A Medical Mystery in Middle China

China has launched a massive effort to stamp out Kashin-Beck disease, including moving populations from affected areas, but the cause of this crippling ailment remains elusive

XI'AN, CHINA—Since he was a boy, the short middle-aged man with gnarled fingers and misshapen legs has suffered from deformed joints. But in the past couple of years, even taking a step has been agony for Ma Ming-An, a 50-year-old farmer. "I stay home and cook. Now my wife works in the fields," says Ma, sitting on a gurney at Shaanxi Provincial Institute of Endemic Disease Control (SEDC) in Xi'an. Last month, Ma traveled from his home in southwest Shaanxi to the province's capital to receive treatment for Kashin-Beck disease (KBD)—a little-known ailment that has crippled and stunted the growth of hundreds of thousands of people in China's heartland.

A surgeon at SEDC, Yu Yue-Xiang, leans over Ma and presses a lump above his right knee. It is deteriorated cartilage that has dislodged from its moorings at the end of the femur—a symptom of advanced KBD. A week earlier, Yu removed four bullet-sized chunks from Ma's left knee. He's about to wash out the right one. The arthroscopic surgery is no cure, but within a week Ma should be back on his feet.

Yu and his colleagues are on the front lines of a battle against a baffling ailment. Although fungal toxins, tainted water, and trace-element deficiencies have all been implicated in the debilitating disease, KBD's precise cause is an enduring mystery. "We have no idea what triggers it," says SEDC vice director Bai Guanglu, who leads Shaanxi's KBD response.

China has mounted an aggressive attack against KBD, including providing millions of people with supplements and clean water—and condemning whole villages as part of the biggest relocation effort in history to combat a disease. Over the past 2 years in Shaanxi, some 85,000 people were relocated from land considered irredeemably tainted. Since 1995, several hundred thousand people in five provinces have been uprooted and resettled. "I don't know of any similar response to an issue like this," says Ellen Silbergeld, an environmental scientist at Johns Hopkins University in Baltimore, Maryland. The measures are gaining traction: KBD incidence is falling, as is the rate of dwarfism, the disease's most-severe manifestation.

Earlier this year, China launched a 5-year, $240 million initiative that aims to stamp out the disease altogether. The State Council–led program will first fine-tune intervention and treatment strategies in a pilot area—an ethnic Tibetan enclave in Sichuan Province—before expanding them to other regions. Some researchers say victory is near: Liu Yunqi, a professor at Harbin Medical University's Kashin-Beck Disease Institute in China, predicts that by 2020, there will be no new KBD cases. "The disease will be totally under control," he says.

At the front line. Pediatrician Philippe Goyens of the Kashin-Beck Disease Foundation examines a girl in Tibet.

CREDIT: FRANÇOISE MATHIEU

Kashin-Beck disease may be fading, but the riddle of its origin is as potent as ever. Researchers have made strides in unraveling how KBD warps skeletal growth, and the hunt is on for genes that confer susceptibility or resistance. Figuring out the ailment's cause could offer insights into cartilage metabolism and common degenerative maladies of the Western world, such as osteoarthritis. "Understanding this disease will have global significance," says Virginia Kraus, a rheumatologist at Duke University in Durham, North Carolina.

But KBD is no easy target. "Researchers have been grappling with this disease for a long, long time," says Bruce Caterson, a connective-tissue biologist at Cardiff University, U.K. "The harder we look, the more frustrating it gets."

Molecular carnage

The first inklings of an endemic blight came in a report in 1849 by a Russian surveyor who noted that people in villages along the Urov River, east of Siberia's Lake Baikal, suffered bone deformities. A few years later, Nikolai Kashin, a doctor with a Cossack military detachment in Russia's Far East, described Urov disease and sketched crippled patients. A second Cossack doctor, Evgeny Beck, documented cases in a 1906 monograph *Osteoarthritis Deformans Endemica*. The disease later came to light in what is now northern North Korea—where it had long been known as *tojiru*—and in China, where it's called *da gu ji bing*, or "big joint disease."

KBD afflicts at least 1 million people in 14 provinces, according to the Chinese Center for Disease Control and Prevention's surveillance of sentinel sites. Other estimates put the affected population in China and neighboring parts of Russia and North Korea as high as 2.5 million. Prevalence peaked in the late 1950s, when in many severely hit villages 60% to 90% of children showed signs of KBD, says Liu. Now, he says, the incidence is about 5%. In comparison, some 60% of adults over age 65 have symptoms of osteoarthritis.

