PASPCR Commentary:
Hunting disease genes in the wilds of the genome

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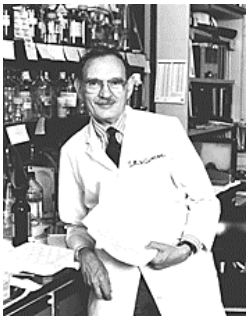
I've always been a science geek. Other kids wanted to be firemen, pilots, battleship captains. I wanted to be a scientist. As a kid, I rode the train 'downtown', to Philly's Franklin Institute, peering at sunspots through a grainy telescope, or to the Academy of Natural Sciences, a grinning *Giganotosaurus* welcoming visitors into the lobby. One chilly night, I watched Sputnik inch across the sky, silently beeping America to a postwar frenzy. The space race catapulted my father from teaching TV repair in a dingy warren of flickering green oscilloscopes to designing missile guidance systems in a spiffy building full of gleaming computers. I was about seven when, early one Sunday morning, I plugged in a device I'd built of cardboard and stiff wire, the ensuing flash and bang blowing fuses and sending me scurrying back to bed. Back then, you could buy real chemistry sets, colorful metal cabinets crammed with square, glass vials containing the ingredients for gunpowder or low-yield nuclear weapons, rather than just baking soda, colored vinegar, and a diagram showing how to build a 'volcano'. I've always been impressed by my mom's equanimity when I burned down the house with my chemistry set. Truthfully, not the entire house, just my part of it. Still, I was impressed. Thanks, mom.

My medical school, Penn State, required a 'problem-solving project' of each graduate. My roommate gave a questionnaire to friends during a marijuana-induced mental haze the last week of classes. I spent two years doing tumor virology and somatic cell genetics. Neither project worked, giving me a foretaste of how science really goes. However, I did blow up a Sorvall superspeed centrifuge, and again, most of those involved showed a sense of humor. Thanks, John.

My last year of med school, I did a wonderful project with the late Bill Mellman at Penn. Using somatic cell hybrids, Ching Chern, Bev Emanuel, and I mapped the human *GOT1* gene to chromosome 10q24. Later, while a Pediatrics Resident at CHOP, I designed and carried out a study that disproved a then-current theory of the etiology of I-cell disease, an inborn error of mucolipid metabolism. Those were hectic times in the lab, dropping pipettes when my beeper went off, sprinting to the bedside of a sick kid. And no, I was not the one who admitted the newborn orangutan to the ICU in the dead of night.

Certainly, my most important scientific training was my fellowship at Yale, working with Sherm



Weissman and Bernie Forget. It was the right place at the right time, the dawn of recombinant DNA, and our goal was to clone and sequence the globin genes, the first human genes ever studied. Introns were new and mysterious beasts, and every issue of Cell brought amazing new discoveries. I was analyzing the first mutant gene, from a patient with β-thalassemia, at a time when DNA sequencing was more art than science—maybe one in ten sequencing reactions worked. I'll never forget the moment when Sherm and I realized we'd found 'it', the very first gene mutation, a 'Eureka' moment that could only ever happen once. Thanks, Sherm and Bernie.

In 1981 I began my first faculty position, at the University of Wisconsin, a strange déjà vu, as I'd been an undergrad there in the late sixties, dodging tear gas grenades and police batons. Now I was a kid Assistant Professor, in a Genetics department with ten National Academy members. Sewall Wright had the office next to mine. I spent the next half-decade working on globin genes, mutations, and RNA splicing. But I grew restless; I was looking for something new and different.

What does any of this have to do with pigmentation biology? Well, back when I was a medical student, blowing up a centrifuge, other folks in the lab were working on amazing cells that turned black when you added tyrosine to the media. Melanoma cells. One night, around 1984, I had a bizarre dream, involving Dean Hamer cloning the tyrosinase gene by complementation of amelanotic melanoma cells. When I woke up, it still seemed a good idea. Unfortunately, I wasted two years and two postdocs trying to make my good idea work, until Takuji Takeuchi cloned a mouse tyrosinase cDNA and generously sent it to Dick King and me. I drove around Wisconsin

collecting blood samples from several huge families with OCA, and Kate Strunk and Lutz Giebel in my lab quickly used the mouse *Tyr* cDNA to discover RFLPs in the human gene, demonstrate genetic linkage with OCA1A and OCA1B, clone and sequence the human gene, and identify pathological mutations in the linked families, all this using a homemade PCR machine controlled by an Atari Gameboy. Thanks, Dean (though I've never met you).

