NATURAL HISTORY OF ALKAPTONURIA

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ABSTRACT

Background Alkaptonuria, caused by mutations in the *HGO* gene and a deficiency of homogentisate 1,2-dioxygenase, results in an accumulation of homogentisic acid (HGA), ochronosis, and destruction of connective tissue. There is no effective therapy for this disorder, although nitisinone inhibits the enzyme that produces HGA. We performed a study to delineate the natural history of alkaptonuria.

Methods We evaluated 58 patients with alkaptonuria (age range, 4 to 80 years), using clinical, radiographic, biochemical, and molecular methods. A radiographic scoring system was devised to assess the severity of spinal and joint damage. Two patients were treated with nitisinone for 10 and 9 days, respectively.

Results Life-table analyses showed that joint replacement was performed at a mean age of 55 years and that renal stones developed at 64 years, cardiac-valve involvement at 54 years, and coronary-artery calcification at 59 years. Linear regression analysis indicated that the radiographic score for the severity of disease began increasing after the age of 30 years, with a more rapid increase in men than in women. Twenty-three new *HGO* mutations were identified. In a 51-year-old woman, urinary HGA excretion fell from 2.9 to 0.13 g per day after a 10-day course of nitisinone (7 days at a dose of 0.7 mg per day and 3 days at 2.8 mg per day). In a 59-year-old woman, urinary HGA fell from 6.4 g to 1.7 g per day after nine days of treatment with nitisinone (0.7 mg per day). Plasma tyrosine levels in these patients rose from approximately 1.1 mg per deciliter (60 μmol per liter) in both to approximately 12.8 mg per deciliter (700 μmol per liter) and 23.6 mg per deciliter (1300 μmol per liter), respectively, with no clinical signs or symptoms.

Conclusions The reported data on the natural history of alkaptonuria provide a basis for the evaluation of long-term therapies. Although nitisinone can reduce HGA production in humans with homogentisate 1,2-dioxygenase deficiency, the long-term safety and efficacy of this treatment require further evaluation. (N Engl J Med 2002;347:2111-21.)

THIS year marks the 100th anniversary of Sir Archibald Garrod's description of alkaptonuria (Mendelian Inheritance in Man number 203500) as the first disorder in humans to be found to conform to the principles of mendelian autosomal recessive inheritance.[1] In his Croonian lectures of 1908,[2] Garrod coined the term "inborn error of metabolism" and proposed that alkaptonuria results from a deficiency of an enzyme that normally splits the aromatic ring of homogentisic acid (HGA) (Fig. 1), a tyrosine-degradation product known to accumulate in patients with alkaptonuria.[3] Indeed, alkaptonuria is associated with deficient homogentisate 1,2-dioxygenase (HGO) activity in the liver,[4] and the gene for HGO is mutated in patients with alkaptonuria.[5] HGO deficiency causes excretion of large quantities of HGA daily in the urine,[6,7] which turns dark on standing. In urine, as in tissues, HGA oxidizes to benzoquinones, which in turn form melanin-like polymers. Accumulation of HGA and its metabolites in tissues causes ochronosis, with darkening of cartilaginous tissues and bone, arthritis and joint destruction, and deterioration of cardiac valves.[3,6,7] Treatment with vitamin C to enhance HGA degradation has not proved helpful.[3,8] However, 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione, or nitisinone (Orfadin), has been proposed as potential therapy[9] because it inhibits the enzyme that produces HGA (i.e., 4-hydroxyphenylpyruvate dioxygenase) (Fig. 1).[10] We performed a clinical, biochemical, and molecular evaluation of 58 patients with alkaptonuria.

METHODS

Patients

Sixty-four patients (33 male and 31 female; age range, 4 to 80 years) from 54 different families were enrolled in the study. The protocol was approved by the institutional review board of the National Institute of Child Health and Human Development, and all patients

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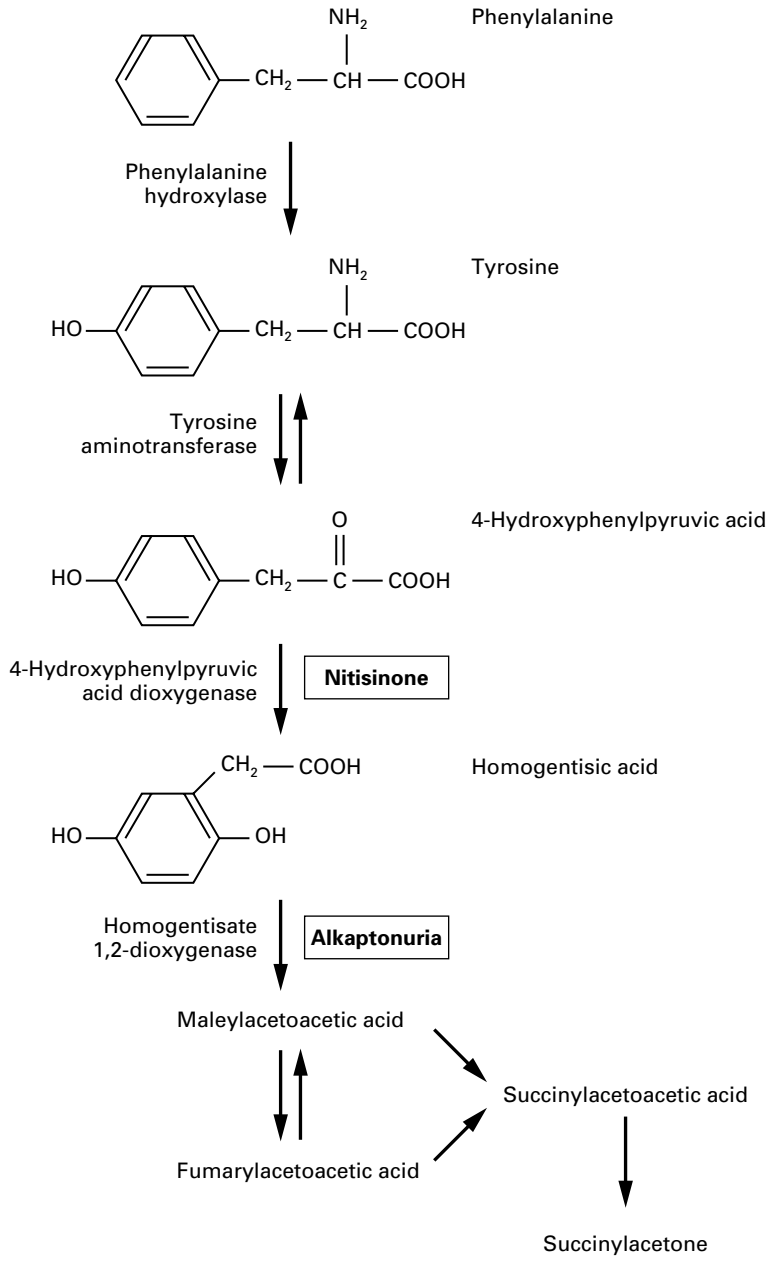