The clinical picture is in sharp focus. "The disease severely erodes health," says Xiong Yongmin, vice director of the Institute of Endemic Diseases at Xi'an Jiaotong University (XJTU). Initial signs include cracking or popping sounds in the finger joints indicating loss of cartilage, and ankle and knee stiffness and pain. As KBD progresses, joints deform, muscles wither, and mobility decreases. (Inexplicably, cartilage padding the spine is not affected.) Many patients are unusually short, have stubby fingers and a waddling gait, and suffer chronic fatigue and weakness. The younger a victim is at onset, the worse the symptoms tend to become.

Scientists are beginning to unravel the damage that KBD unleashes at a molecular level. It begins at the epiphyseal growth plate: the nexus of growing bone and cartilage. Cartilage is a simple tissue—it has a single cell type,

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Hallmark symptoms. Like many KBD patients, Ma Ming-an has severely deformed joints.

chondrocytes—but it takes a biochemical balancing act to maintain it. Chondrocytes produce proteoglycans, which pull water into a collagen mesh. That gives cartilage its elasticity and resilience to the pounding meted out to our joints. Aggrecan, the major proteoglycan in cartilage, binds to a link protein and hyaluronic acid, which in turn is anchored to chondrocytes by the protein CD44. A healthy body is constantly swapping in new aggrecan for old but is much less adept at replacing collagen.

In KBD patients—as well as in the tens of millions of people with osteoarthritis and rheumatoid arthritis—replacement of aggrecan and collagen lost to disease is inadequate, and joints degrade. The cartilage matrix collapses, and pressure piles up on the chondrocytes. Cartilage begins to buckle under daily wear and tear. "In osteoarthritis, this triggers repair responses that go awry and tends to chew up the joints. We suspect the same occurs in KBD," says Caterson.

Although osteoarthritis is a disease of aging, in KBD the mechanical breakdown of cartilage often starts early, in children as young as 2 or 3. As victims grow, "their joints just go in all directions," says Caterson. Other KBD abnormalities include disturbed CD44 metabolism and elevated interleukin-1β and tumor necrosis factor-α, associated with inflammation. It's unclear whether these anomalies contribute to or are a consequence of cartilage erosion.

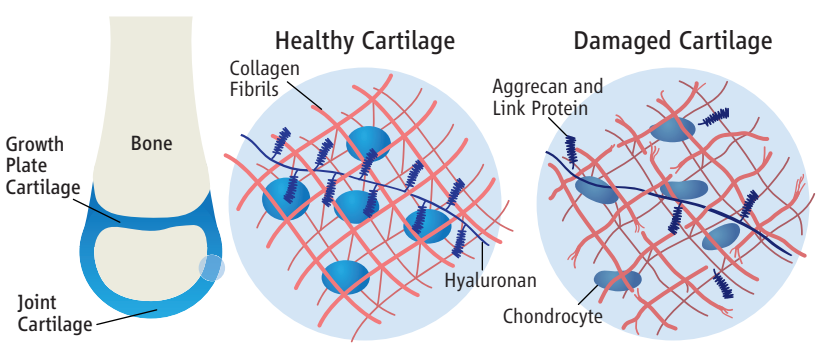
Unmasking the chondrocyte killer is critical to solving the KBD puzzle. As with Viliuisk encephalomyelitis, another disease that emerged in Siberia and continues to confound experts (*Science*, 26 April 2002, p. 642), the culprit appears to be lurking in the environment. "There are many theories," says Feng Qinghua, SEDC's provincial disease director.

Gallery of rogues

In 1992, Françoise Mathieu, a physical therapy specialist, was working for Médecins Sans Frontières (MSF) in the Philippines when colleagues at the nonprofit's newly opened office in Lhasa, Tibet's capital, encountered dozens of people in two counties in Lhasa Prefecture with a severe form of osteoarthritis. "They were wondering what kind of disease it is. They had never seen anything like it before," Mathieu says. That spring, after MSF physicians understood they were dealing with KBD, they invited Mathieu to Lhasa for a 6-week stint to explore whether physical therapy would ease symptoms. She has since devoted her career to KBD.

Early on, Mathieu and her colleagues were struck by how KBD was rife in certain valleys in Tibet, especially east of Lhasa, and rare or absent elsewhere. Peasants are vulnerable, they found, but urban populations and nomads are spared.

