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Reflections on my daughter's DNA



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1. Prolegomena

While the flow of information from the genome to the arrangement and function of cells, the relationship between genotype and phenotype, will be the occupation of biologists for another century, for the physician the order is reversed. Patients present with a clinical phenotype and we ask our doctors four questions:

- What is the name and cause of my condition?
- What will happen to me?
- What can be done about it?
- Who can help me?

I have spent the last 11 years seeking those answers on behalf of my daughter Beatrice.

Our third child, Beatrice or Bea, was born in 2003 with contracted fingers (arthrogryposis) and wide eyes (hypertelorism). The attending pediatrician noted she was a bit floppy. Yet no connections among these or other clinical findings were known to medicine. Those highly directed, system-specific physical exams by various specialists added to the list of physical findings but yielded no plausible comprehensive diagnosis and left me, a physician trained in medical genetics, concerned that something was being overlooked. Scared is the better word.

Bea was not gaining weight principally because the growth of her muscles was not keeping up with the growth of her skeleton. Furthermore, she did not achieve in a timely fashion her gross motor milestones such as holding her head up, sitting up, cruising and walking. This was ominous and distressing because we had little idea why this was the case, nor what to expect or what to do. Lisa, my wife, breastfed and did all she could to get calories into Bea, to no avail. Nothing is more frustrating to a mother than an apparent inability to grow her children.

At the age of 6 months Bea was admitted to the hospital with a diagnosis of failure to thrive; the differential diagnosis of is very long and frightening. There seemed an overwhelming number of possible explanations: was it an acquired condition caused by a parasite or infection? Was it birth trauma? Was it genetic? I favored a genetic cause simply because it seemed unlikely that the numerous different signs and symptoms we observed in Bea would have different etiologies. I also knew that many syndromes manifest at birth have as their cause a genetic variation. Her hospital course was characterized by confusion over who was driving the case: were the neurologists primarily in charge,

or the gastrointestinal doctors, or was it the developmental expert? They all seemed to be working at cross-purposes each stabbing in the dark with their preferred work-up. But each expert had no name for Bea's condition or strong reasons to biopsy this nerve or radiograph that organ. Bea was home safe within 48 h with a prescription for flax seed oil.

After two years, we knew we had hit a dead end with the local experts. Lisa managed logistics: taking Bea to occupational therapy, physical therapy, the orthotic maker, and Bea's doctor. I focused on tactics: how to identify a unifying cause of Bea's syndrome. As Bea's daily care reached equilibrium and we settled into the belief that Bea was not acutely ill or going downhill, I realized I had to assume responsibility for the search rather than wait patiently for the science to catch up to Bea. All parents in this situation, where the medical authorities have essentially given up, learn the same lesson. They are their children's most dogged advocates. And each does as much as is within his or her power, nothing less. So what *could* I do?

2. Telltale toes

Early on I had a hunch Bea's condition was related to Marfan syndrome because her long feet and long, thin fingers and toes closely resembled those of Marfan patients, though she had none of the essential diagnostic features of that syndrome (Loeys et al., 2010). When Bea was three, I took her to be examined by my former teacher and Marfan expert, Dr. Victor McKusick (McKusick, 1960). A training fellow in clinical genetics examined Bea and noticed that she had a bifid uvula (a mild cleft in the soft palate). A Japanese and independently an Italian group had identified in Marfan-like patients mutations in the *TGFβ* receptor genes (Disabella et al., 2006; Mizuguchi et al., 2004). The *TGFβ* growth factor hormones named for their ability to transform normal cells to cancer cells were also known to be involved in many aspects of development including the soft palate (Kaartinen et al., 1995; Proetzel et al., 1995). Her bifid uvula further supported the notion of *TGFβ* involvement though there were many potential candidate proteins involved in the *TGFβ* signaling pathways (Derynck and Miyazono, 2008). Nevertheless, this greatly focused my efforts despite the spooky thought of cancer on the horizon or the complications of a Marfan-like disease.

I soon generated a hypothesis based on the work of Dr. Se-Jin Lee, a professor at Johns Hopkins, a graduate student when we first met. Se-Jin had discovered that muscle development, specifically muscle size, was regulated by a hormone he named myostatin (aka GDF-8) that was

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from the same family of growth and differentiation hormones as the three TGF β factors (McPherron et al., 1997). The TGF β hormones even used the same receptors as myostatin (Lee et al., 2005). Se-Jin showed in mice that too little myostatin allowed muscles to grow abnormally large. He also reported on one Herculean child with mutations in both myostatin genes (Schuelke et al., 2004). There were, however, no reported cases of excess myostatin signaling that might limit muscle development—could this be my Bea's problem?

