2017 Curt Stern Award: The Complexity of Simple Genetics[1]

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Leo Tolstoy famously remarked that happy families are all alike but that every unhappy family is unhappy in its own way. The adage that applied to Anna Karenina is just as apt a description for most Ph.D. students. A smooth transition to a doctorate can be described uniformly as one where experiments work more frequently than not, papers get published in high-quality journals, and students matriculate on time and with a minimum of stress or fuss. Alas, that utopian journey is seldom found, and such was the case when I found myself struggling a year or two into my Ph.D. at St. Mary's Hospital in London. I was unhappy in my own unique way and was considering leaving science altogether; even the simplest experiments were not working, large swaths of my research seemed or were uninspiring, and I had no evidence that the end of my turmoil was anywhere near in sight. Fortunately, I was blessed, through no design or skill of my own, with a wonderful mentor, Lizzy Fisher, who showed remarkable patience (Gandhi would have been proud!), measured guidance, and appropriate support to see me through the tempest of these early days. Indeed, as I reflect, as we all do upon receiving recognition for our work, I cannot help but recognize my disproportionate good fortune in having extraordinary mentors, without whom this journey would have been much different.

As a grateful recipient of this year's Stern Award, an unfathomable honor given the stature of those who were recognized before me, I must also thank both the awards committee and my nominators. Most importantly, however, it is critical that we recognize that the concept of an individual honor in science is fundamentally an artifact. This is because no discovery is the feat of an individual but rather represents the amalgam of thought, industry, and serendipity on a road strewn by many. Thus, even though I am humbled to receive this award, it is difficult to differentiate my contributions from those who came before me, those who worked alongside me, and those who will hopefully carry the torch forward.

As it became apparent that I would be getting a Ph.D. after all, Lizzy encouraged me to look for postdoctoral training in the United States. And so, through yet another series of delicious coincidences and happenstances, I ended up doing my postdoc with Jim Lupski in Houston, an event that has gifted me a mentor and a friend. By late 1997, through the industry of my ophthalmologist colleague Richard Lewis, who set out to collect families with rare ophthalmic disorders and convinced Jim to pursue their study; of the work of Kent Anderson, a previous graduate student in the lab; and of significant, key contributions from the labs of Val Sheffield and Ed Stone in Iowa, Mark Leppert in Utah, and many others, the first loci were mapped for Bardet-Biedl syndrome (BBS),[1–4] a mysterious clinical entity that captured my imagination. My fixation with this disorder, in addition to focusing on the human cost to BBS individuals and their families, which I understood very well, also centered on two key questions: (1) Given a "simple" recessive disease, why was there as much intra- as inter-familial clinical variability? and (2) How could it be that a defect in a single molecule could give rise to such broad yet specific organ pathologies, which included both structural developmental defects

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and progressive degenerative features? Determined to answer these conundrums, I plunged into positional cloning of BBS genes by using progressively improved genomic tools (alighting next to a highly collaborative Genome Center run by Richard Gibbs was certainly a boon to the effort). After a miserable first year of no progress, my psyche was rescued by the arrival of Phil Beales, who shared my passion for this disorder,[5] as a visiting scholar in Jim's lab. Another year passed, and from a discovery perspective it was just as miserable in terms of progress, but at least it was infused by a sense of camaraderie and shared purpose. By May 2000, at the end of our patience, a pair of eerie coincidences happened. First, our colleague Willie Davidson at Memorial University Newfoundland told us about his group's new linkage data that mapped a sixth BBS locus on chromosome 20. At the same time (i.e., the same week!), Les Biesecker and his group reported the positional cloning of *MKKS*, the gene related to McKusick-Kaufman syndrome. We quickly realized that (1) the *BBS6* linkage interval encompassed *MKKS* and (2) the two syndromes shared numerous similarities. A few months later, a pair of papers establishing *MKKS* as a bona fide BBS gene were published.[6,7]

Amid the euphoria of the discovery and no small sense of relief after numerous false positives (including a particularly cruel Christmas Eve in 1999), two interesting observations arose from the sequencing of a 96-well plate of DNAs from unrelated individuals with BBS. First, we found several samples with a heterozygous p.Ala242Ser allele in *MKKS* and no evidence of a second pathogenic allele; this was intriguing, especially given that p.Ala242Ser had been reported in homozygosity in individuals with MKKS.[8] Even more strikingly, one of these families was a consanguineous family who was predicted to map to the yet uncloned *BBS2* locus by virtue of identity by descent.