Figure 1. The Tyrosine Degradation Pathway.

The basic defect in alkaptonuria is deficient homogentisate 1,2-dioxygenase activity. Nitisinone inhibits 4-hydroxyphenylpyruvic acid dioxygenase.

(or their parents) gave written informed consent. Elevated urinary HGA levels confirmed the diagnosis in 58 patients; 6 women had normal urinary HGA levels and were therefore excluded. Two patients were also enrolled in a separate study of nitisinone (Investigational New Drug exemption number 46865, held by Swedish Orphan International), for which they also gave written informed consent. Capsules containing nitisinone were prepared by the Pharmaceutical Development Service of the National Institutes of Health.

Joint Assessment

The Medical Outcomes Study 36-item Short-Form General Health Survey (SF-36)[11,12] was completed by each patient. Each patient also underwent a comprehensive musculoskeletal examination. Axial and appendicular joints were palpated for pain and swelling and assessed for range of motion and effusions. Spinal flexion was measured with the use of the Schober test.[13,14] Small joints of the hands and feet were evaluated by visual inspection and palpation.

A radiographic scoring system was used to assess the severity of

disease in the joints and spine. In each of the cervical, thoracic, and lumbar segments, the disk spaces were scored as normal (0), minimally narrowed (1), or substantially narrowed (2). Calcification was scored as absent (0), mild (1), or marked (2). For each spinal segment, the maximal score (the combined scores for disk-space involvement and calcification) was 4; for all three segments of the spine, the maximal total score was 12. For large joints, involvement of the joint spaces was scored as absent (0); minimal, with osteophytes, subchondral cysts, or both, but no narrowing of joint spaces (1); mild, with minimal joint-space narrowing (2); moderate, with marked joint-space narrowing (3); or severe, with bone on bone or replacement of a joint (4). Joint calcification was scored as absent (0), mild (1), moderate (2), or extensive (3). The maximal combined score for joint-space involvement and calcification was 7 for each of 12 joints (hips, knees, shoulders, elbows, wrists, and ankles).

Assay of Homogentisic Acid

We measured urinary and plasma homogentisate levels according to the spectrophotometric method of Lustberg et al.,[15] with linearity of the assay in the range of 6 to 60 μM.

Mutational Analysis

Genomic DNA was extracted from whole blood according to standard methods.[16] Polymerase-chain-reaction (PCR) amplification of the 14 exons of *HGO* was performed as described elsewhere,[17] and the product was screened for mutations with the use of denatured high-performance liquid chromatography. Heteroduplex PCR products were obtained by heating the products to 95°C for five minutes, followed by gradual cooling to room temperature. Each PCR product (5 μl) was analyzed with the use of the WAVE DNA Fragment Analysis System (Transgenomic) at various temperatures. Fragments exhibiting an abnormal retention pattern were sequenced on an automatic sequencer (CEQ 2000, Beckman Coulter) with the use of a kit (CEQ Dye-Terminator Cycle Sequencing), according to the manufacturer's protocol. Direct DNA sequencing was performed on all exons when fewer than two mutations were detected by denatured high-performance liquid chromatography.

Statistical Analysis

Kaplan–Meier analyses and Student's t-tests were performed as described elsewhere.[18] Pearson's correlation coefficient[19] was determined for measurements of lumbar spinal flexion and for analyses of SF-36 scores according to age.

RESULTS

Diagnosis

Alkaptonuria was diagnosed before 1 year of age in 12 of the 58 patients (21 percent); the mean age at the time of diagnosis in the other 46 patients was 29 years. Thirty-two patients (55 percent) received the diagnosis because of dark urine, and 26 (45 percent) because of chronic joint pain (12 of the 26 were female [mean age, 46 years], and 14 were male [mean age, 37 years]). Two patients were given an incorrect diagnosis of porphyria and four underwent ear or skin biopsies before the correct diagnosis was made.