In the hunt for environmental clues, one startling peculiarity stood out early on. If you overlay a map of KBD incidence on a map of soil poor in selenium (a trace element) in China, the correspondence is striking. Nowhere in the world are selenium levels as low as in a swath of land that arcs from Tibet in the southwest to Heilongjiang Province in the northeast (see map, p. 1380). In this region's population, the mean serum selenium concentration is roughly 20 nanograms per milliliter—one-tenth the U.S. level. KBD occurs almost exclu-



Misery in the joints. In Kashin-Beck victims, damage begins at the epiphyseal growth plate and progresses to joint surface articular cartilage. As the disease progresses, the body fails to adequately swap in new aggrecan for old, the collagen mesh frays and fibrils break, and collagen degrades. Chondrocytes die and lose their nuclei, persisting as ghost cells before the dead tissue is replaced by scar tissue. Surviving chondrocytes cannot meet demand for molecules to repair cartilage battered by daily wear and tear.

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sively in this selenium-poor belt. A 1991 study by researchers at the Shanghai Institute of Metallurgy found that people in KBD-endemic areas in Shaanxi ingested 4.6 micrograms of selenium per day on average. Since then, selenium intakes have increased, XJTU researchers say, but are still far below the U.S. recommended daily intake of 70 mg.

Selenium is a compelling suspect. The element is a component of a couple of dozen human proteins, including a key enzyme—glutathione peroxidase—that defends the body against oxygen-free radicals, molecular wrecking crews that corrode anything in their path. Adequate dietary selenium may help ward off cancers and diseases of aging presumed to arise from accumulated free-radical damage. That appears to be true for osteoarthritis: In 2007, a team led by rheumatologist Joanne Jordan of the University of North Carolina (UNC), Chapel Hill, reported that low selenium levels increased the odds of severe knee and hip osteoarthritis in U.S. women. And animal disease suggests a link to KBD, Caterson says: Epiphyseal-plate malformations occur in sheep in selenium-poor parts of New Zealand.

But selenium deficiency alone does not explain KBD, researchers say. Although many villages in selenium-poor areas have high KBD rates, nearby villages are often disease-free. In surveys in Tibet in the mid-1990s, Mathieu's team found that children with or without KBD have equally low selenium levels. They also observed many children with goiter: KBD villages have high rates of hypothyroidism. Many of China's selenium-deficient areas, it turns out, are also iodine-poor.

In Tibet, says Mathieu, KBD is more strongly correlated with iodine deficiency than selenium deficiency. Acute iodine deprivation harms thyroid function. Because severe hypothyroidism in children impairs the epiphyseal plate and stunts growth, it can masquerade as KBD. Yet in most other KBD-endemic areas in China, iodized salt is widely available and hypothyroidism is rare.

Another bane of Kashin-Beck country is fungi. In some Shaanxi villages, for example, people have lived for centuries in moldy underground dwellings, says Caterson, who collaborates with XJTU's Cao Junling and has visited KBD hot spots four times since 2002. "It's an unhealthy environment," he says. Geography may be a factor: The KBD belt is a climatic crucible in which cold, dry continental air mixes with humid air from the Pacific Ocean.

In the 1960s, Russian scientists linked KBD to consumption of cereals tainted with *Fusarium*, a common fungal genus. Throughout KBD-endemic areas of China, researchers have detected extensive fungal contamination of grains and bread by genera such as *Fusarium*, *Trichothecium*, and *Alternaria*. These may affect two Tibetan staples: *tsampa*—roasted barley dough balls—and *chang*—fermented barley beer. "In preliminary studies, we saw a very strong correlation between fungi in the barley and KBD," says Mathieu.

Mycotoxins produced by *Fusarium* and its brethren are especially nasty. Scientists have zeroed in on three trichothecenes: nivalenol, butenolide, and T-2 toxin. Caterson's lab has found that nivalenol inhibits proteoglycan synthesis in cultured chondrocytes. Similarly, butenolide damages chondrocytes and engineered cartilage, Cao and colleagues reported in the February issue of *Toxicology in Vitro*.

T-2, the most abundant mycotoxin in KBD-endemic areas, may be the worst of the lot. In cell culture, T-2 triggers apoptosis of chondrocytes, revs up synthesis of an enzyme that degrades aggrecan, and inhibits CD44 production. Guinea pigs fed T-2 develop cartilage damage similar to that seen in KBD patients. There may even be a connection between mycotoxins and selenium. In cell culture, selenium blocks T-2-induced chondrocyte apoptosis, a team led by XJTU's Wang Zhilun reported in 2006 in *Food and Chemical Toxicology*.

