PROFILE

Profile of Haig H. Kazazian Jr.

Beth Azar, *Science Writer*

When geneticist Haig H. Kazazian Jr. was 16, he overheard a conversation between his father and a family friend. "What do you think? Is he going to be a doctor?" asked his father. "I think he'll be a scientist," replied the friend. Kazazian went on to become both. Trained in pediatrics, he developed a love of genetics early on and took up research. At first, he focused on blood disorders, with an eye toward characterizing the molecular basis of thalassemia and hemophilia. Then, a discovery in his laboratory on human jumping genes shifted his focus to questions that have taken him on a 30-year journey to understand what mobile pieces of DNA do in the human genome and how they work. Elected to the National Academy of Sciences in 2018, Kazazian is Professor of Pediatrics, Molecular Biology, and Genetics at the Johns Hopkins University School of Medicine. His Inaugural Article (1) reports the search for jumping genes in gastrointestinal tumor cells.

Fulfilling a Father's Dreams

Kazazian's success is all the sweeter because of the hardships his parents endured. They were Armenian immigrants fleeing the Turkish genocide. His mother arrived in Racine, Wisconsin in 1920. His father was the only member of his immediate family to survive life in a concentration camp in northeastern Syria after a forced march from Anatolia. In 1917 his father escaped, via Damascus and Cuba, to Toledo, Ohio, where an uncle owned an oriental rug store. The elder Kazazian, having lost his opportunity for an education, worked alongside his uncle, eventually taking over the business when his uncle died in 1951.

Haig H. Kazazian Jr. Image credit: Courtney J. Barbour.

This is a Profile of a member of the National Academy of Sciences to accompany the member's Inaugural Article on page 32215.
First published December 3, 2020.

"My father was very smart, and he had wanted to become a physician," recalls Kazazian. "I feel like I was fulfilling his dreams in the next generation. I wasn't pressured, but I felt that he was proud that I was pursuing a medical career."

Kazazian excelled in school and relished intellectual challenges, all but ensuring that he would attend a good college. Encouraged by the rector of his church, an alumnus of Dartmouth College, and by his love of the outdoors, Kazazian chose Dartmouth. There, he took premed classes alongside philosophy and French. At the time, Dartmouth Medical School accepted only 24 undergraduates a year and most began medical school after their junior year. Kazazian was no exception. In June of his junior year, he sought the advice of the medical school dean concerning summer preparation, thinking that the ideal arrangement would be working as a hospital orderly. After reviewing his excellent performance in chemistry, the dean suggested he work in a laboratory and arranged a position with a biochemistry professor. Kazazian continued laboratory research for the next three summers.

At that time, Dartmouth Medical School had a two-year preclinical program, and most students finished at Harvard University. Kazazian, who preferred the weather in Maryland, where he had rowed at the Naval Academy, chose Hopkins instead.

Discovering Genetics

Toward the end of medical school, Kazazian participated in a seminar in genetics with pediatrics professor Barton Childs. The experience solidified Kazazian's interest in pediatrics and stimulated what would become a lifetime passion for human genetics. He went off to Minnesota for a 3-year pediatrics internship and residency but soon decided to pursue genetics research, joining Child's laboratory at Hopkins after his second year. There, Kazazian worked with fruit flies on a mechanism called dosage compensation, trying to uncover how females with two X chromosomes compensate for males who have only one X chromosome. Mammals inactivate one of the female's X chromosomes in every cell. Kazazian discovered that in fruit flies both X chromosomes are active in every cell (2). And, "the X in the male is turned up to compensate," he explains.

In 1966, Kazazian found a position in Harvey Itano's laboratory at the National Institutes of Health and entered the Public Health Service. Itano's focus was hemoglobin regulation, and he suggested an interesting project. "My task was to figure out why the δ-chain of hemoglobin A_2, which makes up 2% of the hemoglobin in the adult red [blood] cell, is produced at one-fiftieth the rate of the β-chain of hemoglobin A," he recalls. Itano thought it was because of a difference in protein translation, but Kazazian used an indirect assay to show that it was instead due to a difference in the amount of messenger RNA (3). "There was 40 or 50 times as much β-globin messenger as δ-globin messenger," he says.

After 2.5 years at the NIH, Kazazian was invited to return to Hopkins as a faculty member. He had married in 1962 and by then had two children. His wife asked him to postpone joining the faculty until he completed his last year of pediatric residency, just in case his research career failed to launch. That year, Kazazian wrote his first NIH grant. "I got that grant, and it started a little over 50 years of continuous NIH R01 support," he says.