Joints

Among the 47 patients who were more than 30 years old, low back pain began before the age of 30 in 23 (49 percent); among the 35 patients who were more than 40 years old, low back pain began before the age of 40 in 33 (94 percent). Symptoms in the lumbar and thoracic spine preceded those in the cervical spine. Narrowing of the disk space was followed by disk calcification and fusion of the disk. Of the 35 patients who were more than 40 years old, 15 had lost height, with a mean decrement of 7.9 cm (range, 3.8 to 12.7). Kyphosis was present in 31 of the 58 patients. The Schober test of the extent of lumbar spinal flexion,[20] which is normally 15 cm but gradually decreases with age,[14] was clearly abnormal (<13 cm) in 34 patients. Characteristic radiographic findings are shown in Figure 2A.

Arthropathy was common. Seventeen patients had undergone knee replacement, 10 hip replacement, and 2 shoulder replacement. Fifty percent of the patients had undergone at least one knee, hip, or shoulder replacement by the age of 55 years (Fig. 2B). Eight patients had had three or more joints replaced. The range of motion was reduced by at least 21 percent in the hips of 33 patients, the shoulders of 2 patients, and the knees of 7 patients. Small-joint involvement, determined by pain on palpation, was present in only 10 patients. The composite radiographic score for the severity of spinal and joint disease was 0 until the age of 30 years, after which it rose in a roughly linear fashion with age (Fig. 2C). The severity increased more rapidly in men than in women.

The overall score for physical health, derived from the SF-36 questionnaire, declined with age ($P<0.001$), whereas the overall score for mental health did not change significantly (data not shown).

Connective-Tissue Involvement

Pigmentation of the sclera and ear cartilage occurred only after the age of 30 years and was extremely variable in severity (Fig. 3). Thirty-three patients (57 percent) had tendon-related findings, including 12 with thickened Achilles tendons. Three had muscle tears after minimal trauma (2 in the hamstring and 1 in the quadriceps), and 23 had joint effusions, 18 of which involved the knee. Six patients had suprapatellar bursa effusions, and three had synovitis involving the knee, ankle, or metacarpal phalangeal joint. Two patients had ligament tears involving the anterior cruciate ligament or the ankle.

Stone Formation

Kidney stones were documented on the basis of the history or ultrasonographic studies in 13 male and 3 female patients. The mean age at the time that kidney stones appeared was 64 years (Fig. 4A); the median age was 48 years. Of the 27 men who were 31 to 60 years old, 8 (30 percent) had prostate stones (Fig. 4B). The development of prostate stones was not associated with the development of kidney stones.

Cardiac Involvement

The mean age at the time that aortic dilatation or cardiac-valve involvement was detected (i.e., aortic- or mitral-valve calcification or regurgitation on echocardiography) was 54 years (Fig. 4C); the median age was 52 years. Three patients, each over the age of 50 years, had undergone aortic-valve replacement. No patient had coronary-artery calcification before the age of 40 years, but 50 percent had computed tomographic (CT) evidence of coronary-artery calcification by the age of 59 (Fig. 4D). There was no correlation between coronary-artery calcification and an elevated serum cholesterol level.

Laboratory Values

Only 1 of the 58 patients had a reduced creatinine clearance (29 ml per minute; normal range, 90 to 125); that patient had diabetic nephropathy and a plasma HGA level that exceeded the levels in the other patients.[17] One patient, with low-grade hepatitis, had an elevated alanine aminotransferase level (88 U per liter; normal range, 6 to 41). The mean (±SE) total cholesterol level was 190.1±37.9 mg per deciliter (4.92±0.98 mmol per liter; normal range, 100 to 200 mg per deciliter [2.59 to 5.17 mmol per liter]), the mean hemoglobin level was 139±14 g per liter (normal range, 115 to 167), and the mean alkaline phosphatase level was 105.2±58.1 U per liter (normal range, 37 to 116). Eleven patients had elevated erythrocyte sedimentation rates, ranging from 55 to 110 mm per hour (normal range, 0 to 42). Levels of osteocalcin, reflecting new bone formation, were elevated in 3 patients, whereas levels of urinary collagen N-telopeptide, reflecting bone resorption, were elevated in 24; the mean urinary collagen N-telopeptide value was 73.6±20.1 nmol per millimole of creatinine (normal range, 19 to 66). The mean plasma tyrosine level was 1.4±0.3 mg per deciliter (79±18 μmol per liter; normal range, 1.2±0.3 mg per deciliter [68±17 μmol per liter]). The mean plasma HGA level was 6.6±2.6 μg per milliliter (range, 3.0 to 27.8); in normal subjects, it is undetectable.[21] Urinary HGA levels were increased by a factor of approximately 300 (3.12±1.11 mmol per millimole of creatinine; normal value, <0.01), and total daily excretion ranged between 0.4 and 12.4 g. HGA excretion was not correlated with sex, age, or age at the time of the first joint replacement. The urinary HGA level varied even among family members with the same *HGO* mutations; in one family, the urinary HGA values in three affected siblings were 2.8, 2.8, and 4.7 mmol per millimole of creatinine.

HGO Mutations

In 57 patients, at least 1 *HGO* mutation was identified (GenBank accession number AF045167); 23 of these mutations had not previously been reported (Fig. 5). Forty-six patients were compound heterozygotes. In total, mutations were identified in 104 of 116 alleles (90 percent). There were 3 different nonsense mutations, 24 different missense mutations, 3 different frame-shift mutations, and 5 different intronic mutations resulting in splice-site abnormalities. The mutations involved exons 1 through 13 but not exon 14. Fourteen patients had at least one M368V mutation. Seven patients were also either homozygous or heterozygous for H80Q, which is considered a common polymorphism.[22] We found no correlation between the presence or absence of any type of *HGO* mutation and either the level of HGA excretion or the severity of disease.

