

## ORIGINAL ARTICLE

# Phenotype and Course of Hutchinson–Gilford Progeria Syndrome

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## ABSTRACT

**BACKGROUND**

Hutchinson–Gilford progeria syndrome is a rare, sporadic, autosomal dominant syndrome that involves premature aging, generally leading to death at approximately 13 years of age due to myocardial infarction or stroke. The genetic basis of most cases of this syndrome is a change from glycine GGC to glycine GGT in codon 608 of the lamin A (*LMNA*) gene, which activates a cryptic splice donor site to produce abnormal lamin A; this disrupts the nuclear membrane and alters transcription.

**METHODS**

We enrolled 15 children between 1 and 17 years of age, representing nearly half of the world's known patients with Hutchinson–Gilford progeria syndrome, in a comprehensive clinical protocol between February 2005 and May 2006.

**RESULTS**

Clinical investigations confirmed sclerotic skin, joint contractures, bone abnormalities, alopecia, and growth impairment in all 15 patients; cardiovascular and central nervous system sequelae were also documented. Previously unrecognized findings included prolonged prothrombin times, elevated platelet counts and serum phosphorus levels, measured reductions in joint range of motion, low-frequency conductive hearing loss, and functional oral deficits. Growth impairment was not related to inadequate nutrition, insulin unresponsiveness, or growth hormone deficiency. Growth hormone treatment in a few patients increased height growth by 10% and weight growth by 50%. Cardiovascular studies revealed diminishing vascular function with age, including elevated blood pressure, reduced vascular compliance, decreased ankle–brachial indexes, and adventitial thickening.

**CONCLUSIONS**

Establishing the detailed phenotype of Hutchinson–Gilford progeria syndrome is important because advances in understanding this syndrome may offer insight into normal aging. Abnormal lamin A (progerin) appears to accumulate with aging in normal cells. (ClinicalTrials.gov number, NCT00094393.)

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SOME ASPECTS OF HUMAN AGING APPEAR to be dramatically accelerated in the Hutchinson–Gilford progeria syndrome, an extremely rare sporadic disorder (Fig. 1).<sup>1–3</sup> Within approximately 13 years after birth, affected children die from cardiovascular disease. The cause is abnormal lamin A (denoted “progerin,” to distinguish it from normal lamin A), which is produced by an activated cryptic splice donor site created by a change from glycine GGC to glycine GGT in codon 608 of exon 11 of the lamin A (*LMNA*) gene.<sup>4,5</sup> Progerin disrupts the structural integrity of the inner nuclear membrane in a dominant negative fashion.<sup>6–10</sup> We prospectively characterized the clinical characteristics of Hutchinson–Gilford progeria syndrome in 15 affected children, seeking insights into the pathophysiological process of the disease, elucidating previously unappreciated

aspects of the phenotype, and proposing outcome variables for evaluating therapeutic interventions.

## METHODS

### PATIENTS

We enrolled 15 unrelated white children (including 4 Hispanic children) in a protocol between February 2005 and May 2006. Race was self-reported. This study was approved by the institutional review board of the National Human Genome Research Institute, and written informed consent and assent were obtained from all the patients and their parents, respectively. The molecular mutations in four children were previously reported,<sup>4</sup> Patient 9 was Patient 3 in another report,<sup>11</sup> and Patient 7 was pictured in a review of Hutchinson–Gilford progeria syndrome.<sup>12</sup> The weights of our patients, as record-



**Figure 1. Physical Findings in Children with Hutchinson–Gilford Progeria Syndrome.**

Panel A shows short stature in Patient 2 at 21 months of age. Panel B shows alopecia in Patient 5 at 4 years of age. Panel C shows progressive aging in Patient 9 at 7 years of age. Panel D shows prominent veins, knee joints, and contractures under maximal passive extension in Patient 12. Panel E shows tufting of fingers in Patient 13. Panel F shows phalangeal joint contractures in Patient 14. Panel G shows dimpling in the left leg of Patient 4. Panel H shows areas of hypopigmented skin in Patient 2. Panel I shows abdominal outpouching and reticulated hyperpigmented skin interspersed with hypopigmented skin in Patient 8. Panel J shows circumoral cyanosis in Patient 5.

