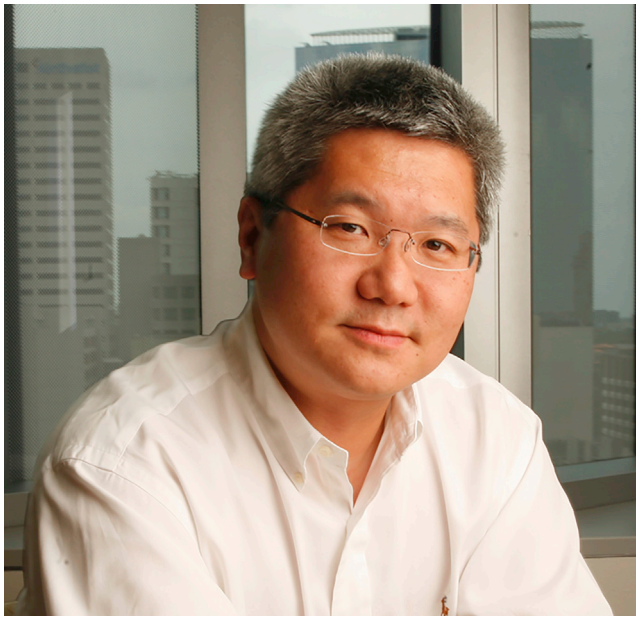
2016 Curt Stern Award Address: From Rare to Common Diseases: Translating Genetic Discovery to Therapy[1]

Brendan Lee[2,*]



Receiving the Curt Stern Award and being introduced by my mentor, colleague, and friend Art Beaudet are the greatest honors I could imagine. I attended my first ASHG meeting in 1991, and ASHG is the scientific community in which I grew up. I remember the excitement of discovery at my first meeting because I was a graduate student hot on the trail of cloning the gene for Marfan syndrome, so it is so apt that our ASHG president this year is Hal Dietz, someone whom I have known and admired since that time because of our shared origins in Marfan syndrome.

It is really an exciting time in human genetics, and my career has reflected the tremendous scientific transformations and transitions over the past 30+ years. As I mentioned, it very much started for me with the primary goal of cloning the first genes for human connective-tissue disease. However, shortly thereafter, it became our hope that discovering these rare-disease genes would inform us about common disease. I remember when I chaired the Program Committee for the 2004 ASHG meeting in Toronto a little more than 10 years ago, the hot topic of the Distinguished Speaker Symposium was about moving from rare to common disease in human genetics. At that time, the first examples of such were just being elucidated. These last 10 years have not so much been about proving this premise but instead about translating it to have an impact on therapy and patient lives directly. For pediatricians and geneticists, the fact that rare diseases would inform common disease was viscerally obvious, although the proof has been two decades in the making. The first scientist who taught me about this was the late and great David Rimoin. He showed me how studying human skeletal dysplasias informed us about common morbidities such as osteoporosis and osteoarthritis. By understanding the pathways that are dysregulated in skeletal dysplasias (i.e., chondrodysplasias, which affect primarily cartilage and skeleton growth, and osteodysplasias, which affect primarily bone and skeleton strength), we could also understand common diseases and even cancers intrinsic to the skeleton. It has taken over two decades, but we now recognize that rare alleles with strong effects do contribute to common phenotypes. The history of human genetics of the skeleton reflects this scientific journey. It started with clinical phenotyping, and in the case of the skeleton, the advent of skeletal radiography drove the underlying nosology that informed molecular studies. The advent of molecular biology led us to cloning the first disease genes via a candidate gene approach in conjunction with linkage, such as fibrillin in Marfan syndrome,[1–3] type I collagen in osteogenesis imperfecta (OI),[4,5] and type II collagen in spondyloepiphyseal dysplasia.[6] More recently, next-generation sequencing has led us to a phase of phenotypic expansion.[7] The increasing genotype-phenotype correlations have led to a mechanistic understanding of pathogenesis and, importantly, a new appreciation of disease complexity, which by informing phenotyping will lead to the development of clinical endpoints that can be used for quantifying mechanism-targeted therapy.

In our own work, the discovery that mutations affecting type I collagen cause OI, the most common form of brittle bone disease (BBD), by Peter Byers and others in the 1980s was the state of knowledge for over two decades. In fact,

[1]This article is based on the address given by the author at the meeting of the American Society of Human Genetics (ASHG) on October 18, 2016, in Vancouver, Canada. The audio of the original address can be found at the ASHG website.