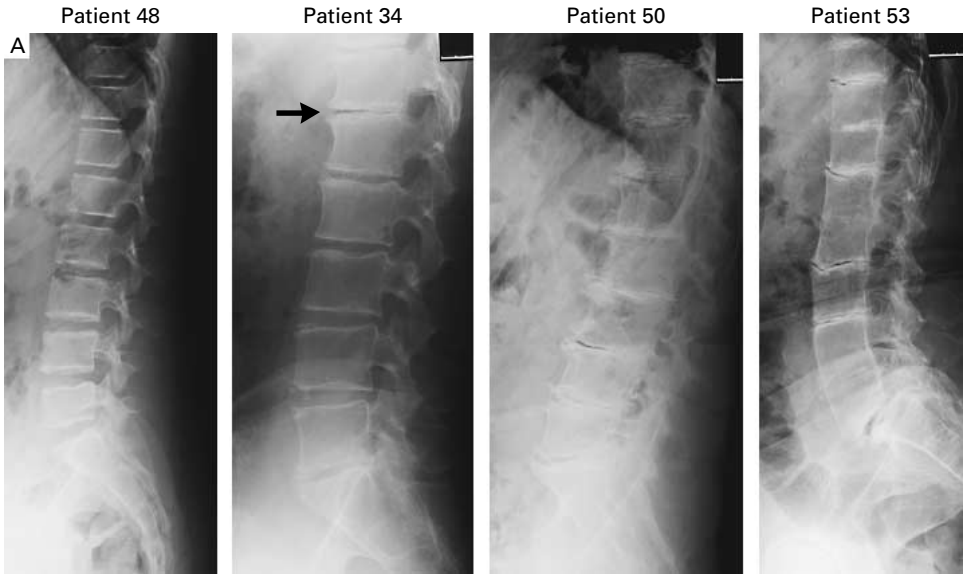
Treatment

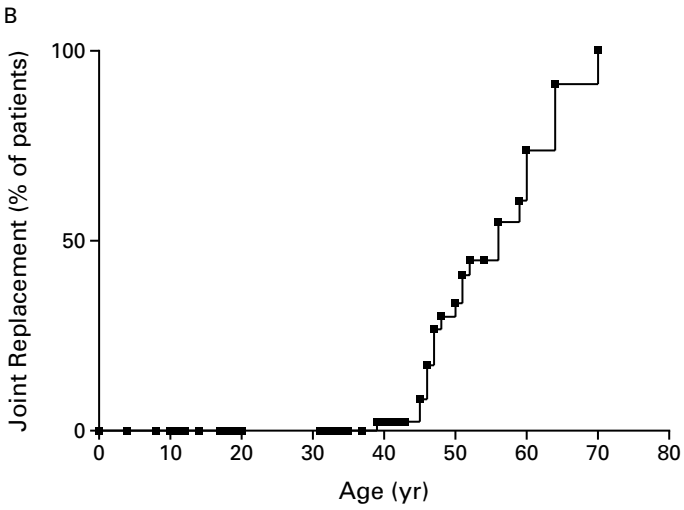
Thirty patients (52 percent) had not received any treatment for alkaptonuria. Twenty-two (38 percent) had taken or were taking vitamin C, in doses ranging from 0.25 to 4 g per day. Twelve of the 22 had stopped taking vitamin C. In the 10 patients who were still taking vitamin C (dose range, 0.25 to 4.0 g per day), the mean urinary HGA level was 3.17±1.11 mmol per millimole of creatinine (range, 1.8 to 6.1), which was similar to the level in 45 untreated patients (3.00±1.01 mmol per millimole of creatinine [range, 1.0 to 5.5]). Six patients adhered to a low-protein diet; the mean urinary HGA level in these patients was 3.93±1.63 mmol per millimole of creatinine (range, 1.8 to 6.6). Two patients were taking oral glucosamine.

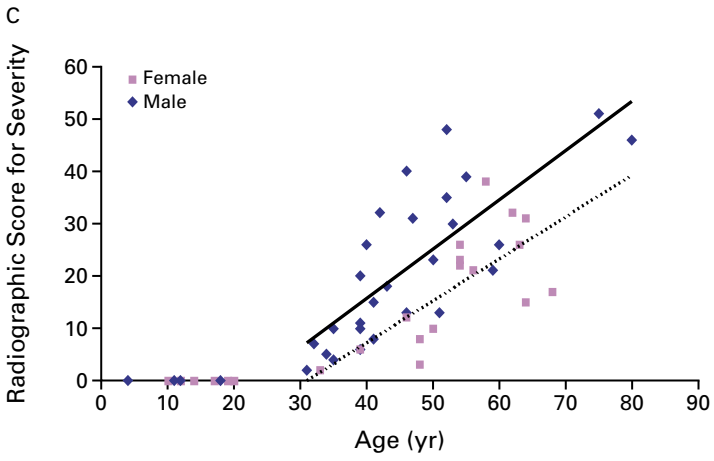
Two patients received nitisinone on an investigational basis, without dietary modifications. One of the two,

Figure 2 (facing page). Stages of Disk Disease, Joint Replacement, and Severity Scores in 58 Patients with Alkaptonuria.