With a neat hypothesis in hand and the proverbial laboratory “help from my friends”, we sequenced the myostatin receptor genes in Bea's DNA reasoning that a single new gain-of-function mutation in a myostatin receptor might enhance signaling and diminish muscle size. As enamored as I was with the hypothesis, a year into that work, it was clear Bea's myostatin receptor genes were genetically normal. But the effort was not fruitless. I had met with or spoken to many of the experts in TGF β signaling. I was now familiar with their literature and could discuss with researchers the issues beyond what had been published as only those who have first-hand experience generating and analyzing data can. But making and testing serial hypotheses looked like a long and winding and maybe endless road.

3. Ome sweet ome

When I found myself stumped, slightly dejected or a year from my last visit, I e-mailed Professor Andy Fire at Stanford down the road from my house. Andy has a calming and unassuming way of walking through a problem and seeing where potential solutions lie. There is an uncommon clarity to his thinking always rendered in an understated manner. Explaining that it had taken me an inordinate amount of time to get sequence data on only four genes, all of which were unperturbed, he suggested what seemed preposterous: look at 100 genes at a time as he was doing with the then-new massively parallel sequencing technology he was prototyping. “Go genomic old man,” was the advice I heard.

It was 2007; Bea was four years old. Clinically she was stable so I felt I had time to take a more exhaustive approach. My needs were beyond what I could do with my own hands. Now was the time to move the project out of my attic and into the wider world. The idea to scale-up sequencing was appealing but technologically beyond my capability. I thought my best chances of accessing such technology and the knowhow to use it were with the biotech companies, but the few I contacted were consumed with their own projects.

That summer of 2007 I inadvertently stepped into the public spotlight when I gave a talk at Google on what I called The Bea Project: parents dealing with a genetic unknown and do-it-yourself (when nobody else will) biology. I presented my quixotic efforts to identify a mutation in the myostatin receptor genes. Brendan Maher, a reporter from *Nature* interested in personal genomics got wind of my talk. He saw in Bea and me a story of very personal genomics and an era that had come of age because of the broad accessibility of technology to study DNA. His article and a cover picture of Bea appeared in October, coincident with a major genetics meeting and Bea became a microcelebrity (Maher, 2007).

That same month I attended a small biotech meeting. Present was Jay Flatley, CEO of Illumina, the leading manufacturer of sequencing equipment. I had known Jay informally from eight years earlier. I reintroduced myself; he had read the story about Bea. I screwed up my courage to ask the big question: would Illumina help sequence my daughter's DNA? At that time a whole genome sequence cost \$350,000. Jay made no promises but said if Dr. Gary Schroth, one of Illumina's sequencing gurus, was interested, he was okay with helping.

That night I emailed Schroth. Over the next three years Gary and his team sequenced the family's transcriptomes (all of our expressed genes), our whole genomes (at low coverage), and lastly our exomes. Why? In part because the data sets were useful to Illumina to cross-validate its own technology platforms, refine its software algorithms with two generations of data, and to test sequencing technologies not

yet ready for the market. Not that Illumina was uninterested in Bea's mystery and being part of a discovery—they were; Gary and his team had already made many technological breakthroughs collaborating with legions of investigators. Jay had picked the right person: Gary was a terrific collaborator because of his unwavering enthusiasm and his scientific creativity.

Eighteen months later, in 2009, Gary had lightly sequenced the entirety of the family's genomes looking for major changes in the DNA and our transcriptomes with high fidelity. These were very formidable datasets to analyze but they yielded nothing that smacked of an answer to me or to Irina Khrebtukova, a computer scientist *cum* bioinformaticist on Gary's team. We observed many interesting bits of biology in what was probably the first family so extensively studied genomically. But if medicine is the art of assessing probabilities, all the new hypotheses seemed like long shots. Nothing directly connected with TGF β popped up, though we tried hard to make those connections. We felt compelled to follow-up on many variants and had long discussions with scientists who had real expertise in one gene or another. Gary and I presented our data at the first Cold Spring Harbor meeting on Personal Genomics hoping someone might see something we didn't. Except for kind words of encouragement from George Church, a loyal fan of patient initiatives, we were ignored.