[2]Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza Room R814, Houston, TX 77030, USA
*Correspondence: blee@bcm.edu

http://dx.doi.org/10.1016/j.ajhg.2017.02.001.

I was a fellow and junior attending physician in the late 1990s and early 2000s, when the standard for diagnosing patients with OI was detecting either qualitative or quantitative defects in collagen via SDS-PAGE electrophoresis. However, now 10 years ago, Roy Morello, a talented postdoctoral fellow in my lab, showed how post-translational modification of collagen by the *CRTAP-P3H1-CYPB* complex was important because mutations in these genes led to the first autosomal-recessive forms of OI.[8] It is appropriate that the venue of this meeting is in Canada because the first characterized patients in that study were from the First Nations community in Quebec, and they carried hypomorphic *CRTAP* variants leading to a rhizomelic form of OI. That discovery led to an explosion of gene discovery in OI and genotypic expansion by us and by other groups, and now over 13 loci cause BBD.[9] Although extremely important from a diagnostic perspective for our patients, the work raised important unanswered questions about the mechanisms of common bone diseases. Clinically, it was difficult to distinguish most severe OI patients both histologically and radiographically, leading us and others to hypothesize that common pathomechanisms, irrespective of genotype, might lead to qualitatively brittle bones. We became focused on matrix-cell signaling as one possible such pathomechanism for many reasons, including the work that Hal Dietz had done in Marfan syndrome. In short, we found that increased TGFβ signaling was important in the pathogenesis of multiple OI forms relating to collagen structure and/or post-translational modifications.[10] Importantly, we found that in preclinical models, anti-TGFβ treatment could dramatically restore bone mass in these forms of OI. This is critical because the effect size of treatment in the skeletal system often predicts the translational potential in humans, and this is among the strongest effect sizes we have seen in comparison with other approaches. This mechanistic finding also predicted that pure signaling defects should also contribute to this phenotypic spectrum. In fact, we and others identified that loss-of-function mutations affecting the WNT1 ligand can cause recessively inherited forms of OI.[11] However, even more importantly, semi-dominant, heterozygous mutations could cause early-onset, dominantly inherited forms of osteoporosis. This proves the link between a rare bone dysplasia and a common disease presentation of osteoporosis. In collaboration with Richard Gibbs and Eric Orwoll, we have since found that there is in fact an increased burden of such mutations in severe forms of osteoporosis in the general population (unpublished data).

What about therapy? These studies suggested the possibility of anabolic therapy, i.e., building bone in the context of OI as opposed to the standard of preventing bone breakdown, i.e., anti-resorptive therapy. In collaboration with Eric Orwoll and Jay Shapiro, we performed the largest randomized placebo-controlled trial of an anabolic therapy, teriparatide, in adults with OI.[12] Surprisingly, we observed a differential response between OI patients with mild forms caused by haploinsufficiency of type I collagen (who experienced significantly improved bone mass over 18 months of treatment) and patients with severe forms of OI due to qualitative defects of type I collagen (who did not respond). This suggested a genotype-specific response to therapy. This beautifully coincided with our discovery of increased TGFβ in severe OI given that this drug, teriparatide, can stimulate TGFβ production, perhaps explaining why severe OI patients who already had high TGFβ did not respond. This could in fact form the basis of personalized therapy. Currently, we would propose that in pediatric OI, anti-resorptive therapy with bisphosphonates remain the first line of treatment given the high turnover state of pediatric OI bone and children's responsiveness to this approach. Our work suggests that adults with mild OI should be treated with teriparatide, especially given that anecdotal reports suggest a much decreased response to bisphosphonates. The WNT1 discovery also led us to show that blocking WNT signaling by using an anti-sclerostin therapy currently in phase III clinical trials for osteoporosis is highly efficacious in that form of OI (unpublished data). Finally, of course, our work also suggests that anti-TGFβ could be a mechanism-specific form of targeted therapy in patients with collagen-related severe OI. We are now testing the safety of an anti-TGFβ reagent in a phase I trial in patients with severe OI as part of the Brittle Bone Disorders Consortium of the NIH Rare Disease Clinical Research Network (see Web Resources).