Panel A shows radiographs in 4 of the 58 patients. Patient 48 (18 years old) has normal, wide disk spaces. Patient 34 (34 years old) has minimally narrowed disk spaces and calcification (arrow). More extensive calcification and more marked narrowing at all visualized disk spaces are evident in Patient 50 (46 years old). (Lumbar kyphosis is also present.) Patient 53 (62 years old) has similarly marked disk narrowing and calcification, with osseous ankylosis at L1–L2 and L4–L5. Panel B shows the results of a Kaplan–Meier life-table analysis of joint replacement. Panel C shows radiographic scores for the severity of spine and joint disease as a function of age in the 58 patients. The maximal score (indicating the most severe disease) was 4 for each segment of the spine and 7 for each of 12 joints. The straight lines are the regression lines (for male patients, $y = 0.94x - 21.96$ [$r = 0.78$]; for female patients, $y = 0.80x - 24.80$ [$r = 0.70$]).







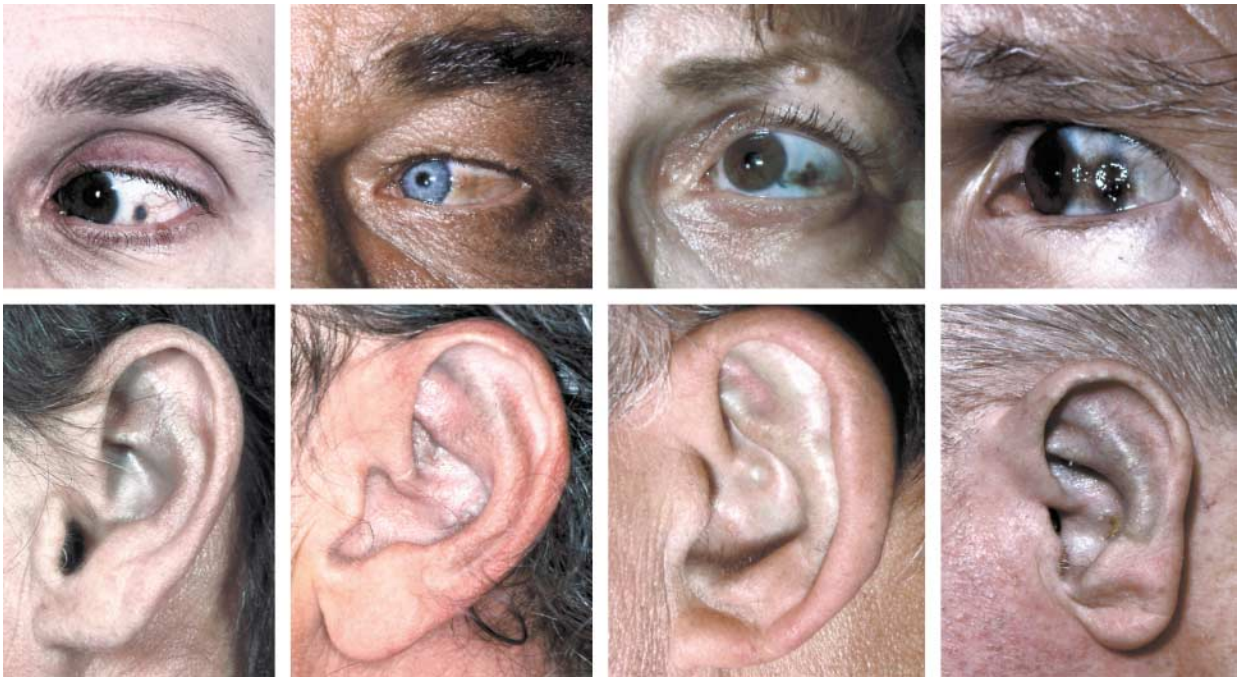


Figure 3. Ochronotic Pigmentation of Sclerae and Ear Cartilage at Various Ages.
These are examples of the most extensive involvement for each age group.

a 51-year-old woman who was a compound heterozygote for the W97R and F136Y mutations, received 0.35 mg of nitisinone twice a day for seven days. The urinary HGA level decreased from approximately 2.9 to 0.9 g per day (Fig. 6A). The dose was increased to 1.4 mg twice a day for three days, after which the urinary HGA level fell to 0.13 g per day and the urinary hydroxyphenylpyruvate level became very elevated. The plasma tyrosine level rose from approximately 1.1 mg per deciliter (60 μmol per liter) to 12.6 mg per deciliter (697 μmol per liter) on day 7 and to 13.0 mg per deciliter (719 μmol per liter) on day 10. At that time, laboratory tests performed on day 7, showing an elevated plasma tyrosine level, became available, and the treatment was stopped. There were no symptoms of photophobia, and a slit-lamp examination of the corneas showed no abnormalities. The plasma tyrosine level fell to 2.5 mg per deciliter (140 μmol per liter), and the urinary HGA level increased to 3.0 g per day over the next 14 days (Fig. 6A).

The other patient, a 59-year-old woman who was a compound heterozygote for the G161R and S305F mutations, received 0.35 mg of nitisinone twice a day for nine days. Her urinary HGA level fell from 6.4 g per day on day 0 to 1.7 g per day on day 9 (Fig. 6B). The plasma tyrosine level rose from 1.2 mg per deciliter (67 μmol per liter) to 20.8 mg per deciliter (1147 μmol per liter) on day 8 and to 23.3 mg per deciliter (1288 μmol per liter) on day 9. At that time, laboratory tests performed on day 6, showing an elevated plasma tyrosine level, became available, and the treatment was stopped. The plasma tyrosine level fell to 2.5 mg per deciliter (137 μmol per liter) over the next 25 days. The patient remained asymptomatic.