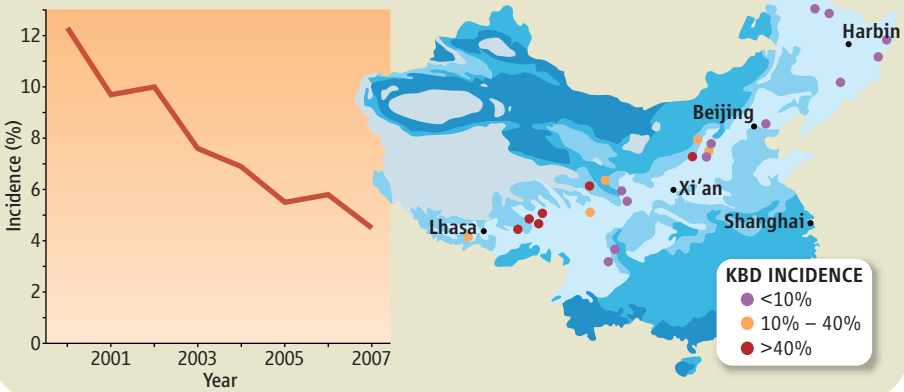
But like selenium or iodine deficiency, fungal toxins fall tantalizing short of solving the riddle: Many villages that consume mycotoxin-tainted grain do not have elevated KBD risk. "That's the thing with this disease," says Caterson. "The jigsaw puzzle seems to fall into place, then something mixes up the pieces."

Another suspect lurks in the drinking water. In many KBD-endemic villages, springs, streams, and wells are chock-full of organic matter. As the material decomposes, it releases humic acid and fulvic acid. Mathieu and colleagues have found that families with at least one KBD-afflicted member are more likely to store drinking water in small containers, which may not allow organic matter to easily settle. Fulvic acid, in cell culture, inhibits collagen formation, blocks selenium uptake, and triggers chondrocytes to make the corrosive compound hydrogen peroxide. Rats fed a selenium-poor diet laced with fulvic acid have impaired bone and collagen formation.

In a stab at a grand unified KBD theory, Chinese researchers, as well as Mathieu and her colleagues in a 2008 monograph *Big Bone Disease*, suggest that free radicals generated by mycotoxins and fulvic acid damage chondrocytes in people with an impaired antioxidant defense due to trace-element deficiencies or malnourishment. But to many experts, things still don't add up. If unquenched free radicals were the sole culprit, smokers should be at higher KBD risk—but they aren't as far anyone knows, and the disease often strikes children, says Duke's Kraus. "There could be an unknown environmental factor," adds Feng.

One dark horse is a virus. In 2004, Li Guang-Sheng and colleagues at Jilin University in Changchun, China, isolated Coxsackie B3 virus from the hearts of victims of Keshan disease, a rare heart malady that occurs in the same region as KBD. Coxsackie B3 is known to destroy heart tissue. And XJTU's Wang Zhilun and Bi Huayin have detected human parvovirus B19 in patients in Shaanxi. A common scourge, B19 triggers periodic outbreaks of so-called fifth disease in schools and nurseries. In adults, the virus can cause arthritis-

Cause or coincidence? Most KBD cases (dots represent incidence in representative villages) occur in a swath of China with extremely low selenium levels, depicted on map in lighter blue. KBD incidence in endemic regions, according to cases confirmed by x-ray diagnosis, has declined steadily since 2000.



SOURCES (LEFT TO RIGHT): CHINESE CENTER FOR DISEASE CONTROL AND PREVENTION; COURTESY OF XJTU

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like symptoms in the hands, wrists, and knees. It's unclear how widespread B19 infections are among KBD patients in Shaanxi, let alone other endemic areas, says XJTU's Xiong.

Undercutting a connection, B19's mild symptoms usually clear up quickly. But selenium deficiency might be a cofactor: Influenza and Coxsackie virus are more virulent in animals fed a selenium-depleted diet, Melinda Beck and her colleagues at UNC Chapel Hill have found. "The same virus might result in a quite different clinical picture according to the nutritional environment," says Jean Vanderpas, a doctor at the Scientific Institute of Public Health in Brussels who has studied KBD.

