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Medical & Biological Sciences
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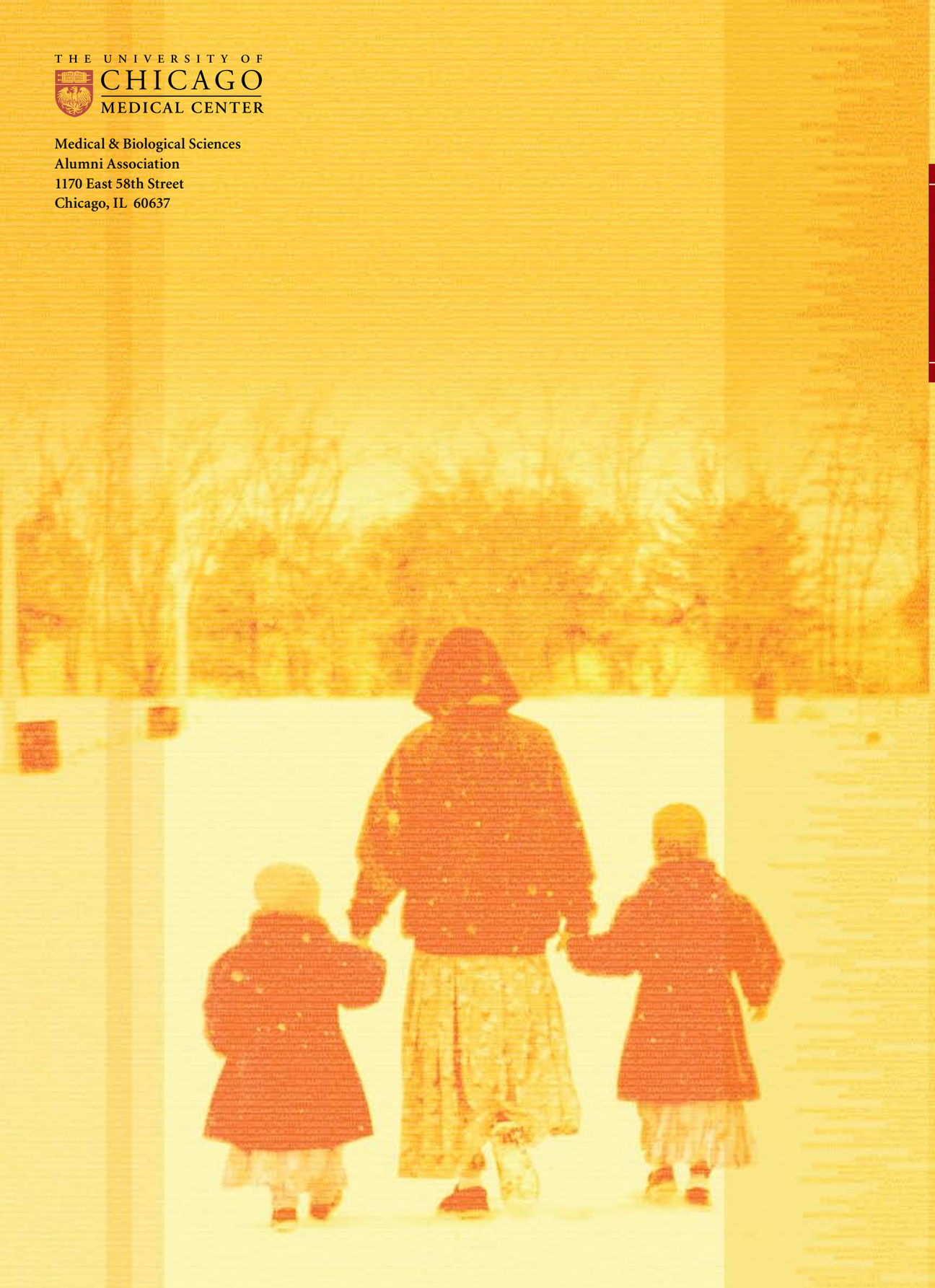
Chicago, IL 60637



UNIVERSITY OF CHICAGO MEDICINE
Biological Sciences Division
ON THE MIDWAY

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GENETIC ISOLATION

Studying the Hutterites

A Hutterite boy has blood drawn by technician Rob Stanaker at one of 11 stations set up in the school's gymnasium.