Another idea was when I realized that white spotting in patients with deletions of chromosome 4q, the location of the *KIT* gene at 4q12, and the involvement of *Kit* in murine *W* spotting, made *KIT* a good candidate gene for human piebaldism. Just a week or so later, from a phone booth in Yellowstone National Park, I returned a chance call from an inquiring patient who said he could trace piebaldism through 15 generations of his very large family. I spotted the gene mutation from across the room as Lutz Giebel brought the autorad into my office.

Among the many physicians I contacted for piebaldism patients was Kazuyoshi Fukai, in Japan. Kazu later came to my lab and discovered that autosomal recessive ocular albinism, thought to be a distinct disease, represented a clinically mild variant of OCA1. More about Kazu anon.

At the 1991 meeting of the American Society of Human Genetics, Roy Breg, a Yale cytogeneticist, wondered to me what was going on with OCA and chromosome 15q, and he

dragged me over to meet Rob Nicholls, who'd discovered imprinting in Prader-Willi syndrome. That meeting led to the *P* gene, and a wonderful collaboration with Rob, Gene Rinchik, Bernhard Horsthemke, and Seung-Tak Lee in my lab, finding a gene deletion in patients with PWS plus OCA and gene mutations in patients with OCA2, and patients with AROA. I think I heard about Seung-Taek's results from a phone booth in Yosemite, where I was climbing El Capitan.

I once had what I thought was a brilliant idea, realizing that inbreeding might be used to map recessive disease genes. Jim Crow told me it was such a good idea that Lander and Botstein had it a decade before, and CAB Smith a decade before that. But it was a good idea, so in 1995 I flew to Puerto Rico where, with Carmelo Almodovar of NOAH, we collected blood samples from patients with Hermansky-Pudlak syndrome, and with Edgar Frenk in Lausanne samples from HPS patients from a village high in the Swiss Alps. Kazu Fukai and Jangsuk Oh then set about mapping and positionally cloning the *HPS1* gene in a truly Herculean pre-Genome Project effort. At the same time, working with Karen Moore at Millennium, Kazu, Jangsuk, and Mohammad Karim in my lab set out to identify the gene for Chediak-Higashi syndrome in much the same way. That time, I took the phone call announcing simultaneous success of both projects from Aruba, where I was windsurfing. Guo Feng and Tu Bailin in my lab then found that human *HPS1* was homologous to murine pale-ear (*ep*), and my desire to test the phenocopy mutant light ear (*le*) as a possible lead to additional human HPS genes led to a wonderful long-term collaboration with Dick Swank, whose work I'd admired for many years and whose contributions to pigment biology cannot be overstated. With Dick and his lab, Tamio Suzuki and Naoki Oiso went on to identify several new HPS genes, largely thanks to man's real best friend, the laboratory mouse

Meanwhile, Dot Bennett, with whom I'd collaborated on and off, had been urging me for years to "get involved with vitiligo". But vitiligo was a disorder with messy genetics, completely unlike the clean single-gene diseases I'd studied thus far. Finally, around 1996, we decided to proceed, with the enthusiastic participation of the Vitiligo Society. I knew I was moving to Colorado, so I asked Pam Fain, an expert in statistical genetics, to join our collaboration. Dot and I rented a little car and drove around back lanes in the British countryside getting blood samples of VitSoc members, and together Dot, Pam, and I set out to map genes. Just as we were getting started, I was amazed to receive a FedEx'd letter of inquiry from an American family with 13 affected by vitiligo. Asem Alkhateeb and Gary Stetler in my lab quickly mapped the gene in that family to chromosome 1p, which Asem later tentatively identified as *FOXD3*. We eventually mapped a number of other vitiligo susceptibility genes, and Ying Jin recently identified one on chromosome 17p as *NALP1*, a regulator of the innate immune system. The most exciting thing about finding "disease genes" is that they are involved in causation, if we can only understand how. It is my fondest goal to identify genes that teach us about the biology of vitiligo and other autoimmune diseases, identify their triggers, and give us clues to new approaches to treating and even preventing these mysterious diseases.

There were many more scientific adventures I haven't told you about, some not involving pigmentation, and there will be many more. Just today, I was contacted by a gentleman from Pakistan who says that almost all males in his very extensive family have developed vitiligo, over many generations—who knows where this might lead? Through it all, I've been incredibly fortunate to share my adventures with my many wonderful teachers, colleagues, collaborators, postdocs, students, lab techs, and fascinating patients. Each made (and many are still making) their own important contributions, and many have become lifelong friends. You know who you are. I thank you all, and I look forward to many more years of scientific adventure with you.

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