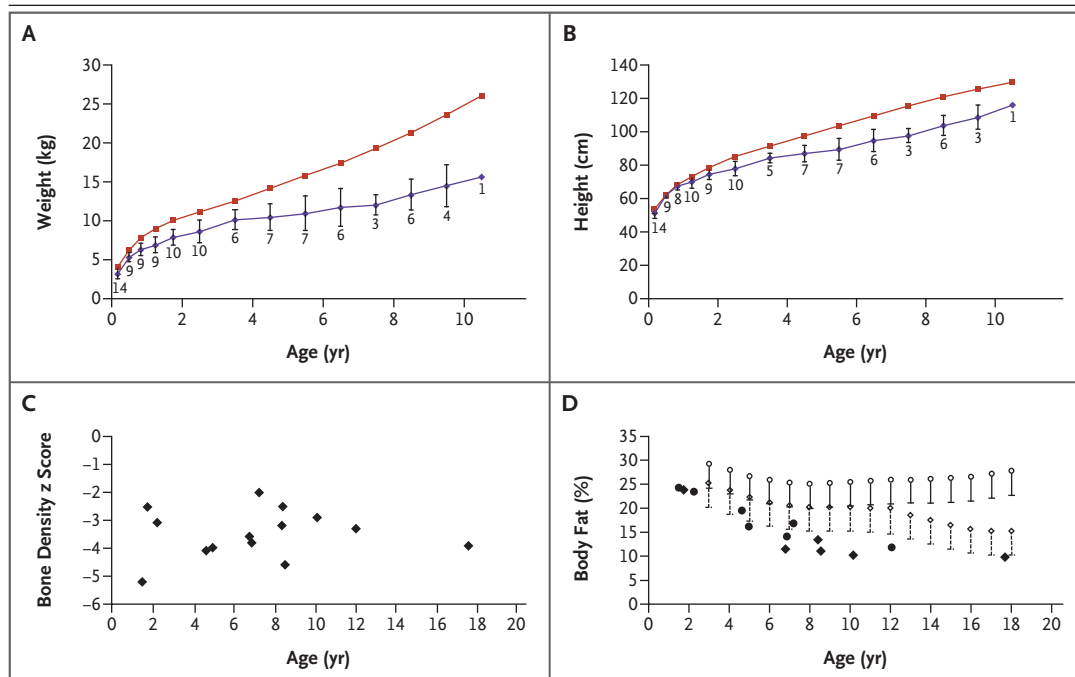
ed at the National Institutes of Health (NIH), were included in an article presenting a statistical predictive model of weight gain in Hutchinson–Gilford progeria syndrome,<sup>13</sup> and the previous weights of five of our patients (Patients 5, 7, 9, 10, and 14) were reported in the aforementioned review of this disease.<sup>12</sup> We measured the weight, height, bone density, and percent of body fat as a function of age in the patients in this study (Fig. 2).

#### CARDIOVASCULAR STUDIES

Transthoracic echocardiography was conducted with the use of the Philips 7500 system (Philips Medical Systems) and according to the American Society of Echocardiography guidelines<sup>14</sup> and established protocols for children. Electrocardiograms were obtained with the use of standard procedures. Treadmill stress testing was performed with the use of a modified Bruce proto-

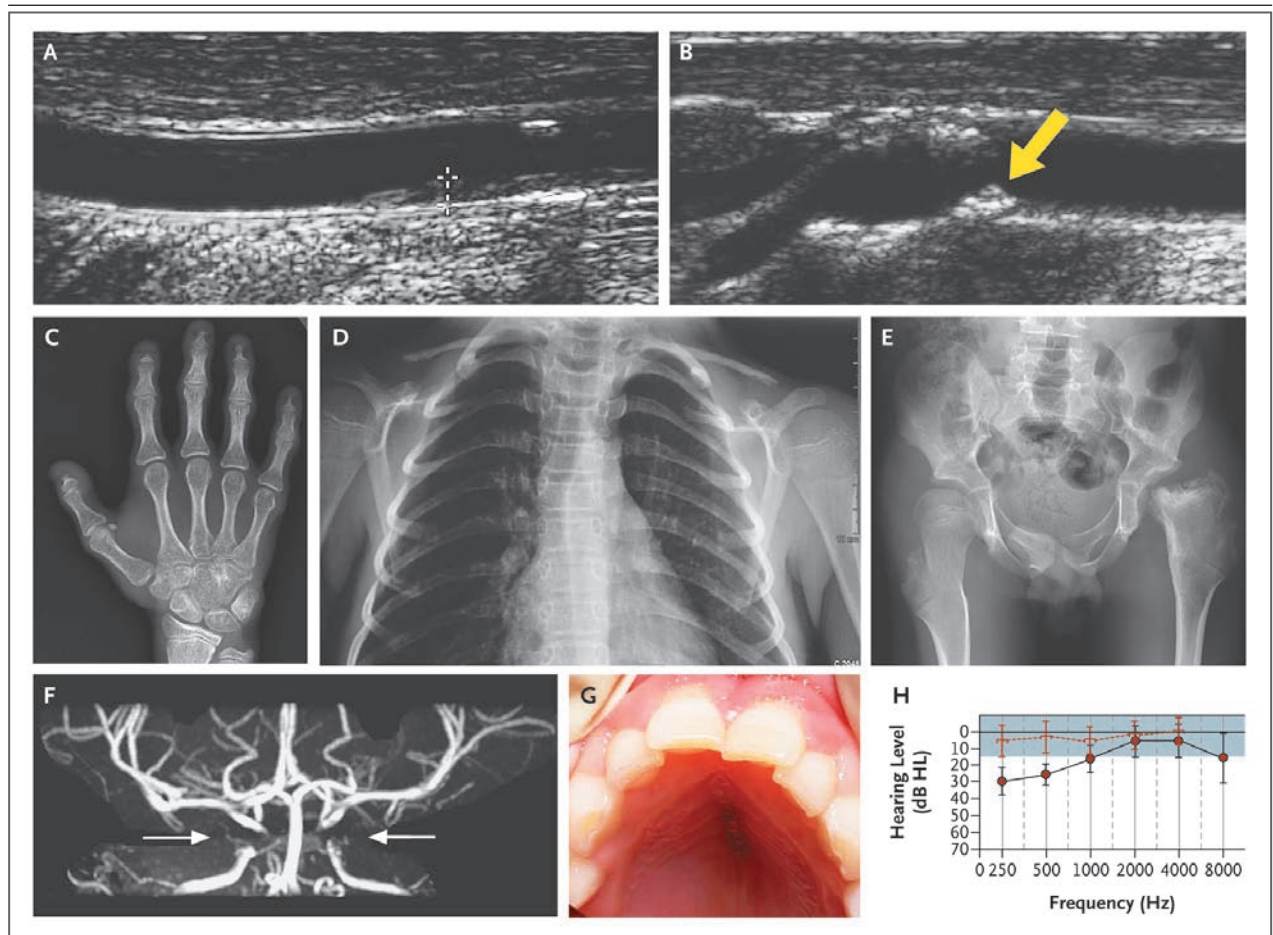
col<sup>15</sup> with continuous electrocardiographic monitoring. Carotid ultrasonography was performed with the Acuson Sequoia ultrasound machine (Siemens), which was equipped with a high-frequency linear-array transducer.