Story by Katie Scarlett Brandt
Photos by Jason Smith

GENETIC ISOLATION

A small community in South Dakota could hold the key to understanding many common diseases

Sanjiv Shah, MD, glanced up from his arterial tonometer as his next subject approached. "Give me two marks on the paper, Doctor," Donny Waldner entreated as he took his seat. "No, I can't cheat," Shah replied, and returned to his work with the tonometer, a non-invasive method to measure the central coronary artery.

Shah's was one of 11 stations spread like the face of a clock around the school gymnasium, and Donny would have to collect check marks on a form showing he had visited each of them before the day's end. Physicians and researchers would test his sense of smell, his vision, his lungs and, of course, his central coronary artery.

The battery of tests brought a range of medical care that isn't always available to Donny and his neighbors in this remote corner of South Dakota. But that's not the only reason Donny—and Shah and his medical colleagues—had gathered in the drafty gym this cold winter morning.

These tests would be more than a thorough checkup for the families in this farming community: They would add to over a half century of research that has amassed medical and genetic data on the Hutterites, a small religious community whose very isolation is helping scientists make discoveries that could affect the health of millions.

For one weekend in December, Carole Ober, PhD, a University of Chicago human geneticist, led a group of researchers from the University of Chicago, Northwestern University and Children's Memorial Hospital to the Millbrook Colony of Hutterites for a follow-up to a study she began a decade ago, funded by the National Heart Lung and Blood Institute (NHLBI).

In three days, physicians and researchers tested 130 of the 150 people who live in the colony's rows of connected gray houses. The closest town is Mitchell, S.D., population 14,558. Roughly 15 minutes away by car, it lays claim to the world's only Corn Palace, the Dakota Discovery Museum and the Enchanted World Doll Museum.

Millbrook is one of more than 30 colonies scattered across South Dakota that Ober has visited to collect data on asthma and fertility. Though the different research teams in Ober's group study various aspects of the Hutterites' health, all their findings will join a Hutterite gene bank that builds on almost 60 years of research.

Science meets Hutterites

The Hutterites' relationship with scientific visitors goes back to the 1950s when biologist Arthur Steinberg from Case Western Reserve University first visited the colonies. He saw the isolated population as harboring excellent potential for genetic study. He knew that 1,265 of them had arrived in North America in 1874, and could trace their ancestry to fewer than 90 people.

The Hutterites could tell Steinberg virtually every detail of their ancestors' trip overseas because those ancestors had kept meticulous records. They recorded them all in a single book, *Das grosse Geschichtsbuch*. All 612 pages of it survive today in a South Dakota colony not far from Millbrook.

Steinberg considered the Hutterites an ideal population for biological research. Such a small population limits genetic diversity and their communal lifestyle eliminates environmental variability.

"The Hutterite environment is so remarkably uniform," Ober said, adding that its members eat the same food, share all goods and receive the same education.

So for two more decades, Steinberg continued his research, handwriting family trees in dozens of notebooks.

After years of continuing Steinberg's work, Ober now keeps those notebooks. She first encountered them in 1979, when she was looking for a post-doc position. Then at Northwestern, geneticist Alice Martin, MD, offered Ober a job in her lab studying genetics and population structure among the Hutterites. Having written her dissertation on the social structure and gene distribution of a Rhesus monkey population, Ober's background in anthropology and genetics fit Martin's needs perfectly. Ober first traveled to the colonies later that year.

Ober took a position in obstetrics and gynecology at the University of Chicago in 1988. And now she organizes her own trips—roughly three a year to conduct these follow-up studies. Her group goes during the winter months, when the earth is gray and dry and the Hutterites aren't so busy with farm duties.

On this trip, Shah took Donny Waldner's blood pressure as he held very still, his back straight, and watched.

122 over 72.

"What happens if you get excited? Wouldn't it be off? If somebody would be scared or something?" Donny asked.

"Yeah, that would make it higher," Shah said.

A few seconds passed as white lines against a black background peaked and valleyed across Shah's computer screen. Beeps rose occasionally from the carotid artery monitors. From behind partitions in the room's back corner, there was the soft swoosh of blood flowing through hearts on the echocardiogram machines. Suddenly, Donny interrupted the lull with a forceful throat clear, and with his head slightly lowered, raised his eyes toward Shah. "I did it on purpose, to see if it's gonna go up," he said, referring to the line dancing across the screen.