Kazazian spent his early years at Hopkins working out the regulation of hemoglobin synthesis to understand the human blood disorder β-thalassemia. Working with colleagues interested in other hemoglobin disorders, such as sickle cell anemia, Kazazian helped develop methods for prenatal diagnosis (4). During that time, two other research teams found DNA polymorphisms in the β-globin gene cluster, and Kazazian's group used the data to improve prenatal diagnosis of sickle cell anemia, from a diagnostic success rate of 36% to 80% (5). After Stylianos Antonarakis, a research fellow in Kazazian's laboratory, found three more β-globin polymorphisms, Kazazian hypothesized that he could use them to help find mutations in β-thalassemia.

"We found that the polymorphisms had specific patterns for which we coined the term 'haplotypes' (6). There were five polymorphisms upstream of the β-globin gene, and, instead of finding 2^5, or 32, different patterns of interaction, we found only three. Similarly, the polymorphisms downstream of the β-globin gene were going together. There were only three of those. Between these two regions was a hotspot for recombination."

Kazazian postulated he could use the haplotypes to precisely classify thalassemia patients. He contacted Stuart Orkin, a researcher at Harvard University who was cloning mutant globin genes, and set up a collaboration. Pat Giardina at the Cornell University Thalassemia Clinic provided them with a diverse group of blood samples from 30 patients and all 60 parents. "They were [various] ethnicities: Chinese, Asian Indian, and Mediterranean," he says. "For the first analysis we studied Mediterraneans. Among nine different haplotypes we found a different mutation on all but one" (7). Over the next 5 years, Orkin and Kazazian used haplotypes to discover β-thalassemia mutations in people from India, China, Egypt, Mexico, Brazil, Taiwan, and Israel, in effect determining the multimutational basis of a single-gene disease (8–13). In 1987, Kazazian's group used direct sequencing after PCR to find rare mutations causing β-thalassemia (14).

Delving into "Jumping Genes"

By 1984, Kazazian was looking for his next challenge. The blood-clotting gene, factor 8, which was defective in the blood disorder hemophilia A, had just been cloned. This gene was a major target, and hemophilia A was present at high frequency across populations. The gene seemed ideal to explore the variety of disease mutations in humans. Kazazian's group approached molecular biologist Tom Maniatis, who provided factor 8 cDNA clones. From 240 blood samples analyzed, they found many small deletions and nucleotide substitutions. Then, in May 1987, research fellow Hagop

Youssoufian found two patients with what looked to be large insertions. In one week, Youssoufian succeeded in finding a LINE (long interspersed nuclear element) insertion, a transposable element, known colloquially as a "jumping gene" (15). These sequences copy themselves and move around inside genomes. "I decided instantly to shift the focus of my entire group to work on LINEs," recalls Kazazian. "We knew from Barbara McClintock's work that transposons existed in maize, and we knew from a 1985 *Cell* paper (16) that there were retrotransposable elements in yeast. But we had not seen them in humans or other mammals."

With initial help from molecular biologist Maxine Singer, Kazazian's team began a 30-year journey to understand how retrotransposons—specifically LINE-1 (L1) retrotransposons—in humans and mice work and to find new ones. While his earlier research had been loosely focused on diagnosing and treating diseases, the transposon work was largely basic research. They found a number of human L1 insertions that cause diseases, including hemophilia and muscular dystrophy. Then, John Moran, Kazazian's postdoctoral fellow, devised a cell culture assay for retrotransposition, which they used to show that the average human genome has 80–100 active L1 retrotransposons (17, 18) and that most of the activity resides in a handful of very active "hot" elements (18). Soon after, Eric Ostertag, a graduate student in Kazazian's laboratory, discovered a new human transposon, SVA, a SINE (short interspersed nuclear element) that is an active jumping gene (19).

"Research in transposable elements is fascinating," Kazazian says. "There are sequences in our DNA that can jump around, and they have multiplied to the point that there are 1.6 million mostly dead or inactive ones among L1s, Alus (other active SINEs), and SVAs. They occupy nearly 50% of the genome. When I started, we thought the whole genome was composed of protein-coding sequences. Now we know that those sequences account for only 1.5%."

Through studies in rodent models, Kazazian's laboratory determined that retrotransposition is unlikely to occur in germ cells but instead happens during early embryonic development. This means that the embryo becomes a mosaic of cells with different genomes (20).

Kazazian spent most of his adult life at Hopkins, but in 1994, the University of Pennsylvania School of Medicine lured him away to become chair of its genetics department. At the University of Pennsylvania, he helped build the department and moved further away from clinical research and firmly into basic research. Kazazian stayed there until 2010, when he returned to Hopkins to complete his career where it had started. At Hopkins, he continued his work on basic questions about L1s and began examining whether L1 insertions play a role in gastrointestinal cancers. The work is the subject of Kazazian's Inaugural Article (1). His group found insertions in single tumor cells, but the role of the insertions in tumor development is unclear. "We studied 10 cases; five of them had no insertions, either in the normal cells or the cancer cells. The other five had highly variable numbers of insertions, 2–28 insertions per cell. As far as the insertions go, it was hard to know whether any of them were involved in the etiology of the cancer."