DISCUSSION

The Egyptian mummy Harwa, dating from 1500 B.C., had alkaptonuria,[23] but the term "alcapton" was first used in 1859 to describe a patient's urinary reducing compound,[24] later identified as 2,5-dihydroxyphenylacetic acid, or homogentisic acid.[25] By 1908, Garrod had proposed that alkaptonuria was an inborn error of metabolism,[2] and by 1909, Neubauer had mapped the complete tyrosine-degradation pathway.[26] A review of cases of alkaptonuria throughout the world noted that approximately 600 cases had been described through 1962.[7] We estimate that the current incidence of alkaptonuria is 1 case in 250,000 to 1 million live births.

Clinically, alkaptonuria resembles ankylosing spon-

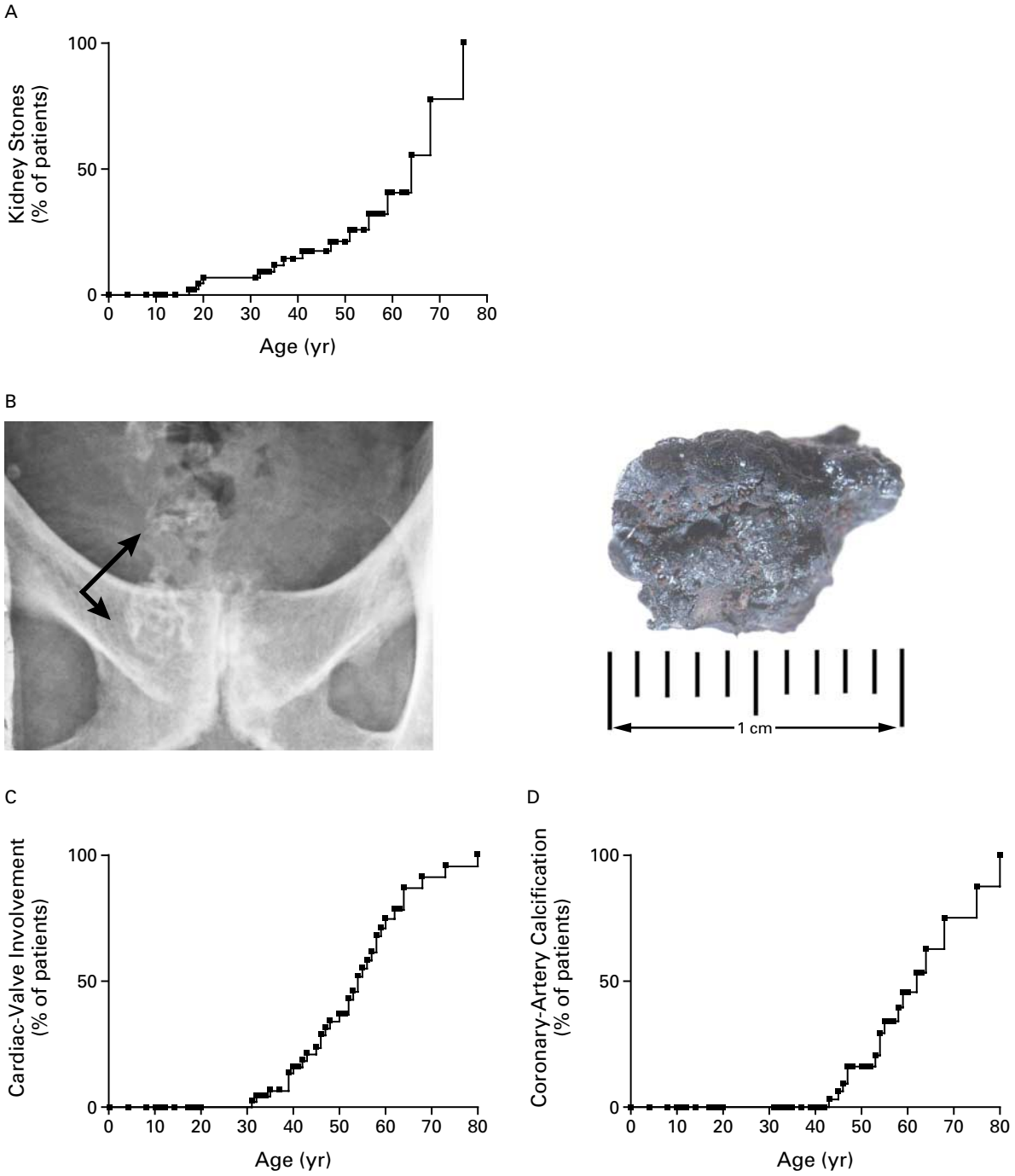


Figure 4. Kidney and Prostate Stones and Cardiac Involvement.

Panel A shows the results of a Kaplan–Meier life-table analysis of kidney stones. The pelvic radiograph on the left-hand side of Panel B shows collections of prostate stones (arrows) in a 46-year-old man with alkaptonuria. The photograph on the right-hand side of the panel shows a black prostate stone passed by a 47-year-old man. The longest dimension is 1 cm. The results of Kaplan–Meier life-table analyses of cardiac-valve involvement and coronary-artery calcifications are shown in Panels C and D, respectively.