Intima–media thickness in the common carotid artery was measured from the intima–lumen border to the media–adventitia border,<sup>16</sup> and the presence of plaques was evaluated (Fig. 3A). The intima–media thickness of the near and far wall was determined over a 2-cm segment of the distal common carotid artery, where the walls are parallel, with the use of edge-detection software (Medical Imaging Applications).<sup>17</sup> Six children underwent applanation tonometry of the carotid artery with the use of SphygmoCor, version 7.01 (AtCor Medical). The augmentation index (the difference between the first and second peaks of the central arterial wave form divided by the pulse



**Figure 2.** Weight, Height, Bone Density, and Percent of Body Fat as a Function of Age in 15 Children with Hutchinson–Gilford Progeria Syndrome.

Panel A shows weight plotted according to age. Red squares represent the normal third percentile for both boys and girls, which is approximately 2 SD below the mean. Blue diamonds and I bars represent means ( $\pm$ SD) for patients with Hutchinson–Gilford progeria syndrome. Points are centered for each time period. For example, all values between 0 and 4 months are averaged and plotted at 2 months. The numbers of patients with Hutchinson–Gilford progeria syndrome for whom data were available are given below each point. Data obtained while patients were receiving growth hormone treatment were excluded. Panel B shows height plotted according to age, as in Panel A. Data obtained during growth hormone treatment were excluded. Panel C shows bone density as a function of age. Each z-score unit represents 1 SD from the normal mean for age. Panel D shows body fat as a function of age. Circles represent girls, and diamonds represent boys. Open symbols represent normal values, with bars for 1 SD; closed symbols represent values for children with Hutchinson–Gilford progeria syndrome.



**Figure 3. Vascular, Bone, Dental, and Auditory Findings in Children with Hutchinson–Gilford Progeria Syndrome.**

In Panel A, the markers show the borders of plaque in the common carotid artery in Patient 15. Panel B shows the vascular lesion (arrow) in the carotid artery in Patient 15. Panel C shows acro-osteolysis in Patient 14 at 12 years of age. Distal phalanges show resorption to tufts. Panel D shows clavicular resorption and the conical chest in Patient 13 at 10 years of age. Panel E shows the coxa valga in Patient 13. The angle of the acetabulum with respect to the femur is reduced. The left hip joint is characterized by destruction and displacement. Panel F shows high-grade stenosis (arrows) at the level of the carotid siphons bilaterally in Patient 14. Panel G shows the ogival palate in Patient 9. Panel H shows the composite audiogram of the right ear, indicating low-frequency hearing loss in 11 patients with Hutchinson–Gilford progeria syndrome. The black line indicates the mean air-conduction threshold for the right ear, and the orange line indicates the bone-conduction threshold for the right ear.

pressure) was corrected for a heart rate of 75 beats per minute. The ankle–brachial index was determined with the use of an 8-cm Hokanson TMC7 pediatric cuff at the ankles and an 8-MHz Doppler probe. Studies of brachial-artery endothelial reactivity<sup>18</sup> were performed with the use of 12-MHz ultrasonography (HDI 5000, Philips Medical Systems).

#### SPECIALTY EXAMINATIONS

Musculoskeletal evaluations included a medical history, physical examination, measurement of range of motion of the axial<sup>19</sup> and peripheral joints, and a 6-minute walk test.<sup>20</sup> Parents com-

pleted the Child Health Assessment Questionnaire,<sup>21</sup> which measures activities of daily living. Assessments of oral sensory and motor function, swallowing, articulation, and speech were performed. Audiologic testing included pure-tone evaluation, tympanometry, and distortion-product otoacoustic emissions.

#### LABORATORY AND DEMOGRAPHIC ASSESSMENTS

The Fanconi Syndrome Index measured the daily urinary excretion of 21 amino acids.<sup>22</sup> For the oral glucose-tolerance test, children fasted for 8 to 10 hours before receiving 1.75 g of dextrose per kilogram of body weight. Glucose, insulin, and

free fatty acids were measured at 0 and 2 hours. Hologic (Bedford, MA) provided normal values for bone mineral density and the percentage of body fat. To create graphs depicting the patients' height and weight as compared with those of healthy children, the means of heights and weights were obtained for each 4-month period in the first year, each 6-month period in the second year, and each 12-month period thereafter.

## RESULTS

### GENERAL FINDINGS

In all 15 patients, the clinical diagnosis was made at a median age of 19 months (range, 3.5 months to 4.0 years), based on failure to thrive and skin abnormalities (in all patients), alopecia (in 13 patients), sleeping with eyes open (in 11), circumoral cyanosis (in 9), prominent scalp veins (in 8), and decreased joint range of motion (in 7). Every patient was heterozygous for the G608G mutation in *LMNA*. Patients 14 and 15 died of myocardial infarction at 13 years 5 months and 17 years 11 months of age, respectively.

