In genetics, stay open to the unexpected

William Check, PhD

July 2015—When Uta Francke, MD, received the Association for Molecular Pathology Award for Excellence in Molecular Diagnostics at the 2014 AMP meeting, she titled her lecture "Adventures in Disease Gene Identification and Characterization of Mutations." Her title was appropriate for a research clinician who, during her 35-year career, while working on several major human genetic disease challenges, contributed in significant ways to our understanding of important genetic disease mechanisms and whose laboratory identified the gene for Wiskott-Aldrich syndrome.

Iris Schrijver, MD, who is one of her past trainees, speaks of Dr. Francke's "passion for research" and her "many groundbreaking discoveries."

"Her research has advanced our understanding of molecular mechanisms of many inherited conditions," among them Marfan, Rett, Prader-Willi, and Williams-Beuren syndromes, says Dr. Schrijver, director of the molecular pathology laboratory at Stanford University Medical Center and a professor of pathology and pediatrics, Stanford University School of Medicine. She tells CAP TODAY: "Uta pioneered how research testing could be translated to the clinical arena. In that sense she helped shape molecular diagnostics."

Dr. Francke

In a CAP TODAY interview, Dr. Francke, now professor emerita of genetics and pediatrics at Stanford University School of Medicine, explained the discoveries she had chosen for her lecture, focusing on two major genetic mechanisms: mosaicism and synonymous variants. She also discussed the need for better communication of genetic information to the public and her view of the future of the molecular laboratory.

"In genetics, mosaicism results from mutation during mitosis after fertilization," Dr. Francke explains. Dr. Francke was working in the early 1970s at the University of California, San Diego, on Lesch-Nyhan syndrome in the group of William Nyhan, MD, co-discoverer of the gene responsible for that rare X-linked recessive disorder, caused by deficiency of hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1), an enzyme critical in purine metabolism. Lesch-Nyhan syndrome has a pleiomorphic phenotype that includes self-mutilation. In particular, affected boys have a strong urge to bite themselves.

At the time, Dr. Francke says, they were confirming the diagnosis by measuring enzyme activity in blood cells, not analyzing DNA directly. With this method it was not possible to detect the carrier state in the mother because the blood enzyme level is normal in female carriers, due to non-random X-inactivation in blood cells.

Dr. Francke realized that because of the way hair follicles arise in the embryo, hair root cells are clones. So each hair follicle will have mostly one or the other phenotype, while some are mixed. Dr. Francke performed HPRT1 enzyme analysis on hair follicles picked from different sites. As expected, carrier women had a significant number of hair roots that were completely negative for HPRT1 (Francke U, et al. *J Pediatr.* 1973;82:472–478).

"This test for carrier detection of Lesch-Nyhan syndrome is relevant to today's mosaicism problem because it used independent 'biopsies' to study mosaic composition in the body," Dr. Francke says. "It is just another noninvasive

way to get samples from human beings. Newer analytical methods are sensitive for low-level mosaicism," she continues, citing whole-exome and whole-genome sequencing. "So it is important to have access to other tissues to assess whether the mosaicism is limited to blood or not."

Her second example of mosaicism occurred when Dr. Francke was at Yale in the 1980s and working on X-linked muscular dystrophy (DMD). After Louis Kunkel, PhD, of Harvard, had discovered the dystrophin gene, Dr. Francke's lab used still-novel cDNA probes to detect intragenic deletions and analyze the mechanism of transmission in multi-generation pedigrees. In one family, two affected cousins were born to mothers who were sisters, and both were DMD deletion carriers.

However, another three sisters were not carriers. Mosaicism in the grandmother would have explained this finding, but she did not contribute the Xp haplotype that included the dystrophin gene deletion. The mutant X chromosome was derived from the grandfather who was postulated to have germline, or postzygotic, mosaicism for the condition while remaining unaffected (Darras BT, Francke U. *Nature.* 1987;329:556–558).