While his work has focused on how jumping genes may cause disease, other researchers have suggested that jumping genes may have specific functions. Kazazian is not convinced. "I really don't believe that they are functional," he says. "I think they're parasites. I will need strong evidence showing that they actually have a function. Perhaps, one of these days, the reverse transcriptase of L1 will be shown to be important in early development, but not yet."

Although Kazazian closed his laboratory in July of this year, he is in a phased retirement, writing an annual review of human genetics and working on a book. "I'm done in the lab, but I'm still enthusiastic and interested in the biology of transposable elements."

1 K. Yamaguchi *et al.*, Striking heterogeneity of somatic L1 retrotransposition in single normal and cancerous gastrointestinal cells. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 32215–32222 (2020).
2 H. H. Kazazian Jr., W. J. Young, B. Childs, X-linked 6-phosphogluconate dehydrogenase in drosophila: subunit associations. *Science* **150**, 1601–1602 (1965).
3 H. H. Kazazian Jr., H. A. Itano, Studies on the quantitative control of polypeptide synthesis in human reticulocytes. *J. Biol. Chem.* **243**, 2048–2055 (1968).
4 C. D. Boehm, S. E. Antonarakis, J. A. Phillips III, G. Stetten, H. H. Kazazian Jr., Prenatal diagnosis using polymorphic DNA restriction sites: Report on 95 pregnancies at risk for sickle cell disease or β-thalassemia. *N. Engl. J. Med.* **308**, 1054–1058 (1983).
5 J. A. Phillips III *et al.*, Prenatal diagnosis of sickle cell anemia by restriction and endonuclease analysis: HindIII polymorphisms in γ-globin genes extend test applicability. *Proc. Natl. Acad. Sci. U.S.A.* **77**, 2853–2856 (1980).
6 S. E. Antonarakis, C. D. Boehm, P. J. V. Giardina, H. H. Kazazian Jr., Nonrandom association of polymorphic restriction sites in the β-globin gene cluster. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 137–141 (1982).
7 S. H. Orkin *et al.*, Linkage of β-thalassaemia mutations and β-globin gene polymorphisms with DNA polymorphisms in human β-globin gene cluster. *Nature* **296**, 627–631 (1982).
8 H. H. Kazazian Jr. *et al.*, Molecular characterization of seven β-thalassemia mutations in Asian Indians. *EMBO J.* **3**, 593–596 (1984).
9 T. C. Cheng *et al.*, β-Thalassemia in Chinese: Use of in vivo RNA analysis and oligonucleotide hybridization in systematic characterization of molecular defects. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2821–2825 (1984).
10 E. P. Economou *et al.*, Molecular heterogeneity of beta-thalassemia in Mestizo Mexicans. *Genomics* **11**, 474 (1991).
11 I. R. Hussein *et al.*, Molecular characterization of β-thalassemia in Egyptians. *Hum. Mutat.* **2**, 48–52 (1993).
12 G. Garewal *et al.*, The molecular basis of β thalassaemia in Punjabi and Maharashtran Indians includes a multilocus aetiology involving triplicated α-globin loci. *Br. J. Haematol.* **86**, 372–376 (1994).
13 D. Rund *et al.*, Evolution of a genetic disease in an ethnic isolate: β-thalassemia in the Jews of Kurdistan. *Proc. Natl. Acad. Sci. U.S.A.* **88**, 310–314 (1991).

14 C. Wong *et al.*, Characterization of β-thalassaemia mutations using direct genomic sequencing of amplified single copy DNA. *Nature* **330**, 384–386 (1987).
15 H. H. Kazazian Jr. *et al.*, Haemophilia A resulting from de novo insertion of L1 sequences represents a novel mechanism of mutation in man. *Nature* **332**, 164–166 (1988).
16 J. D. Boeke, D. J. Garfinkel, C. A. Styles, G. R. Fink, Ty elements transpose through an RNA intermediate. *Cell* **40**, 491–500 (1985).
17 J. V. Moran *et al.*, High frequency retrotransposition in cultured mammalian cells. *Cell* **87**, 917–927 (1996).
18 B. Brouha *et al.*, Evidence consistent with human L1 retrotransposition in maternal meiosis I. *Am. J. Hum. Genet.* **71**, 327–336 (2002).
19 E. M. Ostertag, J. L. Goodier, Y. Zhang, H. H. Kazazian Jr., SVA elements are nonautonomous retrotransposons that cause disease in humans. *Am. J. Hum. Genet.* **73**, 1444–1451 (2003).
20 H. Kano *et al.*, L1 retrotransposition occurs mainly in embryogenesis and creates somatic mosaicism. *Genes Dev.* **23**, 1303–1312 (2009).