4. Pay dirt

Negative data are dark clouds and heavy rain on any parade and the words “keep looking” is a trying refrain. But perhaps because I was so convinced there had to be a causative variation that “explained” Bea's condition, Gary sequenced our exomes, ostensibly to test Illumina's exome capture kit in development. As the data flowed to Irina, now skeptical of my ideas after two years of mining data, I reminded her to be on the lookout for any gene variant specific to Bea with the letters “TGF” in the name. On the Saturday afternoon 7th of November 2010, Irina emailed me saying: “Looks like you were right.” Bea alone among the family had a highly damaging variant in the TGF β 3 gene. By the end of the weekend it was clear from the literature that such a variant would disrupt the structure of the TGF β 3 protein (Daopin et al., 1992; Mittl et al., 1996). In fact, two decades earlier someone had mutated that very codon and showed that the resultant hormone was inactive (Brunner et al., 1992). Another paper showed TGF β 3 was essential for fusion of the soft palate during fetal development (Nawshad and Hay, 2003). From that moment on it was all about TGF β 3.

The next few years were occupied with the next logical step—understanding the effects of Bea's TGF β 3 mutation. It was not enough to know that Bea had this variant. There were no reports of mutations in the coding region of the TGF β 3 gene. As well, there was a very scant usable literature on the TGF β 3 protein.

Like Diogenes, I looked for an honest man who also was a TGF β expert. A friend and scientific confidante studying muscle development suggested I meet Professor Malcolm Russell Whitman at Harvard, a *bona fide* TGF β maven. We struck up a collaboration to study the effects of the mutation on the function of the TGF β 3 protein. Over the next year, he and his colleagues in Korea were able to show very clearly that the mutant protein was by itself inactive—no downstream molecular signaling could be detected—but because the TGF β 3 hormone has two identical components (a dimer), the mutant version also inhibited the wild-type protein. Bea's variant protein reduced TGF β 3 signaling by about 75% qualifying the mechanism as dominant-negative.

Phenotypes are fluid and as the body grows or as it ages, signs and symptoms come and go. Bea's widely spaced eyes grew close enough over the years to be called “normal”; one leg became manifestly weaker than the other while all of her cardiac parameters and great vessel dimensions remained well within the normal range. The extent of a phenotype is also dependent on how hard one is willing to look—brain biopsies are not a routine part of the work-up. Bea at seven years still

had substantially less muscle mass than her peers, but she was not the same little girl Dr. McKusick had examined.

Bea had not been re-examined by a geneticist since she was three years old. In December of 2011, I was asked by a friend to present the story of Bea to medical students in Vancouver. I obliged for two reasons. First, I felt the need to convey the dual perspective of physician and father. Second, I also knew of Dr. Judy Hall at the University of British Columbia, who had spent some of her celebrated career studying amyoplasia congenita, a mix of heritable conditions involving muscle development. I had read some of her work when Bea was very young and did not see the connection so I put amyoplasia in the back of my head. But Dr. Hall was a legendary clinician trained under Dr. McKusick, so I called to ask if we could meet.

I gave my talk to the students and on the way to the airport met Judy in her crowded office. Her enthusiasm and willingness to help almost overwhelmed me. We suggested she examine Bea while attending a pediatric meeting in Carmel, California. Perfect. Two months later, Bea and I trundled off to meet Judy in her hotel where she proceeded to examine Bea in fantastic detail providing the best physical exam Bea had ever had. I took notes while Judy narrated the entire landscape of Bea from crown to heel. She measured the length of her limbs and the angles of her joints. She examined Bea's hand like a palm reader. "This distal digital crease is formed at Week 12 of fetal development and its absence tells us when the problem began," she said. It was a marvel to behold and a ringing affirmation of what keen clinical observation can yield.

In 2013, Judy and I, along with Malcolm, Gary, their respective teams and others published a case report describing Bea in clinical and molecular detail. The paper identified the variation in the *TGFβ3* gene likely responsible for her phenotype and the histological, cell biological and biochemical data to support a likely mechanism of action: a hypomorphic hormone providing inadequate TGFβ3 signaling during *in utero* muscle and skeletal development (Rienhoff et al., 2013). The primary purpose of the report was to alert clinicians to this new syndrome so we might find other Beas... or *TGFβ3* variants. It was the clearest science and the most carefully crafted piece of writing I had ever produced. The work was done by a band of people who had never met, who did not even know each other professionally and who, for the most part, spent their own time and money to do the work. That in itself seemed miraculous.