The second important mechanistic discovery that pertains to today's molecular laboratory world concerns synonymous mutations. "Next-generation sequencing generates millions of variants, some of which are de novo," Dr. Francke notes. When there is no affected parent for comparative genetic analysis, it is more difficult to determine which variant causes a specific disease phenotype. To help with this task, filters are applied. Dr. Francke illustrates this process as a funnel—a huge amount of information is poured in the top and successive filters reduce the amount of information to a small trickle at the bottom. This process aims to retain deleterious mutations, those that cause a change in amino acid sequence such as nonsense, missense, frameshift, or changes in a splice junction. What's typically removed, Dr. Francke says, are intronic variants, genes known to cause a different disease, and synonymous substitutions, those that do not cause an amino acid change. "The common assumption is that a synonymous mutation cannot be disease causing," she says. The current success rate for finding disease-causing mutations in people with unknown diseases by use of whole-exome or whole-genome sequencing is only about 25 percent.

In her lecture, Dr. Francke focused on the missing mutations. She described two examples from the work of her laboratory in the 1990s in which disease-causing mutations were disguised as synonymous substitutions.

One situation concerned Laron syndrome, a type of dwarfism due to growth hormone insensitivity. Dr. Francke and her collaborators analyzed the growth hormone receptor gene (GHR) in a cohort of individuals with growth hormone insensitivity syndrome in an inbred population from Ecuador. The only GHR mutation they found, however, didn't cause a change in the receptor amino acid sequence. Rather, it created a new donor splice site and produced abnormal pre-mRNA splicing leading to an in-frame deletion of eight amino acids (Berg MA, et al. Am J Hum Genet. 1993;52:998–1005). "Not every synonymous mutation is to be thrown away," Dr. Francke concluded.

The second example of the importance of synonymous variants was identified in a patient with Marfan syndrome, which results from an abnormality in the very large gene for fibrillin-1. By using cDNA from skin fibroblasts in a long-range RT-PCR approach, Dr. Francke's lab, then at Stanford, found an exon-skipping mutation that caused the disease (Liu W, et al. *Nat Genet.* 1997;16:328–329). "The splice junctions were intact and a single nucleotide substitution was present in the middle of the exon," Dr. Francke says. "It did not cause a change in the amino acid sequence. Now people know that splicing enhancers and splicing silencers can be present within coding exons. This mutation may have eliminated a splicing enhancer necessary for the exon to be included and translated into protein."

Dr. Francke also pointed out that filtering out mutations already known to cause another disease may result in missing a responsible gene. Different mutations in the same gene can cause different phenotypes depending on the location and type of mutation, perhaps increasing or decreasing the activity of a protein.

Dr. Francke's example of this phenomenon starts with Wiskott-Aldrich syndrome (WAS), an X-linked immunodeficiency characterized by eczema, thrombocytopenia, small platelets, and recurrent infections, for which

Dr. Francke's laboratory discovered the responsible gene by positional cloning (Derry JM, et al. Cell. 1994;78:635–644). The WAS gene encodes a critical molecule in the actin cytoskeleton, called WAS protein (WASp), which is necessary for phagocytes to migrate.

Besides the classic WAS phenotype, there is a group of patients with congenital X-linked thrombocytopenia (XLT) who have small platelets but only transient eczema, if any, and minimal immune deficiency. Patients with the XLT phenotype also have mutations in the WAS gene. However, Dr. Francke and her colleagues found patients with classic WAS have more complex mutations, resulting in termination codons, frameshifts, and early termination (Zhu Q, et al. Blood. 1995;86:3797–3804).

On the opposite side are variants that have no pathogenic significance. Current human databases contain known mutations for which some people have both copies knocked out, Dr. Francke says. "Yet these people are walking around apparently normal. So these genes are not necessary or other genes compensate." One example Dr. Francke cites is a gene acting in fast-twitch muscle fibers. "If you don't have it, you have no fast-twitch muscles," she says. "You therefore are better at marathons than at sprinting." People are increasingly identifying these innocuous knockouts and including them in publicly available databases. In the future, the interpretation of sequencing results will be a lot easier, Dr. Francke predicts. "A lot more will be known about variations in human genes and more innocent variants will be found that can be ignored."