From behind his glasses, Shah returned Donny's guilty glance. "OK, try to hold still. You want to get out of here, don't you?"

Sickness, inside and out

The Hutterites are private people, but Steinberg and now Ober have offered them a chance for tailored health exams and discussions that would otherwise cost thousands of dollars. While the Hutterites receive individual medical attention, Ober's group reaps the benefits on a much grander scale: They maintain a nearly 13,000-person database, and they have studied more than 1,000 people related to each other in a single 13-generation pedigree. That information has led to dozens of important discoveries, one of which proves that Hutterites and the larger European-derived populations have very similar gene frequencies and common phenotypes.

"It makes the studies we do in Hutterites very relevant to other populations," Ober said. "No one [on the outside] will care if we find a gene for heart disease in the Hutterites," unless it's applicable to outside populations.

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In April, Ober's group published their most significant finding yet related to asthma research. The *New England Journal of Medicine* ran the article, about a protein called YKL-40. Researchers already knew that YKL-40 is elevated in people with asthma and poor lung function; Ober's collaborators at Yale University had published on that in *NEJM* last November.

But at Chicago, Ober's group measured YKL-40 in Hutterite blood they'd collected 10 years ago, and performed a genome-wide study to find the gene that caused YKL-40 elevation. Within months, they identified a variation in the gene that encodes the protein and showed that the variation was associated with asthma and reduced lung function among the Hutterites—a finding that the researchers successfully replicated in non-Hutterite populations as well.

"This actually went pretty fast because of the infrastructure we have in place," Ober said.

After they collect data from the colonies, Ober's group searches the Hutterites' genotypes for indicators of certain diseases—also called genetic markers or SNPs (single nucleotide polymorphisms). These mapping and association studies involve places in the DNA sequence where people can have one or two different nucleotides. Certain nucleotides at specific positions in the DNA are associated with the prevalence of particular diseases.

Ober's lab uses homegrown software to run these studies. Geneticist Mark Abney, PhD, and geneticist and statistician Mary Sara McPeek, PhD, developed the program on-site at Chicago.

"In my lab, you can do molecular work at the bench, patient recruitment and evaluation in the field, and statistical and theoretical work at the computer," Ober said. "It's really nice because they get to meet the subjects whose blood samples they study in the lab and they're involved in all components of the research through to the end."

Research for Ober's lab team involves more than just a coded number on a tube. During the Millbrook trip, graduate student Gülüm Kosova sorted, labeled, spun and prepped blood samples, but back in the lab, she also ran the analysis programs and interpreted the data. She was looking specifically at fertility patterns among the women, which was Ober's original interest in Hutterites.

"We're relatively understaffed for a project this huge. Other studies of this magnitude have much larger teams," Ober said. But her researchers—about a dozen in all—are dedicated, and they manage. They've published in *Nature Genetics, Science,* the *American Journal of Human Genetics* and now the *New England Journal of Medicine.*

Masses of data

More research groups have joined Ober's study with independent sources of funding. Some go on the trips with Ober, others put in requests to use the database or blood samples. The researchers with Ober on this trip collect blood; take respiration and heart readings; conduct allergy, smell and vision tests; and ask dozens of questions on items like sleep, restless leg syndrome, Parkinson's disease and migraine.

At right: Hutterite girls stand with their mom in the entrance to the gym, taking a moment to assess the 35 visitors who have commandeered not only their school, but all conversation. *Left:* As they sit for their tests, the Hutterites watch the researchers' every move.

At right: Carole Ober *(standing in background)* talks with a line of girls as they wait their turns at the blood-draw station. Ober, who has visited the colonies regularly since 1979, organized the trip, bringing together research groups from the University of Chicago Medical Center, Northwestern University and Children's Memorial Hospital. *Below:* Graduate students in Carol Ober's lab, Gülüm Kosova *(front)* and Minal Çalişkan, cap and label tubes of blood with assorted colors—purple, red, blue, green and yellow—to denote specific tests.

In his Chicago lab, ear, nose and throat specialist Jayant Pinto, MD, said it has taken his group two years to enroll 80 patients in a clinical trial. In contrast, the 80 patients he saw in merely three days at Millbrook yielded unparalleled data for his allergy, olfaction and sinusitis research.