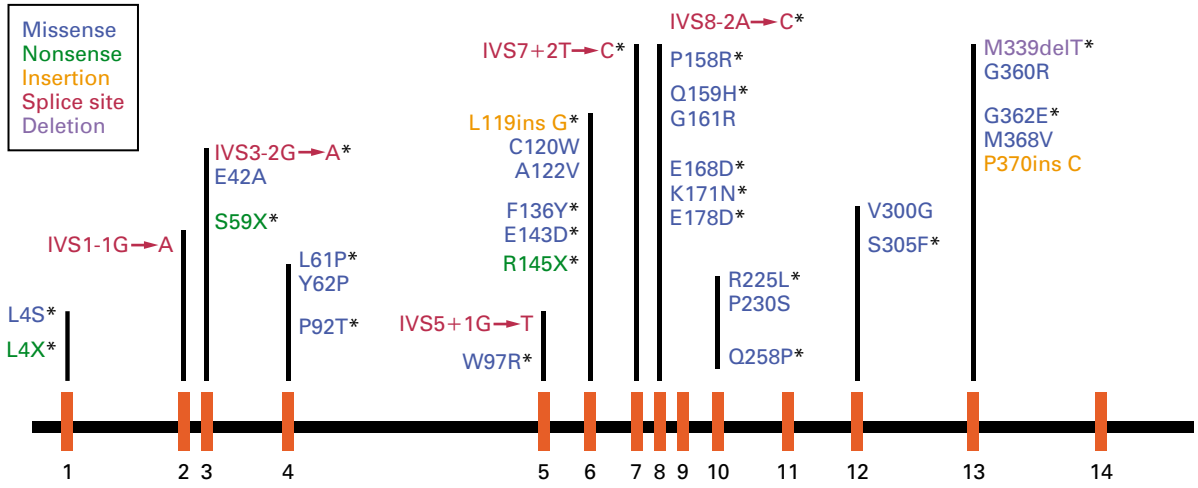


Figure 5. *HGO* Mutations in the 58 Patients with Alkaptonuria.

Exons are represented by vertical red bars, and introns by the horizontal black line. Mutations are listed above each exon and are color-coded according to type. For splice-site mutations, the nucleotide involved is indicated; for other mutations, the amino acid residue is indicated. Deletions and insertions involve a single base pair. Asterisks indicate mutations that have not previously been reported. The GenBank accession number for *HGO* complementary DNA is AF045167; the number for the HGO protein sequence is AA02698.

dylitis[27] in its particular damage to the spine and large joints but differs in sparing the sacroiliac joint. The spinal involvement results in kyphosis, height loss, and decreased lumbar flexion, and the joint disease decreases the range of motion and causes effusions. Ochronotic arthritis resembles a crystalline or inflammatory arthritis in its waxing and waning course. Joint symptoms typically begin in the third or fourth decade of life and progress until chronic pain prompts a knee, hip, or shoulder replacement; on average, this occurs at the age of 55 years. Tendon and ligament ruptures occur with minimal provocation, and kidney stones probably form because of the extremely high levels of urinary HGA excretion (renal clearance rates of 400 to 500 ml per minute).[3] This tubular secretion rids the body of HGA and presumably reduces its accumulation in tissues.

Recent diagnostic innovations have advanced the characterization of alkaptonuria as a disease entity. Magnetic resonance imaging can show thickening of the Achilles tendon. CT and echocardiographic studies can reveal coronary-artery calcifications and cardiac-valve deterioration, respectively. Ochronotic prostate stones are visible on radiographs, and radiolucent kidney stones can be seen on ultrasonograms. Biochemical assays of urinary collagen N-telopeptides indicate that bone resorption occurs in patients with alkaptonuria, probably because of a combination of the primary effects of ochronosis and the secondary effects of arthropathy-related disuse.

Molecular analyses, which have only recently become available, reveal a striking spectrum of *HGO* mutations, reflecting the disorder's clinical variability. Analysis of the *HGO* gene and its 1715-bp transcript showed that 46 of the 58 patients in our study were compound heterozygotes. We also identified 23 new mutations, bringing to 67 the total number of *HGO* mutations reported to date,[5,22,28-32] and indicating that most *HGO* mutations are unique to a family. An exception is the M368V mutation, a common mutation believed to have spread throughout Europe with past migrations[22] and found in 15 of the 116 alleles in our patients. In contrast to studies in Slovakia[29,32] and the Dominican Republic,[33] our study showed no evidence of a mutational hot spot or a founder effect.

Several therapeutic approaches have been used in patients with alkaptonuria. High-dose vitamin C decreases urinary benzoquinone acetic acid but has no effect on HGA excretion,[8] and no credible studies have shown that treatment with vitamin C is clinically effective.[3] In our patients, neither vitamin C supplementation nor protein restriction was associated with a reduction in urinary HGA excretion. Surveillance for cardiac, renal, and prostate complications after the fourth decade of life and strict attention to pain control are also advisable.

We propose that the next therapeutic step be direct pharmacologic reduction of HGA production. Theoretically, this could be achieved with nitisinone, a triketone herbicide that inhibits 4-hydroxyphenylpyruvate dioxygenase by rapid, avid binding (50 percent inhibitory concentration, 40 nM) that is reversible.[34,35] Nitisinone is approved by the Food and Drug Administration for the treatment of tyrosinemia type I.[36] A fa-

