tall stature, a father who is still living at age 85 years, and a mother who died at age 70 of lung cancer.

RESULTS

Sequencing of *WRN* genes in the index patients showed no mutations and Western blot analysis showed the presence of WRN protein of normal size at a level comparable to control fibroblasts (data not shown). Sequencing of the *LMNA* gene in Patient 1 showed a heterozygous synonymous change, c.1968G>A, p.Q656Q (Fig. 2A). This change was also present in the presumably affected mother, and the affected sister but not in the unaffected father, indicating co-segregation of the sequence variant with the phenotype within the family. The maternal grandparents are deceased, and thus unavailable for testing. This change was located at the last nucleotide of exon 11 and could affect its splicing. Fibroblast cell lines from the index case were established and immortalized with the catalytic subunit of human telomerase (hTERT) for further study. Indeed, RT-PCR spanning exons 10–12 showed a product of smaller size in addition to the wild-type product (Fig. 2B). Sequencing of this smaller product showed an in-frame deletion of 150 nucleotides (r.1819–1968del150)

that corresponds to progerin seen in HGPS. Western analysis further demonstrated the presence of progerin (Fig. 2C). The ratio of progerin/lamin A was approximately 0.15 which is one quarter of that observed in HGPS cells.

In Patient 2, sequencing analysis of the *LMNA* gene revealed the alteration c.1968+5G>A in the donor splice site of intron 11 (Fig. 2A). Using an established hTERT fibroblast line from the proband, RT-PCR spanning from exons 10 to 12 again showed a smaller product, containing the 150 nucleotide deletion (r.1819–1968del150) along with the normal sized product, as seen in the first case (Fig. 2B). Western analysis of the second case also showed progerin at a level similar to the first case, which is considerably lower than in classical HGPS patients (Fig. 2C).

DISCUSSION

We report on two pedigrees of adult-onset coronary disease with progeroid features. Although we have not examined loci other than *LMNA* and *WRN*, this constellation of features is most likely caused by the presence of progerin known to be responsible for HGPS.

It has been noted that there may be a relationship between the size of the in-frame deletion of *LMNA* exon 11 and the age of onset as well as the clinical diagnosis. The c.1968+1G>A mutation that gave rise to the skipping of entire exon 11 and the in-frame deletion of 90 amino acids instead of 50 was reported in neonatal-onset restrictive dermopathy (RD) [Navarro et al., 2004]. However, the c.1867A>T (p.Thr623Ser) mutation that leads to an in-frame deletion of 35 amino acids was identified in a 45-year-old Japanese man with CAD and facial features of HGPS [Fukuchi et al., 2004].

There appears to be a relationship between the amount of progerin relative to wild-type lamin A and the age of onset as well as the clinical diagnosis. Moulson et al. [2007] estimated that the ratio of progerin to wild-type lamin A was approximately 1:1 in cells from an HGPS patient carrying the c.1824C>T mutation, the most common mutation found in classical HGPS, while it is close to 3:1 in cells derived from RD patient who carried a c.1968+1G>A mutant case. Interestingly, the c.1968+1G>A mutation could cause either a 50 amino acid deletion resulting in HGPS [Moulson et al., 2007] or a 90 amino acid deletion resulting in RD [Navarro et al., 2004], although the molecular mechanism determining the choice of splicing site has not been elucidated. Our cases illustrate the opposite end of the spectrum, in which a progerin to lamin A ratio of approximately 0.15:1 leads to adult-onset progeroid phenotypes resembling WS.

Our interpretation agrees with the results of the Splice Site Prediction by Neural Network (Berkeley Drosophila Genome Project; http://www.fruitfly.org/seq_tools/splice.html). The normal junction sequence, CCCAGgtgagttg, where uppercase letters are exon 11 and lowercase letters are intron 11, gave a score of 0.99, indicating an optimum splice donor site, whereas the c.1968+5G>A mutant sequence, CCCAGgtgaattg, with the mutated nucleotide underlined, gave a score of 0.44, indicating a weak splice donor site. There are two unpublished reports of a mutation at the same position but involving a different nucleotide,

c.1968+5G>C, reported in the Leiden Muscular Dystrophy Homepage (<http://www.dmd.nl>). This mutant sequence, CCCAGgtgacttg, gives a score of 0.28, which indicates an even weaker splice site and the phenotype is reported as HGPS.