Which brings us to the topic of results reporting for variants of unknown significance. "Some say we should not even tell patients about them," Dr. Francke says. "People who have been trying to get informed consent for exome sequencing for patients with inherited conditions tell the family they might find abnormalities that aren't directly pertinent to the patient but that might relate to conditions that could show up later in life." The key question for patients is: How much do they want to know? Only what causes this child's phenotype? "Not knowing enough, most people say, 'I want to know everything.' I think people should have a right to access that information if they want, but they should be forewarned that they might learn something unexpected. It's their genome, but they share it with family members. There should be discussion in families."

In addition, Dr. Francke notes the limits to genetic influence. For instance, she says, the effects of gene variant are probabilistic, not deterministic. The environment, too, has an influence. Brain development is responsive to stimulation and sensory input. And we are learning more about the role of epigenetics.

Besides her laboratory work in human genetics, Dr. Francke is proud of her vision. "I predicted that genetic services would be provided on the Internet," she says. Her 1999 presidential address to the American Society of Human Genetics was titled "Human Genetics in the Information Age." In it she said: "I have no doubt that interactive Web-based systems will be developed that can provide accurate, timely, and individualized genetic information." People are still arguing about whether this is good or bad and how it should best be done, but no one denies it has happened.

Dr. Francke was a consultant to the Internet-based genetic services company 23andMe from 2007 to 2010. (Jill Hagenkord, MD, chief medical officer of 23andMe, says Dr. Francke "predicted the company before it existed.") "They had bright, educated PhD science writers who would write clear reports that told customers in simple language what their findings meant," Dr. Francke says of the company. In 2010 she became emeritus professor at Stanford and took a part-time position (until October 2013) as senior medical director at 23andMe. In that capacity, Dr. Francke worked with its professionals on several genetic analyses of their consumer database (for example: Tung JY, et al. PLoS One. 2011;6: e23473. doi:10.1371/journal.pone.0023473).

She directed an interview-based study of the experience of clients who received a positive result for one of three BRCA mutations that are present in about two percent of the Ashkenazi Jewish population (Francke U, et al. PeerJ. 2013;1:e8. doi:10.7717/peerj.8. Print 2013). "The bottom line was that no one was extremely upset when they found out," Dr. Francke says. "A lot of people went for confirmation at Myriad Genetics. All results were confirmed." The survey also found that people informed their families of the findings.

Controls in the survey were people who had a negative result. “Those people understood they still had a risk,” Dr. Francke says.

As for the future, Dr. Francke told the AMP audience, “Everyone will have their genome sequenced and interact with it on mobile devices.” She added in the interview: “Perhaps everyone will have a next-generation sequencing screen at birth. It could replace all current newborn screening tests and be cheap, and interpretation will be assisted by sophisticated information technology systems.”

One who was in the audience for Dr. Francke’s talk last year was Federico Monzon, MD, medical director, oncology and Latin America, Invitae. One of the major accomplishments of Dr. Francke and her colleagues, he tells CAP TODAY, was to show that the mechanisms for genetic diseases were more complex than expected. “Mosaicism had not been associated with disease, and synonymous variants were thought to be clinically irrelevant. Her work led to an understanding that silent variants could lead to disease via alterations in RNA splicing.

“What Dr. Francke and colleagues showed us is that you should not take for granted that current knowledge explains everything. You can always learn something new by studying patients who do not conform to established paradigms and by being open to genetics manifesting in unexpected ways. Keeping an open mind often leads to the discovery of new mechanisms of disease.”

Without the understanding that Dr. Francke and her generation provided in terms of causes and mechanisms of human hereditary disorders, Dr. Monzon says, “I don’t think we would have such a rich armamentarium of genetic testing as we do now.”
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