I was moving house with a newborn child when the lab called to tell me that Daryl Nishimura and team in the Sheffield lab had just reported the cloning of *BBS2*.[9] It was thus, on Interstate 59 in a less-than-perfectly-functional rental truck, that I made a decision that, in retrospect, has shaped my thinking henceforth: I asked Ph.D. student José Badano and (super)technician Steve Ansley, my "eye guys" team, to sequence all available BBS samples regardless of our mutational findings in *MKKS*. Remarkably, a couple of weeks later, these two individuals (who would eventually give me the gift of lifelong friendship) and I were able to show that there was an excess of BBS individuals who, in addition to having bona fide pathogenic alleles in either *BBS2* or *MKKS*, had an excess of heterozygous variants at the other locus. On the basis of these data, we proposed an on-off switch model wherein these additional alleles acted as modifiers of penetrance.[10] Over time, with many more genes and alleles discovered,[11–16] we would refine and adapt the model. We now understand, still imperfectly, that such excess rare variation in BBS genes contributes to the overall burden of the disorder and most likely modulates both penetrance and expressivity.[17–19]

The next inflection point occurred shortly after Aravinda Chakravarti enticed me to move to Johns Hopkins University to join the faculty of the newly created McKusick-Nathans Institute of Genetic Medicine. In the spring of 2002, the triumvirate of Steve, José, and myself set up a new lab in Baltimore. Jim was fully supportive of our plans for BBS but expressed some trepidation in us "leaving the nest," which José's thesis committee shared, acutely. Still, we ignored all of them summarily and set out to tackle a substantially harder question: what was the pathomechanism of BBS, and by answering *that* question, could we go on and understand why individuals with BBS had such an apparent excess of rare alleles? Many of us leave our postdoctoral mentor's laboratories armed with aphorisms that resonate for years. During those early stages of the lab in Baltimore, Jim Lupski's adage, "Professa, there is timeliness to science," could not have echoed truer. At the time, the handful of BBS genes cloned gave no real clues to pathomechanism. Thus, we searched further. It was upon the cloning of *BBS8* that an alignment of observations from several laboratories provided invaluable insight. The year before, Brad Yoder and colleagues had shown that defects in mechanosensory cilia drove renal cystic disease in the *Orpk* mouse.[20] Soon thereafter, Joel Rosenbaum speculated in a review that cilia could somehow be implicated in other disorders of renal dysfunction,[21] including syndromes such as BBS. This observation was noted by Phil Beales, who also pointed out to me a possible ciliary link by virtue of the existence of some BBS individuals with heterotaxia, a phenotype known to be caused by defects of cilia in the embryonic node.[22] To complete that puzzle, Willie Davidson introduced us to Michel Leroux, a then newly minted Simon Fraser University assistant professor who had taken an interest into BBS and who had observed that some BBS proteins co-localized with centrioles.[23] We now understand that these were not centrioles but rather basal bodies, structures that anchor the cilium in nearly all mammalian cells. Of note, the BBS-affected family that had piqued Phil's interest was the index family who, as Steve Ansley and I discovered at 2 a.m. on my birthday while slouching over an old 377 sequencer, carried a homozygous 4 bp deletion in *BBS8*; the ciliary hypothesis for BBS causality was developed shortly thereafter.[24]

The years that followed were nothing short of remarkable to us. Aravinda tried in vain to tame our juvenile exuberance with more aphorisms: "discovery is a marathon, not a sprint," he would admonish us. And yet, the field of ciliary genetics and biology seemed so new that foundational discoveries that moved us forward seemed to occur on a weekly basis. In a tour de force that speaks highly of a community of scientists who came together across four continents to solve a problem, we were privileged to play our part in multiple collaborations that helped define the biology of cilia, bore deeper into its contribution to human genetic disorders, and finally,

began to illuminate the biological underpinnings of genetic burden. Crucially, we recognized that dysfunction of primary cilia was largely predictive of the types of phenotypes that would result, an observation from which emerged the concept of ciliopathies.[25] Second, our community began to appreciate that cilia were not simply mechanosensory or "fluid facilitators" but actually partook in a host of regulatory roles in paracrine signaling, most notably sonic hedgehog and Wnt.[26] These data put a new arrow in our quiver: they allowed us to implement physiologically relevant assays to measure ciliary function in the presence of missense mutations found in affected individuals whose functional consequences and contribution to disease were unclear.