5. Modus operandi

What kind of project was—and is—The Bea Project, one with many sequential and technically complex steps, heavily dependent on goodwill and the availability of the right technology, conducted in an intellectual state of affairs where there are more unanswered questions about TGFβ signaling than good answers? How did we make it happen? I have a simple answer: I searched hard for people willing to help. There were some who would not make allowances for my ignorance or my status as a father or my non-academic status but many more said "Yes". It was vital to involve those who knew a great deal about a subject, those who actually did experiments and those who could provide the needed sparks and leaps of imagination to keep the project moving forward. It still astounds me that complete strangers could be moved to pitch in. Some explained that making a small contribution to a collaboration with a face, a name and, indeed, a mission was more gratifying than working alone on some gene or cell line or mouse. For others, it was their curiosity. For a few it was professional development, a no-less important matter in the current academic environment. They were all welcome.

But sheer opportunism played a role, too. My work in drug development takes me to many hospitals and labs around the world. When my schedule permitted, I took the opportunity to introduce myself to those I thought could shed light on Bea-related clinical or biological questions at hand. I prepared for those meetings by reading their work so my

questions were better informed and their answers more useful. Where possible, I got introductions from informal advisors and collaborators. It was hit or miss but most gave me a bit of their time, a few became collaborators and some even friends.

6. Peek moments

There are two paths to understanding the relationship between genotype and phenotype, a reductionistic but necessary first exercise. One is studying the natural experiment of human variation. The other is the unnatural experiment: making in the laboratory a cell, a worm, a fly, a fish or a mouse with the exact genotype of interest. Professor Tom Doetschman had spent his life studying the effects of mutations in the *TGFβ* family of genes. Over the decades of his TGFβ research in mice, he was thoroughly familiar with the complexities of TGFβ signaling including the role "background genetics" of the mouse played in determining phenotype, a potential source of artifact and misinterpretation if not carefully controlled (Doetschman, 2009). His publications after 25 years remain the most durable of the mouse TGFβ literature because he provided a comprehensive and totally transparent description of what he did and what he saw in mice. Dr. Doetschman was the man to call.

To my great fortune, a cold call in December 2010 to Tom and yielded within a year a *TGFβ3⁺/TGFβ3^{C409Y}* mouse. Bea and I visited Tucson in October 2011. We were escorted deep into the bowels of the basic science building to the vivarium, past airlocks and passcodes, to visit the mouse colony. Bea was dressed in a sterile gown that dragged along the floor, gloves, booties and a mask that nearly covered her face. Connie, the *maus haus frau*, introduced Bea to the varieties of mice used in science, most with beautiful long tails and shiny coats but with ears full of punch holes. The chimeras with Bea's gene were running around happily. Bea was besotted. Surely against protocol, we smuggled mouse #17 through the airport and on the plane. A service mouse if anybody asked. Re-christened back to San Francisco as Almond Joy because she liked the nuts and she was an instant joy to Bea, she lived a very generous mouse life of 2.5 years before dying in front of an attentive but teary Bea in a brief spasm of twitches and shakes. Almond Joy is buried in the family graveyard beneath the front lawn, next to two cats, two rabbits and a parakeet.

Almond Joy shone a bright light in Bea's life but a lesser light on our genetic question. Almond Joy and her littermates appeared to our eyes completely normal. She was a little chubby but Bea was an indulgent mouse keeper. Being a *TGFβ3⁺/TGFβ3^{C409Y}* heterozygote did not confer any obvious phenotype involving muscle. It would have been a great boon to the project to get any phenotype, which would then allow us to study in detail the involvement of TGFβ3 in development. We can accept for now that this *TGFβ3* variant has no phenotype as a heterozygote, that mice are able to fully compensate for a significant loss of TGFβ3 activity during development. After all, mice are not always human. But maybe this is not such a great disappointment; maybe this specific variant is relatively benign. This is the seesaw that the father rides on one end with the scientist on the other end.

The second way to understand the phenotypic effects of variants in a gene is to "collect" patients, those that acquired their mutations "naturally". Not surprising, it matters a great deal which patient traits one chooses to select for. An orthopedic clinic may see those with contracted joints, the hand clinic those with clinodactyly, the ENT clinic patients with bifid uvulas, and the cardiovascular clinic those with abnormalities in the aortic vasculature. Thus, each clinic has a bias toward patients with particular features. It is a near certainty, however, that all patients with hand problems will not all have vascular problems and that those with contracted joints will not all have bifid uvulas. In practice, the more severe phenotypes such as vascular disease will initially define the disease. Death gets all the attention and nobody ever died from a bifid uvula or bent fingers. But over time, even a lifetime, the full phenotypic spectrum of a syndrome is revealed.