All 15 children appeared aged and had prominent eyes, micrognathia, decreased subcutaneous fat, alopecia, skin dimpling and mottling, prominent cutaneous vasculature, fingertip tufting, and distal-joint abnormalities (Table 1 and Fig. 1A through 1F). Most patients (Table 1) also had sclerotic skin (Fig. 1G), altered pigmentation (Fig. 1H and 1I), and circumoral cyanosis (Fig. 1J).

### GROWTH, NUTRITION, AND METABOLISM

Mean weight, which was normal at birth for all 15 children, decreased below the third percentile by 2 months of age (Fig. 2A). Between 2 and 10 years of age, healthy children gain 1.80 kg per year ( $r^2=0.996$ ); 10 children with Hutchinson–Gilford progeria syndrome who were not receiving growth hormone gained only 0.65 kg per year ( $r^2=0.981$ ). Five patients (Patients 5, 6, 7, 10, and 13) received growth hormone for a total of 150 months; four patients received growth hormone during this study. On average, these five patients gained 1.01 kg per year. Height for children with Hutchinson–Gilford progeria syndrome decreased below the third percentile for normal height by 15 months of age (Fig. 2B). Between 2 and 10 years, healthy children grow 5.84 cm per year ( $r^2=0.997$ ); 10 children with Hutchinson–Gilford

progeria syndrome who were not receiving growth hormone grew 3.58 cm per year ( $r^2=0.985$ ). Patients who were receiving growth hormone grew 3.98 cm per year. In nine children, the values for the body-mass index (the weight in kilograms divided by the square of the height in meters) for age were below the third percentile.

Ten children had head circumferences below the third percentile; their mean ( $\pm$ SD) z scores (scores expressed in standard-deviation units from a given mean for age-matched controls) averaged  $-3.5\pm 0.9$ . The two oldest children, who were 12 and 17 years of age, were Tanner developmental stage II (first appearance of pubic hair, breast buds, and slight enlargement of penis and testicles); the others were stage I (the stage before pubertal development).

Bone mineral density of the lumbar spine was decreased in all 14 children tested; 11 had z scores in the osteoporotic range, and 3 had scores in the osteopenic range (Fig. 2C). Skeletal age<sup>23</sup> averaged 3 months less than chronologic age, and it was delayed in the eight youngest children and advanced in the three oldest.

The mean ( $\pm$ SE) percentage of body fat was only  $16\pm 1\%$  and decreased with age (Fig. 2D). The mean ( $\pm$ SE) energy intake, assessed by means of completed 7-day food records for 11 children, was  $116\pm 8\%$  of needs according to predicted energy requirements<sup>24</sup> and  $125\pm 5\%$  of needs according to measurements of resting energy expenditure.

### CARDIOVASCULAR CHARACTERISTICS

We observed reduced vascular function with age. Seven children (Patients 4, 5, 6, 7, 10, 12, and 14) had systolic or diastolic blood pressures that were elevated as compared with both age-matched and height-matched healthy children (Table 2). Five of the oldest children had elevated pulse rates for age. Oxygen saturation was more than 95% for all 13 children tested.

Five children, including the three oldest, had long QT intervals (Table 2) on electrocardiographic testing. Two of the five had evidence of biventricular hypertrophy and biatrial enlargement. Patient 14 had a deep Q wave in lead V<sub>1</sub>, and Patient 15 had abnormal ST–T waves and a short PR interval.

Twelve children had normal resting echocardiograms (Table 2), but Patient 10 had a mildly thickened aortic valve with aortic, mitral, and

**Table 1.** Clinical Findings in Children with Hutchinson–Gilford Progeria Syndrome.\*

Patient No.	Sex	Age yr, mo	Lipodystrophy	Alopecia	Finger-tip Tufting	Altered Skin Pigment	Circumoral Cyanosis	Sclerotic Skin	Hypodontia	Ankyloglossia	Ogival Palate	Delayed Tooth Eruption	Double Row of Teeth	Vision	Intraocular Pressure	Corneal Dryness
														Sight† Glasses		
1	F	1, 6	Yes	Yes	Yes	Yes	Yes	Yes	ND	No	No	Yes	No	ND	No	No
2	M	1, 9	Yes	Yes	Yes	Yes	Yes	Yes	ND	Yes	No	Yes	No	Hyperopic	ND	No
3	F	2, 1	Yes	Yes	Yes	Yes	Yes	Yes	ND	No	No	Yes	No	Normal	Normal	No
4	F	2, 3	Yes	Yes	No	Yes	Yes	Yes	ND	Yes	Yes	Yes	No	Normal	ND	No
5	F	4, 8	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	No	No	Hyperopic	ND	No
6	F	5, 0	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Normal	ND	Yes
7	M	6, 10	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	No	ND	ND	No
8	F	6, 11	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	No	Hyperopic	ND	No
9	F	7, 3	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Hyperopic	Normal	No
10	M	8, 5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Hyperopic	Normal	No
11	M	8, 5	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Hyperopic	Normal	Yes
12	M	8, 7	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Hyperopic	Normal	Yes
13	M	10, 2	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Hyperopic	Normal	Yes
14	F	12, 1	Yes	Yes	Yes	No	No	No	No	No	Yes	Yes	No	ND	ND	No
15	M	17, 8	Yes	Yes	Yes	No	Yes	No	No	ND	No	Yes	No	ND	Normal	Yes‡

\* ND denotes not determined.