"This is great for me," Pinto said as he set up his acoustic rhinometer for measuring nose volume in a corner of the gym. "I can see people we studied on prior trips, see how their sense of smell has changed with aging. And it's practical. Everyone has ear, nose and throat problems at some point in their lives—allergies when they're young, hearing and olfactory loss when they're old, sore throats, breathing problems." Finding genetic variation that underlies these common complaints remains Pinto's goal.

Meanwhile, Pinto assessed each nose's volume, looked inside for inflammation, polyps or deviations, and asked questions about allergies, nasal symptoms and smell. He also conducted cognitive analyses.

When Donny Waldner sat down for his survey, he told Pinto he'd never noticed any problems with allergies, though he had tested positive for mugwort and ragweed. They discussed Donny's work as a welder and scraper, and the kinds of chemicals to which he's been exposed, and then Pinto asked him to remember three words: banana, door and baby. This was part of a cognitive evaluation. Loss of smell is an early sign of neurodegenerative diseases, Ober had told the Hutterites during their information session the night before. Pinto was checking for that.

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Pinto first came on the Hutterite trip seven years ago as a postdoctoral fellow in Ober's lab for studies on chronic sinusitis and olfaction prevalence. Now, his research goes hand-in-hand with Ober's, as allergies go hand-in-hand with asthma and sinus problems.

Next door, Gorka Alkorta-Aranburu, a graduate student in human genetics, blindfolded people, held scented markers to their noses and asked them to identify the smells. There were fruits, garlic, fish and 13 other scents.

Next he told Donny to draw the face of a clock and label it 11:10. Donny drew two hands—one pointing to the 11, one to the 10—and then spat back the words Pinto had asked him to remember: banana, door and baby.

"Give me a challenge," he said.

'Organized chaos'

The Hutterite lifestyle falls somewhere between that of the Amish (which they pronounce Ay-mish), who follow stricter rules, and the Mennonites, who live more freely. Women alternate weeks cooking in the common kitchen, wear long dresses with white short-sleeved shirts and black scarves over their hair, and aren't permitted to drive. Though the older generations haven't been educated past eighth grade, a few of the women professed an interest in biology and medicine when speaking to their visitors from Chicago.

Ober was the first of those visitors to arrive. On Thursday, Dec. 6, she flew into Sioux Falls, S.D., on the state's eastern border with Iowa, where she rented a van and drove the 60 miles west to Millbrook. The remaining researchers—32 of them—arrived Friday. Their supplies awaited them in boxes piled across the concrete floor of the Millbrook school gym.

"Organized chaos," Ober said. "It's complete organized chaos and so much fun."

Friday night, Ober presented the schedule for the next three days to the Hutterites. After dinner in their shared dining hall—where men took their food from a spread in the room's middle before sitting on one side, and the women filled their plates in the kitchen—Ober set up her PowerPoint presentation in the chapel, adjacent to the dining hall.

"On the outside, diabetes, asthma and heart disease have skyrocketed," she told them near the beginning of the 30-minute presentation. "Why? Is the same thing happening with the Hutterites?"

Saturday morning, researchers awoke in darkness in their Mitchell hotel rooms. The sky stretched black and starless outside their windows. They wrapped themselves in layers of clothes: The average temperature during the trip reached 14 degrees Fahrenheit, sharp and cold.

The visiting women attempted to dress like the women in the colony—modestly and with scant jewelry or makeup. The men tried, too, and without much difficulty. Millbrook men dress much like any others in the area: black pants, suspenders and long-sleeve button-down shirts.

That morning, Ober's van left first. Instead of going directly to Millbrook, she had to pick up a 15-year-old girl from another colony, Ruth Waldner. (Many Hutterites share the same last name.) The two had met on one of Ober's previous trips, and Ruth had proven so helpful—organizing papers, directing stragglers and running errands—that Ober again requested her assistance.

Down seven miles of straight road, shadowy fields and dark sky straddling either side, Ruth lived with her mother, father and seven siblings in a house they'd moved into the day before Ober arrived. Spotless wooden floors led from the kitchen to the living room. The younger kids shared bedrooms scattered between two levels, while the older siblings had their own rooms. In the room they'd spared for guests, Ruth's mother, Leah, planned to lay a quilt across the bed once they'd cleared out the moving boxes.