Since our initial report of Bea in 2013, there has been a second distinct *TGF β 3* mutation found in another young girl whose phenotype only mildly resembles Bea's (Matyas et al., 2014; Rienhoff, 2014). This year a report was published of 43 patients with *TGF β 3* mutations ascertained on the basis of cardiovascular disease (Bertoli-Avella et al., 2015). None had variants related to those of Bea's, the loss of a cysteine amino acid that disrupts a critical structural feature of the *TGF β 3* protein. This keeps open the view that Bea's clinical trajectory is still a matter of conjecture. One invariant lesson genetics teaches is that different alleles of a gene can beget different phenotypes, sometimes unpredictable, sometimes strikingly different and even paradoxical. So while it is now clear that mutations in *TGF β 3* can cause cardiovascular disease, the obverse, that all mutations in *TGF β 3* cause cardiovascular disease, formally remains unsubstantiated speculation.

7. Hamartia

No one emerges from a project like this unchanged. Looking inward, my strengths and weaknesses as a scientist and clinician became more obvious. The social nature of science, science as a community effort became very real. I have a greater appreciation for what constitutes a scientific fact, the actual burden of scientific proof. I learned the value of a coherent narrative not just for enlisting the help of others but to critically interpret and re-assess the data, ours and others'. I have also learned that a facile narrative sells well but can mask unexplained complexity. Biology is rarely as simple as it is represented (Lazebnik, 2012).

The goals of the academic community in furthering science are different than those of parents. Parents and patients naturally seek eternal truths on which to plot certain courses while academics are more skeptical of facts, regarding them as ephemeral. There is also the need to "tell stories" to publish papers and obtain funding. These pressures often lead the course of investigation away from answering questions parents have. The academic world is highly competitive requiring greater focus, speed and finesse to succeed. My efforts were dismissed by some important scientists to the point where I stopped introducing myself as a father but rather as a clinician with an interesting case. Admittedly, I was unprepared for competition from academics. When first reported, Bea's syndrome was hers alone but it is now subsumed under the name of another despite a chorus of objections (Pyeritz et al., 2014) and unmindful of the complexities and practical consequences of nosological designations (von Kodolitsch et al., 2015), those discussions are among the Professionals, not me. After all, my point in the telling the world about Bea was to set the world watching for other Beas—these reports are the first echoes of her story. But it reminded me I was still a pilgrim in a foreign land.

Though I regard the current paradigm of *TGF β* pathophysiology as overly simplistic, I have great faith science will in the long term refine ideas as well as sift out wrong ones and trend toward a kind of truth. I am both an optimist and the father of Bea. As the former, I have hope that the partiality of Providence favors for her an uneventful course while the latter ensures Bea visits her cardiologist yearly.

8. Desiderata

Bea is a quintessentially normal 11-year-old tomboy—she plays baseball, enjoys skinning squirrels, keeps a lizard, refuses pink clothing, loves school. If she were to voice any complaints about her genetics, it

might be that she is a girl. That psychological phenotype is likely to change.

As to the four questions we ask our doctors, Bea's syndrome has a name (OMIM 615582), indeed a few! As to what the future holds: we don't really know. There will be decades of watchful waiting. To the question, what can we do, the short answer is be vigilant. There are no obvious or safe medicines that build muscles. As to who can help, we also answered that for ourselves: there are legions of people willing to share their knowledge, their knowhow and their creativity.

There are no last steps in a scientific investigation. The horizon is always receding as you walk toward it. We are always left with mysteries. But none for Beatrice. She has transcended her condition despite the attention given to it. She has taught me that a few bent fingers, a weak leg, maybe even the risk of life-threatening vascular disease is less important than living life fully engaged. She has embraced the world adapting to its challenges beginning when she was an infant rolling on the floor when she could not crawl. She has steadfastly insisted on engaging rather than withdrawing as a victim of genetics, self-identifying not by her limitations but by her strengths and interests, insisting to be in a swim meet knowing she will be last, hiking at half-pace but seeing the world less blurred. She is so much more than her DNA. She is remarkable not because she has some rare variant; she is remarkable because it doesn't matter.

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