† Hyperopic is defined as the requirement for more than 2 diopters of refractory correction.

‡ This patient had neurotrophic keratopathy with dense corneal scarring in both eyes and visual acuity of 20/125 in the right eye, and vision was limited to hand motion in the left eye.

**Table 2. Cardiovascular Findings in Children with Hutchinson–Gilford Progeria Syndrome.\***

Patient No.	Sex	Age yr, mo	Blood Pressure (Highest Reading)		Pulse beats/min	ECG	Resting Echocardiogram	Intima–Media Thickness†		Ankle–Brachial Index	
			Systolic mm Hg	Diastolic mm Hg				Right	Left	Right	Left
1	F	1, 6	108	76	103–150	Normal	Normal	0.29	0.28	ND	ND
2	M	1, 9	123	78	115–143	Normal	Normal	ND	0.36	ND	ND
3	F	2, 1	108	60	118–129	Normal	Normal	0.39	0.47	ND	ND
4	F	2, 3	136‡	85‡	125–142	QT, 463 msec§	Normal	0.32	0.32	1.17	1.37
5¶	F	4, 8	107	79‡	95–119	Normal	Normal	0.46	0.36	1.08	1.00
6	F	5, 0	124	64‡	97–115	Normal	Normal	0.30	0.28	1.10	1.13
7	M	6, 10	128‡	80	98–129	QT, 458 msec§	Normal	0.44	0.37	0.99	0.95
8	F	6, 11	117	58	114–123	Normal	Normal	0.36	0.44	1.06	0.98
9	F	7, 3	115	69	101–111	Normal	Normal	0.44	0.41	1.06	1.11
10	M	8, 5	124‡	78	92–123	Normal	Abnormal	0.41	0.41	1.08	1.10
11	M	8, 5	121	64	111–133	Normal	Normal	0.45	0.42	1.03	1.05
12	M	8, 7	128‡	70	69–155	Normal	Normal	0.46	0.49	0.92	1.03
13	M	10, 2	110	72	82–124	QT, 452 msec§	Normal	0.46	0.40	0.97	0.82
14	F	12, 1	169‡	100‡	115–142	QT, 456 msec§	Abnormal	0.53	0.51	ND	ND
15	M	17, 8	132	75	93–112	QT, 458 msec§	Abnormal¶	0.45	0.47	0.91	0.87

\* ECG denotes electrocardiogram, and ND, not determined.

† The normal pediatric value is  $0.43 \pm 0.07$  mm.<sup>16</sup>

‡ This value exceeds the 99th percentile for age-matched and height-matched normal controls. Blood pressures and pulses were measured an average of six times during the course of an admission.

§ The normal QT is less than 440 msec.

¶ Data were obtained on the second visit.

tricuspid regurgitation. Patient 14 had moderate left ventricular hypertrophy with a thickened aortic valve and a subaortic membrane. Patient 15, who had a history of rheumatic fever, had evidence of concentric left ventricular hypertrophy with diastolic dysfunction, aortic and mitral stenosis and regurgitation, and mild pulmonary hypertension. Echocardiographic exercise stress testing in Patients 5 through 14 revealed a mean ( $\pm$ SD) duration of exercise of  $566\pm 90$  seconds, with a mean of  $6.0\pm 0.8$  metabolic equivalents expended. During exercise, the mean heart rate increased from 104 to 164 beats per minute, the mean systolic blood pressure increased from 99 to 112 mm Hg, and the mean diastolic blood pressure remained at 69 mm Hg. Patient 7 had hypokinesis of the distal anteroseptum, suggesting ischemia; the other nine stress echocardiograms were normal. No child had evidence of chest pain or changes on an electrocardiogram associated with ischemia.

Fourteen children had normal carotid-artery intima-media thickness with evidence of adventitial thickening. Patient 12 had complete occlusion of the left internal carotid artery, and stenotic lesions were detected on ultrasonography in two other children (Fig. 3B).

The ankle-brachial index, which is used to measure the difference in blood pressure between the legs and arms, was determined in 11 children. The index was abnormal ( $<0.92$ ) in Patients 13 and 15, indicating arterial disease in the legs. The mean ( $\pm$ SD) augmentation index in the six children tested was  $29.4\pm 8.6\%$ , with a mean corrected augmentation index of  $39.7\pm 10.2\%$ . There was no difference in flow-mediated dilatation of the brachial artery in 14 patients with Hutchinson-Gilford progeria syndrome as compared with 13 controls who were 7 to 16 years of age ( $12.6\pm 1.8\%$  change from baseline vs.  $10.6\pm 0.7\%$ ,  $P=0.34$ ), suggesting that endothelial function was reasonably preserved.

#### MUSCULOSKELETAL FUNCTION

Every patient 18 months of age or older had abnormal range of motion in at least three peripheral joints. The mean ranges of motion for wrist, ankle, and hip rotation were 63 degrees (normal value, 150), 36 degrees (normal value, 70), and 69 degrees (normal value, 90), respectively. Spinal flexion was decreased in all 11 children studied. Eleven children had stooped shoulders, nine had

calcaneovalgus, seven had hands with subluxed finger joints, four had genu valgum, three had kyphosis, and one had calcaneo varus. Muscle strength was preserved. Radiologic examinations showed acro-osteolysis, clavicular resorption, and coxa valga in all 15 children (Fig. 3C, 3D, and 3E).