In addition to searching for suspects in the environment, researchers are pursuing new genetic leads that might explain why some populations are particularly vulnerable. Cases cluster in families, which could be due to environmental or genetic factors. An obvious place to hunt for variants is among the two dozen known human proteins bearing selenium, including glutathione peroxidase and selenoprotein P, which governs selenium metabolism. To test whether there are gene variants that confer susceptibility or resistance to KBD, Caterson and colleagues in 2007 collected spit samples and toenail clippings from KBD patients in Shaanxi. It took a year to satisfy Chinese officials that exporting the samples would not compromise personal information of Chinese citizens. The samples are now in North Carolina, where Jordan's team is analyzing toenail clippings for selenium and other trace elements and Kraus's group is scanning DNA from spit for variants in selenium-related genes.

One promising candidate is *DIO2*, a gene encoding a selenoprotein that converts thyroxine to its active form. Last year, Ingrid Meulenbelt and colleagues at Leiden University Medical Center in the Netherlands identified a *DIO2* variant that increases osteoarthritis risk. KBD, says Kraus, "may very well result from an interaction between genes and the environment."

Pinpointing KBD-related genes would enable vulnerable populations to be screened. "Then we'd know if the disease is all due to a lack of selenium or iodine or if other agents are involved," says Kraus.

A final push

With so many targets to shoot at, KBD interventions, not surprisingly, have been hit or miss. A decade ago, Mathieu's team gave iodized oil to one group of children with the disease, iodine followed by sodium selenate tablets to a second group, and placebos to a third. Correcting iodine deficiency markedly improved symptoms. "Young patients respond well," Mathieu says. But the selenium offered no apparent additional health benefit, the researchers reported in *The New England Journal of Medicine* in 2003. "We urgently need to clarify selenium's role. High doses are extremely toxic," says Kraus.

Recent studies reveal an even more complex nutritional picture, says Mathieu, who cofounded the Kashin-Beck Disease Foundation to carry on work in Tibet after MSF pulled out in late 2002. Her team has documented not only iodine and selenium deficits but also low levels of calcium and vitamins A, D, and E. The biggest clinical improvement, they have found, happens when children with KBD consume a more diverse diet including nettles, a traditional food full of vitamins that is largely eschewed by younger Tibetans. To test whether several deficiencies conspire to give rise to KBD, Mathieu's group just finished a 3-year trial in which 1064 children ages 3 to 10 were given iodine and selenium and either a cocktail of micronutrients—copper, manganese, zinc, and vitamins A, C, and E—or a placebo. Results are expected later this year.

Searching for answers. Françoise Mathieu analyzes grains in Gansu (*top*); a Tibetan boy with severe KBD.

CREDITS (TOP TO BOTTOM): CAMILE CHASSEUR; FRANÇOISE MATHIEU

Experience shows that a strategy that reduces KBD in one village can flop in the next. "There is no single method of primary prevention," says Xiong. In Shaanxi last year, 5.47 million people—nearly 15% of the population—received selenium supplements. Another 672,000 were given uncontaminated wheat. Some interventions may not be feasible, notes Vanderpas. "It seems almost impossible, operationally, to change fulvic acid in drinking water or decrease mycotoxins in cereals at the population level," he says.

When all else fails, authorities in five KBD hot spots—Gansu, Qinghai, Shaanxi, Sichuan, and Tibet—have ordered relocations. Former settlements are converted to farmland or rangeland for livestock. Some Chinese scientists are ambivalent about this approach. "We don't support relocation because it costs a lot of money, but we don't object to it either," says Liu. Relocation is a hardship for many, adds SEDC's Bai. "The elderly especially are reluctant to move," he says. "Moving people is the last resort." Or as Silbergeld suggests, "Perhaps the government needs to consider acting aggressively on food safety and improving diets rather than moving people around."

Although experts differ on the best approach to combating KBD, they concur that it is a disease of deprivation. Heading northeast from Shaanxi, China's population is more affluent, on average, and KBD rates fall off sharply. Xi'an, a city of 3 million people, is in the heart of KBD territory, but the only cases doctors here see are people from the countryside, SEDC's Yu says. KBD, Liu says, "only occurs in China's rural areas."

With that in mind, China's latest KBD initiative, overseen by the State Council's Leading Group of Poverty Alleviation and Development, has an overarching aim of improving people's lives. Measures will include providing untainted grains and selenium supplements, improving drinking-water quality, and relocating people from hundreds of villages. A pilot scheme is being rolled out this year in Sichuan's Aba Prefecture.

China's strategy may be controversial, but it is working. "There are fewer and fewer victims," says Bai. Most patients are now in their 40s or older, he says. That may be a blessing for China but a curse for KBD sleuths: As the disease retreats, so does the likelihood of unlocking its secrets.

–RICHARD STONE

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