Each of Ruth's aunts had contributed a square, and Leah could illustrate her family's recent history through the patches: The sisters had left for different colonies, married and raised children. Angels represented those lost to illness or accident, like Ruth's brother Elias who died in a farming accident. The family donated his liver, kidneys and eyes—every organ that could be saved. And when Leah talked about the girl who received his liver and the letters the girl had written to say she'd gone to school and had a baby, Leah smiled.

Ruth was ready when Ober arrived. After a night of restless sleep, waking every hour out of fear that she wouldn't wake up on time, Ruth wanted to get to Millbrook.

A fresh look

Jake Waldner, a smallish man in bifocals, was one of the first to arrive for his tests on Saturday, and his first stop was the ophthalmologists' corner. The ophthalmologists came from Children's Memorial and tested for color blindness, vision loss, eye length, pressure and curvature.

Ophthalmologist Nils Loewen, MD, handed Jake a pair of glasses and asked him to count how many dots shone from a special flashlight Loewen held.

"What does that tell you?" Jake asked, watching Loewen put the flashlight to one side and pick up a book of numbers.

"That tells us if you have suppression in the eye."

Once Jake had identified the correct numbers in the seas of floating dots, Loewen put drops in his eyes to dilate his pupils.

"Where you're from, is it cold like it is here?" Jake asked. He'd discovered earlier that Loewen originally hailed from Germany.

"Not as cold, no. South Dakota is extreme," Loewen said, smiling.

Jake nodded, but kept his eyes trained on Loewen. "Germany, if you look on the map, is almost the same height as South Dakota," he said, pride in his voice. "Coldest I've seen it here is probably 30 below."

The ophthalmologists are among the more recent groups to join the Hutterite research trips. They'd return home to pore over their data. Other groups, though, never left the lab. Graham Coop, PhD, in human genetics at Chicago has never seen a Hutterite face, but he knows their blood.

Coop recently was lead author on a paper in *Science*. After discussions with Ober, he spent two years looking at the genetics of Hutterite pedigrees to determine where along their chromosomes recombination occurred.

Recombination takes place during meiosis, a special kind of cell division that occurs in the testicles and ovaries. In the process of making sperm or egg cells, the parent-to-be combines the chromosomes inherited from each of their parents and reshuffles them, swapping parts of one chromosome for the matching segments of the other version of that same chromosome. This results in the parent passing down a mosaic of genes, shuffled in new combinations.

"I was interested in doing something like this, but didn't have any idea how. I knew the Hutterites would be a good resource," Ober said

of conversations she'd had with Coop and co-investigators Jonathan Pritchard, PhD, and Molly Przeworski, PhD, also in genetics.

Przeworski, Pritchard and Coop ran the study using Ober's resources. They looked at DNA samples from 725 Hutterites, representing 82 nuclear families. And they found, through looking at half a million genetic markers along each chromosome, that chromosomes from the mother averaged around 40 recombination events and those from the father, 26. Their work confirmed a previous finding that older mothers contribute more recombination events, while the father's age is immaterial, but showed for the first time that heredity determines where recombination occurs along the chromosome.

A delicate balance

Like ophthalmologist Loewen, the Hutterites' speak German, though a dialect not necessarily understood by High German speakers. Their first language is Hutterish, a mishmash of Tyrolean and Austrian dialects with words adopted from Russian. Around age 6 they learn English, and speak it with an accent.

Teachers from outside the colony, dressed in jeans and sweaters, come to teach the 38 students enrolled as part of the local public school district. Paula Pecenka has taught 7th and 8th grade at Millbrook for nine years. Her classroom looks like any other—bright posters and colorful artwork on the wall, a marker board at the front, desks set in rows.

Pecenka teaches the "same exact" curriculum as the local public school, and the students take standardized tests. "Kids are kids," she said. "The thing I like best here is the parental support at home. You get immediate feedback because they're *there*."

In most colonies, children begin working after 8th grade. Girls baby-sit and learn to sew and cook; boys work with farm equipment or in the machine shop. But Millbrook instituted high school three years ago. Students take most courses online, in the school's flat-screen-clad computer room, but for some subjects, such as geography, teachers come in from the local high school to teach. The first graduating class will walk the stage next year.

In Ruth's colony, there is no high school yet. That's why she was able to assist Ober during this round of tests.