The Child Health Assessment Questionnaire identified five children with mild limitations in function and seven children with moderate limitations in function. In addition, two children with severe limitations could not tie shoelaces, fasten buttons, cut their own meat, take a tub bath, turn their necks to look over their shoulders, or open jars. The results of the 6-minute walk test averaged 946 ft (288.3 m) in affected boys (range, 828 to 1296 [252.4 to 395.0]; range in age-matched healthy children,<sup>25</sup> 1043 to 2839 [317.9 to 865.3]) and 1121 ft (341.7 m) in affected girls (range, 928 to 1354 [282.8 to 412.7]; range in age-matched healthy children,<sup>25</sup> 1155 to 2440 [352.0 to 743.7]).

#### NEUROLOGIC INVOLVEMENT

Patient 12 had transient ischemic attacks from 5 years of age, and left-sided motor seizures developed after a cerebrovascular accident. Angiography revealed severe stenosis of the middle cerebral, vertebral, and basilar arteries. Patient 2 had staring spells, diaphoresis, and flushing; a previous electroencephalogram suggested diffuse encephalopathy. Pseudotumor cerebri was diagnosed in this child at the NIH at 21 months of age. On angiography, Patient 10 had irregularities in the anterior, middle, and posterior cerebral arteries suggesting arteriosclerosis. Patient 14 had stenosis of the carotid siphons (Fig. 3F). Patient 9 had occlusion of the carotid artery detected by means of magnetic resonance angiography.

#### ORAL HEALTH AND DENTITION

Oral abnormalities included hypodontia (most often, missing second premolars), ankyloglossia, ogival palate (Fig. 3G), double rows of teeth, and delayed tooth eruption (Table 1). In 14 patients, the mean ( $\pm$ SE) dental age<sup>26</sup> was  $3.3\pm 0.8$  years at a chronologic age of  $7.1\pm 1.2$  years.

#### OPHTHALMOLOGIC STATUS

Eight children had hyperopia (Table 1). Five children had corneal dryness, two with the dry-eye syndrome and one with keratopathy. Intraocular pressures were normal in the eight children tested.

**SPEECH AND LANGUAGE**

Lingual range of motion and strength were reduced in 11 children. Five children had labial weakness (an inability to keep the lips closed against pressure). The children tended to pocket solid food in their anterior and lateral sulci; six children maintained vertical chewing, which should normally evolve to rotatory chewing. Most children distorted their sibilant consonants, with compensatory lisping. Perceived nasal speech affecting speech intelligibility was noted in 4 of the 15 children. Auditory comprehension and expressive language skills were average in the eight children tested.

**HEARING**

All 11 children who underwent pure-tone evaluation had a conductive hearing loss in the 250- to 500-Hz range (Fig. 3H). Patients 14 and 15 also had high-frequency sensorineural hearing loss. Tympanograms were normal in 10 of 11 children tested; Patient 10 had flat tympanograms bilaterally.

**LABORATORY RESULTS**

Normal results of blood tests included the absolute neutrophil and lymphocyte counts and hemoglobin, glycated hemoglobin, C-reactive protein, homocysteine, sodium, potassium, calcium, alkaline phosphatase, aspartate aminotransferase, total bilirubin, lactic dehydrogenase, creatine kinase, uric acid, blood urea nitrogen, total protein, albumin, prealbumin, thyroid-stimulating hormone, free thyroxine, vitamin D, IgM, IgG, and IgA levels. Lymphocyte phenotyping was generally normal. Platelet counts were elevated in 14 of 15 children, and the prothrombin time was prolonged in 8 of 11 children (Table 3). Serum phosphorus was elevated in 8 of 15 children. Some children had elevated levels of serum triglycerides, total cholesterol, and low-density lipoprotein cholesterol, with reduced levels of high-density lipoprotein cholesterol<sup>27</sup> (Table 3); Patient 12 was receiving atorvastatin. The level of insulin-like growth factor I (IGF-I) was below the normal range in Patients 2 and 4, above the normal range in Patients 10 and 13 (both of whom were receiving growth hormone), not tested in Patient 3, and normal in the remaining patients (data not shown). Levels of luteinizing hormone were prepubertal (0 to 1.6 U per liter) except in Patient 1, who had a luteinizing hormone level of 18 U per liter.

Glucose-tolerance tests were performed in all

but the two youngest children. Patient 13 had an elevated fasting glucose level (103 mg per deciliter [5.7 mmol per liter]), and Patient 14 met criteria for diabetes (2-hour glucose test, 207 mg per deciliter [11.5 mmol per liter]). Fasting insulin values were elevated in five children; 2 hours after glucose loading, the mean insulin level increased by a factor of 10 (Table 3). Free fatty acid levels were normal at baseline and decreased appropriately with the insulin elevation at 2 hours.