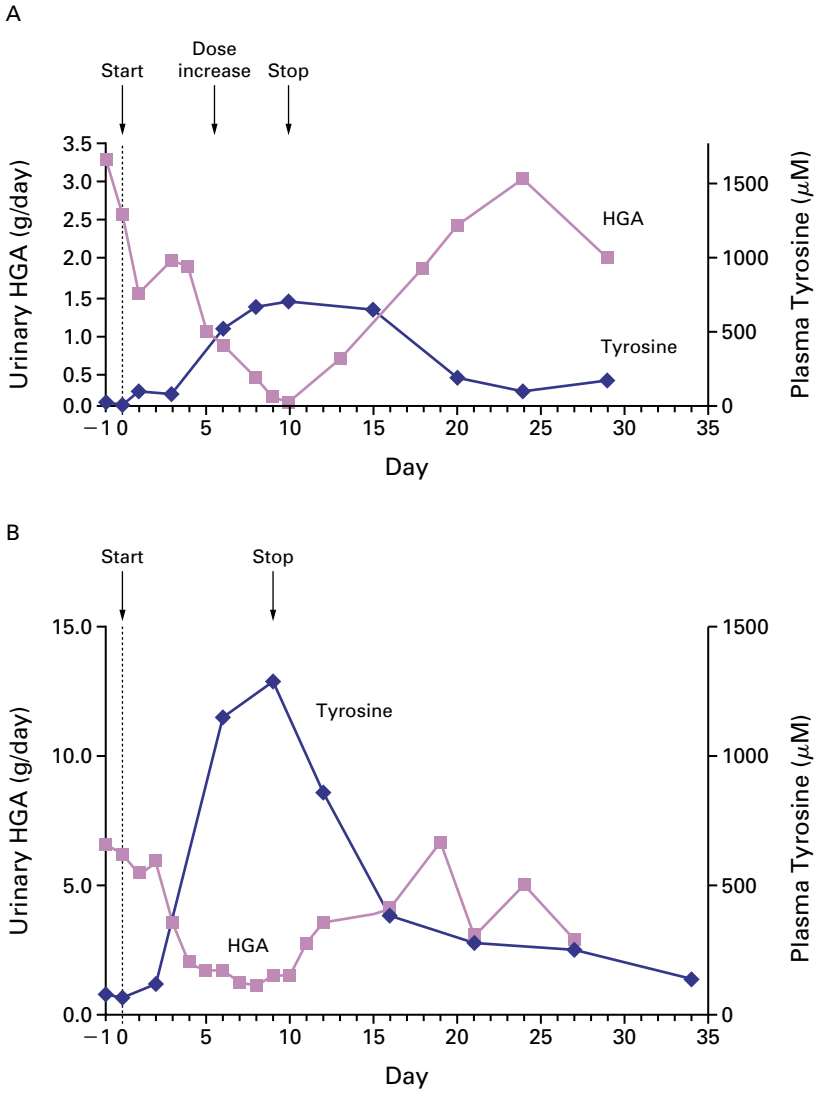


Figure 6. Effect of Nitisinone Treatment on Urinary Homogentisic Acid (HGA) and Plasma Tyrosine Levels in Two Patients with Alkaptonuria.

One patient, a 51-year-old woman, initially received 0.35 mg of nitisinone twice a day; the dose was subsequently increased to 1.4 mg twice a day (Panel A). The other patient, a 59-year-old woman, received 0.35 mg twice a day, with no increase before the treatment was stopped (Panel B). Start denotes the start of treatment, and Stop the cessation of treatment.

tal hereditary liver disease, tyrosinemia type I results from a deficiency of fumarylacetoacetate hydrolase, an enzyme distal to homogentisate 1,2-dioxygenase in the tyrosine catabolic pathway (Fig. 1). The only known side effects of nitisinone are elevated plasma tyrosine levels and resulting corneal irritation, which can be ameliorated by severe restriction of dietary tyrosine and phenylalanine. The recommended dose of nitisinone in children with tyrosinemia type I is 1 mg per kilogram of body weight per day, given in two divided doses. In a study involving healthy men, a single such dose led to plasma tyrosine levels as high as 19.9 mg per deciliter (1100 μmol per liter) — approximately 15 times the normal value; the plasma tyrosine level remained approximately 8 times the normal value for 14 days,[37] returning to the base-line level within 2 months. Oral nitisinone (approximately 0.1 mg) reduced urinary HGA excretion by more than 80 percent in a murine model of alkaptonuria.[38]

We administered nitisinone in two of our patients

with alkaptonuria, at a dose of 0.01 and 0.04 mg per kilogram per day — less than 1/20 the dose used in mice with alkaptonuria or in children with tyrosinemia type I. The drug reduced urinary HGA excretion by 69 percent or more in our two patients, providing proof of principle for the ability of nitisinone to lower HGA production.

However, several key issues concerning nitisinone treatment must be addressed. The ocular effects of long-term treatment must be assessed, since hypertyrosinemia generally does not cause symptoms in the short term. Dietary restriction of either tyrosine and phenylalanine or total protein should be considered as a means of preventing hypertyrosinemia, although compliance would be extremely difficult for adults. In addition, treatment with nitisinone could theoretically lead to the development of tyrosinemia type III, or 4-hydroxyphenylpyruvate dioxygenase deficiency, an extremely rare disorder.[36] Neurologic complications have been reported in patients with this disorder, but a causal relation between biochemical abnormalities and neurologic symptoms has not been established.[36] Children treated with nitisinone have not had neurologic problems but have also been treated concomitantly with dietary restriction of phenylalanine and tyrosine, resulting in minimally elevated tyrosine levels (i.e., 3.6 to 7.2 mg per deciliter [200 to 400 μmol per liter]).

Although our life-table analyses and diagnostic evaluations provide substantial data on the natural history of alkaptonuria, longitudinal studies are needed to determine the course of joint complications. Diagnosis of the disorder, which currently relies on documentation of an elevation in urinary HGA excretion by a factor of 100 to 600, on the basis of gas chromatographic–mass spectrometric analysis of organic acids, must be improved. Screening of newborns should be considered. Finally, treatment with nitisinone warrants further investigation, with attention to the dose, long-term efficacy, tyrosine levels, and ophthalmic, neurologic, and dermatologic complications.

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