David Waldner—"the boss" (secretary and treasurer) of Millbrook and a white-haired man with a voice like Sean Connery—said that finding balance between outside exposure and the inside collective is key. Millbrook's small circle of male leadership deemed high school OK, but they sold a geothermal manufacturing business because it was drawing the colony's members too far from the collective.

"You have to weigh those things. The more our people are exposed to the outside way of life, the more they forget about community," he said. "I enjoy our way of life. Not everyone does. We have our problems, and we work through them all the time."

Even though Ober's research brought whole groups of people in from the outside, the Millbrook Hutterites looked forward to the weekend of tests. The thousands of dollars worth of diagnostic testing they each received didn't require travel beyond a few feet from their front steps.

Ober's current research interest grew from a call the NHLBI put out 15 years ago for genetic studies on allergy and asthma. Ober applied, described the Hutterites as her subjects and received five years' worth of funding. That funding has continued ever since.

But her studies don't end with allergy and asthma. The lab looks at myriad phenotypes. In their study on sex-specific effects on human quantitative traits, Ober, Abney, Lin Pan and former graduate student Lauren Weiss, PhD, analyzed Hutterites' systolic blood pressure, height, fasting insulin, triglycerides, lipoprotein, serotonin, age at menarche and cholesterol.

Thanks to the group's previous findings, they can demonstrate that these thousands of test results could show the outside population something about life, disease and genetics as well.

"We reasoned that because their external environment is so similar, we'd have a good shot at picking up sex-specific effects, if they exist," she said.

It was already well known that in fruit flies genes interacted with their sex, so Ober wanted to reproduce that study in humans. In doing so, they were able to show that the X chromosome plays some role, but even genes on the non-sex chromosomes affect these traits differently in males and females.

Thanks to the group's previous findings, they can demonstrate that these thousands of test results could show the outside population something about life, disease and genetics as well.

Goodbye—for another decade

To the right of the allergy table, blue partitions walled off the pulmonary section. Jim Klocksieben, supervisor of the adult pulmonary function lab at Chicago, performs tests for asthma. His station at Millbrook consisted of a single table—a far cry from the private rooms with giant machines he usually employed in his Hyde Park lab.

Klocksieben put a plug over Verna Waldner's nose and asked her to hold a tube to her mouth and breathe in methacholine, a substance that would cause her lungs to tighten if she was asthmatic, like an asthma attack. After a few breaths, he set a timer and asked her to wait for three minutes in order to let the methacholine take root in her lungs. She then exhaled into another tube so he could see if the methacholine had any affect.

All of the tubes were connected to the computer that displayed arcs corresponding with Verna's breathing. Klocksieben watched the screen, put a hand on her back and encouraged her to "blooooooooooooow" with all her might. "It's like wringing out a sponge," Klocksieben explained. He wanted every last drop.

Verna failed the challenge. The methacholine mimicked an allergic reaction in her lungs that indicated she was asthmatic. In a non-asthmatic's lungs, there would've been no reaction.

Klocksieben replaced the methacholine tube with albuteral, a nebulizer, and Verna leaned back in her chair breathing the clean air. She'd first been diagnosed with asthma in the '90s, during the Ober group's initial study. The doctor then asked her if she felt tightness in her chest when she breathed in cold air or exercised. "Doesn't everybody?" she asked.

Next to Verna, Klocksieben coached an older woman through the challenge. Born in 1929, Elizabeth Waldner was a mother with 13 children, 60 grandchildren and 28 great-grandchildren who she had "counted, but not lately."

Elizabeth sat in her black and white polka dot dress, with her hands crossed in her lap and looked at Klocksieben from under her headscarf with a little smile on her face. "If you feel lightheaded or dizzy, it's not the test," he joked with her. "It's my male magnetism."

The PFT tests were the last. By noon Monday, the researchers had finished their work and packed their vans for the drive back to Sioux Falls.

"I'll see you again, maybe, in 10 years," David Waldner said in his Sean Connery voice. "But I won't forget your faces for a long time."

Clockwise from top left: Scientists study various aspects of the Hutterites' health; their findings will add to nearly 60 years of research. In one of the gym's side rooms, ophthalmologist Marilyn Mets looks into a patient's eyes after they've been dilated. At one of the asthma stations, research assistant Susan Kuldanek measures nitric oxide in the airways of a Hutterite man.