Mean urine osmolality was slightly increased in 3 of 8 patients, and five patients had mild aminoaciduria (Table 3). There was no glucosuria, proteinuria, or calciuria; the mean ( $\pm$ SE) fractional excretion of magnesium was  $2.2\pm 0.3\%$  (normal value,  $<5\%$ ), and the fractional excretion of phosphorus was  $6.9\pm 0.8\%$  (normal value,  $<20\%$ ). Mean urinary creatinine excretion was normal at 19 mg per kilogram per day,<sup>28</sup> indicating complete 24-hour urine collections. The mean ( $\pm$ SE) normal creatinine clearance for all patients was  $175\pm 24$  ml per minute per  $1.73$  m<sup>2</sup> of body-surface area. Urinary deoxyypyridinoline, pyridinoline, and NTX telopeptides were within the normal range for prepubertal children.<sup>29</sup> Urinary organic acids were normal except for nonspecific abnormalities involving moderately increased metabolites such as 3-hydroxyisobutyric acid in Patients 6 and 8.

**DISCUSSION**

New mutations in the human genome occur at a rate of approximately 1 in 100 million base pairs per generation; mutations of cytosine-guanine (CG) to thymine-guanine (TG) occur frequently. When base pairs 154,375,028 to 154,375,029 on chromosome 1 change from CG to TG, the new sequence resembles a splice donor, and the cell's spliceosome deletes 150 nucleotides from the LMNA mRNA. CAAX is the motif cysteine (C), two aliphatic amino acids (AA), and any amino acid (X). Farnesyl groups linked to cysteines of C-terminal CAAX boxes tether the normal and mutant lamin A (progerin, which lacks 50 amino acids near the carboxy terminus) to the inner nuclear membrane.<sup>10,30</sup> Although normal lamin A is released by enzymatic cleavage of 15 C-terminal amino acids, progerin, lacking the cleavage site, remains permanently anchored to the membrane, binding other proteins, causing blebbing of the nucleus, disrupting mitosis,<sup>31</sup> and altering gene expression. These abnormalities are not due to haploinsufficiency, since mice completely lacking

**Table 3. Results of Laboratory Tests in Children with Hutchinson–Gilford Progeria Syndrome.**

Variable*	Normal Range	Study Group	
		Mean (Range)	No. of Patients with Abnormal Results/ Total No.
Platelet count — per mm <sup>3</sup>	150,000–350,000	452,000±34,000 (265,000–714,000)	14/15
Prothrombin time — sec×10 <sup>-3</sup>	10.6–14.7†	13.0±0.2 (11.7–14.0)	8/11
Erythrocyte sedimentation rate — mm/hr	4–20	16±4 (4–40)	3/12
Carbon dioxide — mmol/liter	20–28	21±1 (16–25)	4/15
Magnesium — mmol/liter	0.75–1.00	0.92±0.02 (0.76–1.12)	2/15
Phosphorus — mg/dl	2.7–5.5†	5.5±0.2 (4.5–6.9)	8/15
Alanine aminotransferase — U/liter	6–45†	29±7 (4.5–6.9)	2/14
γ-Glutamyltransferase — U/liter	0–52†	16±2 (5–37)	2/15
Amylase — U/liter	36–143	103±8 (43–200)	2/14
Creatinine — mg/dl	0.2–1.0†	0.3±0.0 (0.2–0.4)	3/15
Vitamin E — mg/dl	3–20†	1.4±0.3 (0.7–4.7)	2/12
Cholesterol — mg/dl			
Total	<170	163±14 (118–308)	4/14
Low-density lipoprotein	<110	112±12 (60–232)	4/14
High-density lipoprotein	>45	43±3 (30–73)	10/14
Triglycerides — mg/dl	<110	108±20 (43–314)	5/14
Oral glucose-tolerance test			
Glucose — mg/dl			
Fasting	<100	85±3 (66–103)	1/13
120 min	<140	121±11 (76–207)	2/13
Insulin — μU/ml			
Fasting	<12	14±5 (2–63)	5/13
120 min	4–160	136±63 (2–825)	4/13
Free fatty acids — mg/liter			
Fasting	70–564	299±40 (100–501)	0/10
120 min	3–85	91±23 (16–250)	5/10
CD3 cells — per mm <sup>3</sup>	800–4500†	2625±452 (782–5909)	3/12
CD4/CD3 cells — per mm <sup>3</sup> ‡	400–2000†	1691±513 (395–3976)	1/12
CD8/CD3 cells — per mm <sup>3</sup> ‡	200–1600†	928±156 (307–1866)	5/12
CD19 cells — per mm <sup>3</sup>	100–600†	863±187 (232–2589)	2/12
Natural killer cells — per mm <sup>3</sup>	70–1400†	556±129 (46–1681)	3/12
Urine osmolality — mOsm/kg	300–900	698±84 (455–996)	3/8
FSI — μmol/kg/day	4–184	172±21 (79–294)	5/11

\* Plus–minus values are means ±SE. To convert the values for magnesium to milliequivalents per liter, divide by 0.5. To convert the values for phosphorus to millimoles per liter, multiply by 0.323. To convert the values for creatinine to micromoles per liter, multiply by 88.4. To convert the values for vitamin E to micromoles per liter, multiply by 23.22. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for insulin to picomoles per liter, multiply by 7.175. FSI denotes Fanconi Syndrome Index, a measure of aminoaciduria.

† These are age-related normal values. Results for each patient were compared with the normal range for that patient's age. The prothrombin times in eight patients were outside the range for their age, even though all the patient values are within the normal range for 1 year of age to adulthood.