Another example of how understanding pathways in rare diseases has led to understanding mechanisms of common disease is my work in urea cycle disorders (UCDs), which I started during my clinical training with Art Beaudet at Baylor College of Medicine. This is a great example where understanding a classical biochemical pathway first described by Hans Kreb on how the body handles excess nitrogen in the form of ammonia from the intake of food protein and intrinsic catabolism can inform about common pathogenetic mechanisms in other diseases, such as hypertension. In addition, it underscores the importance of detailed phenotyping and understanding the natural history of disease in driving important mechanistic hypotheses. In this case, our natural history studies of the second most common UCD, argininosuccinic aciduria (ASA), which is due to argininosuccinate lyase (ASL) deficiency, showed that patients suffered from complex phenotypes including hypertension and neurological decline unrelated to the common morbidity that UCD patients suffer, i.e., hyperammonemia. A simplistic hypothesis would be that ASL is the only enzyme capable of generating arginine, the precursor to nitric oxide (NO), and hence, its deficiency would lead to NO deficiency. However, this was debated for many years given that all patients are treated with pharmacological doses of arginine, and as such, they should not be arginine deficient. In fact, a phenomenon known as the "arginine paradox" describes how all of us respond to the intake of arginine by producing NO in spite of the fact that the arginine concentration in the cell is in fact higher than the K_m of arginine for the

NO-producing enzyme nitric oxide synthase (NOS). Hence, for us to assert that the systemic disease and hypertension in ASL deficiency is due to NO deficiency, we have to hypothesize that ASL deficiency contravenes the arginine paradox and that ASA is an example of a Mendelian form of NO deficiency.

In fact, Ayelet Erez, a talented previous clinical postdoctoral fellow, solved this paradox by showing that ASL has not only a catalytic function, i.e., to generate arginine from ASA, but also a structural function to stabilize an NO synthetic complex that channels extracellular arginine into the cell for NOS action.[13] Hence, loss of ASL leads to loss of a metabolic complex that is required for both endogenous production of arginine from ASA and transport of extracellular arginine into the cell to NOS for NO production. As such, it provides a structural explanation for metabolite channeling as an explanation for the arginine paradox. Importantly, this has translational and therapeutic implications because NO can be generated by both NOS-dependent and -independent pathways. In the latter, NO can be generated via the reduction of primarily dietary sources of nitrate and nitrites by heme-containing enzymes. If ASA patients are hypertensive and NO deficient because of their inability to generate NO from an NOS-dependent pathway, then circumventing this by providing nitrite and/or nitrate should be curative. In fact, in a proof-of-principle case, an ASA patient who had been hypertensive for over a decade and resistant to all forms of standard anti-hypertensive therapies dramatically normalized his blood pressure via simple single monotherapy with either organic nitrates or inorganic nitrites.[14] This is now being tested by us in collaboration with Sandesh Nagamani in the Urea Cycle Disorders Consortium of the NIH Rare Diseases Clinical Research Network (see Web Resources) in a study testing whether NO therapy can effectively treat the hypertension in ASA and improve neurological function. Moreover, this ASL complex constitutes a new target for potentially treating resistant forms of hypertension in the more general population because it directly targets endothelial NO function.

I hope that you have seen how my own career has reflected the transformation of our field from gene discovery to phenotypic expansion to mechanistic understanding and finally to targeted therapy. In any such personal journey, the key elements are environment and mentors. I have had great mentors, such as the late David Rimoin, who introduced me to the world of skeletal genetics, and Arthur Beaudet, who introduced me to not only the world of inborn errors of metabolism but also all the qualities of a great leader in building the Department of Molecular and Human Genetics at Baylor College of Medicine—the department that has been the ultimate model for enabling great colleagues, great science, great trainees, and great collaborations. The most important genetic experiment is the one I conducted with my wife, Maria, which produced my son, Max—together, they have supported this journey. I also want to thank the over 50 trainees whom I have had the honor of mentoring and who were true partners in these and so many studies. I have had the privilege of being supported by great funding institutions, including the NIH and the Howard Hughes Medical Institute. Finally, none of this work could have been possible without the encouragement, support, and participation of families with the genetic conditions that we study and especially the Osteogenesis Imperfecta Foundation and the National Urea Cycle Disorders Foundation.