The present study describes an adult-onset progeroid syndrome closely resembling WS, albeit without the occurrence of cataracts but with accelerated cardiovascular disease. Hyperlipidemia is not a typical finding in HGPS patients, although normal to slightly elevated cholesterol (particularly LDL) are seen in HGPS patients, and we do not have a large enough cohort yet to distinguish whether the lipid abnormalities in the present patients are familial or are a part of the natural history of their laminopathy. Specific *LMNA* mutations that result in the production of higher levels of progerin than normal, but lower than that seen in HGPS, should be considered in the diagnosis of such patients.

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References

- Chen L, Lee L, Kudlow BA, Dos Santos HG, Sletvold O, Shafeghati Y, Botha EG, Garg A, Hanson NB, Martin GM, Mian IS, Kennedy BK, Oshima J. *LMNA* mutations in atypical Werner's syndrome. *Lancet*. 2003; 362:440–445. [PubMed: 12927431]
- De Sandre-Giovannoli A, Bernard R, Cau P, Navarro C, Amiel J, Boccaccio I, Lyonnet S, Stewart CL, Munnich A, Le Merrer M, Levy N. Lamin A truncation in Hutchinson–Gilford progeria. *Science*. 2003; 300:2055. [PubMed: 12702809]
- Eriksson M, Brown WT, Gordon LB, Glynn MW, Singer J, Scott L, Erdos MR, Robbins CM, Moses TY, Berglund P, Dutra A, Pak E, Durkin S, Csoka AB, Boehnke M, Glover TW, Collins FS. Recurrent de novo point mutations in lamin A cause Hutchinson–Gilford progeria syndrome. *Nature*. 2003; 423:293–298. [PubMed: 12714972]
- Friedrich K, Lee L, Leistriz DF, Nurnberg G, Saha B, Hisama FM, Eyman DK, Lessel D, Nurnberg P, Li C, Garcia FVMJ, Kets CM, Schmidtke J, Cruz VT, Van den Akker PC, Boak J, Peter D, Compoginis G, Cefle K, Ozturk S, Lopez N, Wessel T, Poot M, Ippel PF, Groff-Kellermann B, Hoehn H, Martin GM, Kubisch C, Oshima J. *WRN* mutations in Werner syndrome patients: Genomic rearrangements, unusual intronic mutations and ethnic-specific alterations. *Hum Genet*. 2010; 128:103–111. [PubMed: 20443122]
- Fukuchi K, Katsuya T, Sugimoto K, Kuremura M, Kim HD, Li L, Ogihara T. *LMNA* mutation in a 45 year old Japanese subject with Hutchinson–Gilford progeria syndrome. *J Med Genet*. 2004; 41:e67. [PubMed: 15121795]
- Goldman RD, Shumaker DK, Erdos MR, Eriksson M, Goldman AE, Gordon LB, Gruenbaum Y, Khuon S, Mendez M, Varga R, Collins FS. Accumulation of mutant lamin A causes progressive changes in nuclear architecture in Hutchinson–Gilford progeria syndrome. *Proc Natl Acad Sci USA*. 2004; 101:8963–8968. [PubMed: 15184648]
- Gordon LB, McCarten KM, Giobbie-Hurder A, Machan JT, Campbell SE, Berns SD, Kieran MW. Disease progression in Hutchinson–Gilford progeria syndrome: Impact on growth and development. *Pediatrics*. 2007; 120:824–833. [PubMed: 17908770]
- Huang S, Lee L, Hanson NB, Lenaerts C, Hoehn H, Poot M, Rubin CD, Chen DF, Yang CC, Juch H, Dorn T, Spiegel R, Oral EA, Abid M, Battisti C, Lucci-Cordisco E, Neri G, Steed EH, Kidd A, Isley