‡ These cells have two subgroup markers.

lamin A have normal nuclear morphologic characteristics and phenotypes.<sup>8</sup> Rather, progerin acts in a dominant negative fashion, since transfection of a mutant allele into normal cells induces nuclear blebbing.<sup>9</sup>

The result is Hutchinson–Gilford progeria syndrome, one of 11 laminopathies caused by more than 180 known *LMNA* mutations. Our prospective investigation of Hutchinson–Gilford progeria syndrome confirmed the growth impairment, alopecia, sclerotic skin changes, bone-growth abnormalities, cardiovascular and central nervous system complications, abnormal dentition, occasional mild aminoaciduria, and decreased body fat associated with this disorder.<sup>1-3,12,32</sup> We also confirmed the normal findings with respect to hematologic values, serum chemical laboratory values, renal tubular and glomerular function, and humoral and cellular immune function. New findings included prolonged prothrombin times, elevated platelet counts and serum phosphorus levels, hyperopia, specific abnormalities of joint motion, a particular low-frequency conductive hearing loss, and oral motor abnormalities such as decreased lingual range of motion, labial weakness, and vertical chewing. In addition, our patients with Hutchinson–Gilford progeria syndrome were surprisingly active and mobile; despite reduced vascular compliance, in these children the mean distance of the 6-minute walk test (approximately 1000 ft [304.8 m]) was associated with high function, especially in view of their musculoskeletal impairments.

Growth in patients with Hutchinson–Gilford progeria syndrome is clearly abnormal. In the patients in our study, weight began to deviate from normal before height (Fig. 2A and 2B), and the average weight gain between 2 and 10 years of age (0.65 kg per year) was similar to that reported for children with Hutchinson–Gilford progeria syndrome who were studied both retrospectively (0.44 kg per year) and prospectively (0.52 kg per year).<sup>13</sup>

Muscle volume remained proportional to body mass, as indicated by normal production of creatinine per kilogram of body weight. Despite radiologic evidence of bone resorption, laboratory evidence suggests a normal rate of bone turnover. Although our calculated *z* scores suggest osteoporosis or osteopenia, consideration must be given to short stature and small bones in children with Hutchinson–Gilford progeria syn-

drome as compared with age-matched control children.<sup>33</sup> When adjusted for these factors, bone density may be higher than that determined in this study.

Several possible causes of impaired growth were ruled out. Inadequate nutrition was not responsible, since energy intake was sufficient for growth and serum prealbumin levels were normal. Growth hormone production appeared to be adequate, since IGF-I levels were normal. Insulin resistance was at worst mild; levels of both serum glucose and plasma free fatty acids decreased in response to endogenously produced insulin.

Cardiovascular complications generally cause death in Hutchinson–Gilford progeria syndrome. Medial smooth-muscle cells are lost, with secondary maladaptive vascular remodeling, intimal thickening, disrupted elastin fibers, and deposition of extracellular matrix; sclerotic plaques that form in the aorta and coronary arteries are associated with stenosis.<sup>34,35</sup>

A transgenic mouse model recapitulates the vascular pathological features in humans and is useful in the investigation of potential therapies. The mouse model contains the human mutant G608G *LMNA* gene as well as the normal complement of *Lmna* genes. It shows progressive dropout of vascular smooth-muscle cells, collagen and proteoglycan deposition with medial-wall fibrosis and thickening, and relative sparing of the endothelial-cell layer.<sup>36</sup> Loss of medial cells is associated with a blunted vasodilator response.

Our clinical findings also indicate reduced vascular compliance, with elevated systolic and diastolic blood-pressure levels and an increased arterial augmentation rate. Peripheral vascular disease, with reduced ankle–brachial indexes and vessel occlusion, occurred in two children (Fig. 3B and 3F). Endothelial function was reasonably preserved; brachial-artery reactivity was normal.

One possible therapy for Hutchinson–Gilford progeria syndrome would involve inhibition of farnesyl transferase activity to prevent the permanent anchoring of progerin to the inner nuclear membrane. This treatment normalizes the nuclear morphologic features of fibroblasts in Hutchinson–Gilford progeria syndrome<sup>30,37-39</sup>; in the transgenic mouse model,<sup>36</sup> it maintains vascular smooth-muscle cells and decreases proteoglycan deposition in vessel walls.<sup>40</sup> In the *Lmna*<sup>HG/+</sup> mouse knock-in model that expresses normal

lamin A on one allele and progerin on the other, mutated allele, use of the farnesyl transferase inhibitor ABT100 improved body weight, increased fat tissue and bone mineralization, and reduced the fracture rate.<sup>41</sup>

These promising results led to an open-label clinical trial of inhibition of farnesyl transferase in Hutchinson–Gilford progeria syndrome (ClinicalTrials.gov number, NCT000425607). The trial, using weight gain as an outcome variable, has just begun. A concept-based trial such as this requires detailed knowledge of the disease process (for safety concerns) and reliable outcome variables (for efficacy). Measurements of blood pressure and the ankle–brachial index (Table 2) could help to evaluate vascular function, and serum lipid levels could be followed. Long-term measures of improvement could include normalization of low-frequency hearing, bone density, body fat, and range of motion in the wrists and ankles.

Recent molecular evidence indicates that the wild-type cryptic splice site in exon 11 of *LMNA* is recognized occasionally and used by the splic-

ing machinery in normal cells. Indeed, fibroblasts from an older person contain more progerin than fibroblasts from a younger person,<sup>42</sup> and individual cells with increased progerin show nuclear blebbing and other membrane abnormalities.<sup>31</sup> Hence, Hutchinson–Gilford progeria syndrome may serve as a model for the normal aging process.

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