Web Resources

Brittle Bone Disorders Consortium, https://www.rarediseasesnetwork.org/cms/BBD

Urea Cycle Disorders Consortium, https://www.rarediseasesnetwork.org/cms/UCDC

References

1. Dietz, H.C., Cutting, G.R., Pyeritz, R.E., Maslen, C.L., Sakai, L.Y., Corson, G.M., Puffenberger, E.G., Hamosh, A., Nanthakumar, E.J., Curristin, S.M., et al. (1991). Marfan syndrome caused by a recurrent de novo missense mutation in the fibrillin gene. Nature *352*, 337–339.
2. Maslen, C.L., Corson, G.M., Maddox, B.K., Glanville, R.W., and Sakai, L.Y. (1991). Partial sequence of a candidate gene for the Marfan syndrome. Nature *352*, 334–337.
3. Lee, B., Godfrey, M., Vitale, E., Hori, H., Mattei, M.G., Sarfarazi, M., Tsipouras, P., Ramirez, F., and Hollister, D.W. (1991). Linkage of Marfan syndrome and a phenotypically related disorder to two different fibrillin genes. Nature *352*, 330–334.
4. Bonadio, J., and Byers, P.H. (1985). Subtle structural alterations in the chains of type I procollagen produce osteogenesis imperfecta type II. Nature *316*, 363–366.
5. Chu, M.L., Williams, C.J., Pepe, G., Hirsch, J.L., Prockop, D.J., and Ramirez, F. (1983). Internal deletion in a collagen gene in a perinatal lethal form of osteogenesis imperfecta. Nature *304*, 78–80.
6. Lee, B., Vissing, H., Ramirez, F., Rogers, D., and Rimoin, D. (1989). Identification of the molecular defect in a family with spondyloepiphyseal dysplasia. Science *244*, 978–980.
7. Lu, J.T., Campeau, P.M., and Lee, B.H. (2014). Genotype-phenotype correlation–promiscuity in the era of next-generation sequencing. N. Engl. J. Med. *371*, 593–596.
8. Morello, R., Bertin, T.K., Chen, Y., Hicks, J., Tonachini, L., Monticone, M., Castagnola, P., Rauch, F., Glorieux, F.H., Vranka, J., et al. (2006). CRTAP is required for prolyl 3- hydroxylation and mutations cause recessive osteogenesis imperfecta. Cell *127*, 291–304.
9. Marom, R., Lee, Y.C., Grafe, I., and Lee, B. (2016). Pharmacological and biological therapeutic strategies for osteogenesis imperfecta. Am. J. Med. Genet. C. Semin. Med. Genet. *172*, 367–383.
10. Grafe, I., Yang, T., Alexander, S., Homan, E.P., Lietman, C., Jiang, M.M., Bertin, T., Munivez, E., Chen, Y., Dawson, B., et al. (2014). Excessive transforming growth factor-β signaling is a common mechanism in osteogenesis imperfecta. Nat. Med. *20*, 670–675.
11. Laine, C.M., Joeng, K.S., Campeau, P.M., Kiviranta, R., Tarkkonen, K., Grover, M., Lu, J.T., Pekkinen, M., Wessman, M., Heino, T.J., et al. (2013). WNT1 mutations in early-onset

osteoporosis and osteogenesis imperfecta. N. Engl. J. Med. *368*, 1809–1816.

12. Orwoll, E.S., Shapiro, J., Veith, S., Wang, Y., Lapidus, J., Vanek, C., Reeder, J.L., Keaveny, T.M., Lee, D.C., Mullins, M.A., et al. (2014). Evaluation of teriparatide treatment in adults with osteogenesis imperfecta. J. Clin. Invest. *124*, 491–498.
13. Erez, A., Nagamani, S.C., Shchelochkov, O.A., Premkumar, M.H., Campeau, P.M., Chen, Y., Garg, H.K., Li, L., Mian, A., Bertin, T.K., et al. (2011). Requirement of argininosuccinate lyase for systemic nitric oxide production. Nat. Med. *17*, 1619–1626.
14. Nagamani, S.C., Campeau, P.M., Shchelochkov, O.A., Premkumar, M.H., Guse, K., Brunetti-Pierri, N., Chen, Y., Sun, Q., Tang, Y., Palmer, D., et al. (2012). Nitric-oxide supplementation for treatment of long-term complications in argininosuccinic aciduria. Am. J. Hum. Genet. *90*, 836–846.