- W, Showalter D, Vittone JL, Konstantinow A, Ring J, Meyer P, Wenger SL, von Herbay A, Wollina U, Schuelke M, Huizenga CR, Leistriz DF, Martin GM, Mian IS, Oshima J. The spectrum of WRN mutations in Werner syndrome patients. *Hum Mutat.* 2006; 27:558–567. [PubMed: 16673358]
- Leistriz, DF.; Hanson, NB.; Martin, GM.; Oshima, J. GeneReviews at Genetests: Medical Genetics Information Resource (database online). Copyright, University of Washington; Seattle: 2007. Werner syndrome. 1993–2011. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK1514/>
- McClintock D, Ratner D, Lokuge M, Owens DM, Gordon LB, Collins FS, Djabali K. 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. [PubMed: 18060063]
- Merideth MA, Gordon LB, Clauss S, Sachdev V, Smith AC, Perry MB, Brewer CC, Zalewski C, Kim HJ, Solomon B, Brooks BP, Gerber LH, Turner ML, Domingo DL, Hart TC, Graf J, Reynolds JC, Gropman A, Yanovski JA, Gerhard-Herman M, Collins FS, Nabel EG, Cannon RO III, Gahl WA, Introne WJ. Phenotype and course of Hutchinson–Gilford progeria syndrome. *N Engl J Med.* 2008; 358:592–604. [PubMed: 18256394]
- Moulson CL, Fong LG, Gardner JM, Farber EA, Go G, Passariello A, Grange DK, Young SG, Miner JH. Increased progerin expression associated with unusual LMNA mutations causes severe progeroid syndromes. *Hum Mutat.* 2007; 28:882–889. [PubMed: 17469202]
- Navarro CL, De Sandre-Giovannoli A, Bernard R, Boccaccio I, Boyer A, Genevieve D, Hadj-Rabia S, Gaudy-Marqueste C, Smitt HS, Vabres P, Faivre L, Verloes A, Van Essen T, Flori E, Hennekam R, Beemer FA, Laurent N, Le Merrer M, Cau P, Levy N. Lamin A and ZMPSTE24 (FACE-1) defects cause nuclear disorganization and identify restrictive dermopathy as a lethal neonatal laminopathy. *Hum Mol Genet.* 2004; 13:2493–2503. [PubMed: 15317753]
- Olive M, Harten I, Mitchell R, Beers JK, Djabali K, Cao K, Erdos MR, Blair C, Funke B, Smoot L, Gerhard-Herman M, Machan JT, Kutys R, Virmani R, Collins FS, Wight TN, Nabel EG, Gordon LB. Cardiovascular pathology in Hutchinson–Gilford progeria: Correlation with the vascular pathology of aging. *Arterioscler Thromb Vasc Biol.* 2010; 30:2301–2309. [PubMed: 20798379]
- Rodriguez S, Coppede F, Sagelius H, Eriksson M. Increased expression of the Hutchinson–Gilford progeria syndrome truncated lamin A transcript during cell aging. *Eur J Hum Genet.* 2009; 17:928–937. [PubMed: 19172989]
- Saha B, Lessel D, Hisama FM, Leistriz DF, Friedrich K, Martin GM, Kubisch C, Oshima J. A novel LMNA mutation causes altered nuclear morphology and symptoms of familial partial lipodystrophy (Dunnigan variety) with progeroid features. *Mol Syndromol.* 2010; 1:127–132. [PubMed: 21031082]
- Scaffidi P, Misteli T. Lamin A-dependent nuclear defects in human aging. *Science.* 2006; 312:1059–1063. [PubMed: 16645051]
- Yu CE, Oshima J, Fu YH, Wijsman EM, Hisama F, Alisch R, Matthews S, Nakura J, Miki T, Ouais S, Martin GM, Mulligan J, Schellenberg GD. Positional cloning of the Werner's syndrome gene. *Science.* 1996; 272:258–262. [PubMed: 8602509]

FIG. 1.

Clinical features of cases with c.1968G>A *LMNA* mutation. Proband at age 7 (A) and age 37 (B,C). The sister at age 8 (D) and age 30 (E,F). Pedigree (G) with age and genotype. Mother at age 34 and 62 (H,I). Note normal facial appearance in childhood, and prematurely aged appearance of face and hands in young adulthood, with alopecia.

FIG. 2.

Expression of progerin in c.1968G>A and c.1968+5G>A *LMNA* mutants. (A: Sequencing of *LMNA* exon 11. B: RT-PCR result of exon 10–12 of *LMNA*. C: Western analysis of lamin A/C.)