It is my hope that those who read this narrative will be left with one transcending theme: that what we discover and all that we become are truly the product—the amalgam—of those who choose to contribute to our thinking. Case in point: as we transitioned from a genocentric to a systems-based view of BBS, we became confronted with a new reality of massively parallel sequencing and the realization that our functional tools could not scale to help us establish the pathogenic potential of the torrent of variants that the community was reporting in individuals with ciliopathies. The discovery of BBS proteins regulating paracrine signaling had an opportunistic side effect. I recall discussing these "new" findings with my colleagues at Hopkins when Shannon Fisher, who was my direct lab neighbor at the time, suggested that the zebrafish embryo might be the ideal system for modelling some of these defects. And so, in late 2006, she graciously agreed to train a few people from my lab on manipulating and scoring zebrafish embryos. At that time, a key experimental observation became significant in that it defined a process that we have pursued at scale ever since. We found that human mRNA could readily complement the suppression or loss of its zebrafish ortholog and that insertion of human pathogenic mutations in the mRNA provided an efficient and accurate means of assessing the pathogenic potential of alleles.[14] After a steep, hard learning curve and many failures, we were able to scale the tool and begin to study the activity of dozens of mutations, liberating ourselves from the shackles of allele rarity and our inability to discern causality by human genetic arguments alone.[15,16,27]

The paradigm of trans-species complementation has defined the vector of our work during the last decade. During this time, we have invested our energy and our tools to give back to the community that has over the years contributed so much to our own thinking. Under the fervent belief that the whole is greater than the sum of its parts, we have engaged in the systematic modeling of candidate human genetic variants discovered in labs all over the world, we have invited trainees to come learn from us and in turn teach us about their journey in science, and we have shared our successes and failures bereft, to the best of our ability, of a hidden agenda or Kissinger academic politics (which are "so vicious because the stakes are so low").

It is my sincere hope that our community will retain the altruism and the esprit du corps that have always defined it, despite financial and geopolitical headwinds. Looking to the future, I see two threats that could readily be converted into opportunities. First, there is no question that we must learn to merge quantitative science with biological observation. As the pendulum swings, in response to technology and fashion in equal measure, different types of evidence are viewed with variable weight, depending on whether our evaluators have a mathematical or biological penchant. With respect, I must caution that monochromatic views of data are a threat we must work hard to eradicate; in the future, it is critical that biology and data science learn to draw strength from each other's considerable capabilities. Second, it is important that we remind ourselves that ecosystems that lose their diversity face extinction. It is of great concern that a trending message among non-specialists is that human genetics is by and large "done" and that most of what is interesting can now be pursued almost exclusively by computers of ever-increasing sophistication, by their talented operators, and by sheer bulk of petabytes of data. In many contexts, this is true. However, it is important that we maintain our vigil in vocational and skill diversity and ensure that we train and support the careers of young scientists across multiple disciplines. Looking back at the 20 years that led to this amazing recognition, my group and I were the direct beneficiaries of the intellect, intuition, diligence, and hard work of human geneticists, computational biologists, bioinformaticians, medical geneticists, biochemists, model organism specialists, and immeasurable others whose specific skillsets I cannot even begin to define. Had they not been there and had they not had the capacity to donate their skills, most of the discoveries recognized by this year's Stern Award would probably not have happened. To all these colleagues I give my heartfelt thanks; although there are too many to mention individually, the publication record reflects most of the work, albeit imperfectly. Finally, science is a demanding taskmaster that has extracted long hours, tears, and soul-crushing defeats that cannot be survived without the unwavering and selfless support of family and friends. At great personal cost, they have endured and have supported me by encouraging me during the troughs and cheering me during the peaks. Somehow, "thank you" seems grossly inadequate, but I am bereft of better words.

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