Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

UW-Madison Department of Chemistry
1101 University Avenue
Madison, WI 53706-1396
Contact: Prof. Bassam Z. Shakhashiri
bassam@chem.wisc.edu
www.scifun.org

LINKING MASS SPECTROMETRY AND GENETICS FOR STUDYING THE VARIOUS BIOLOGICAL ROLES OF LIPIDS

by

Vanessa Linke

A dissertation submitted in partial fulfillment of
the requirements for the degree of

Doctor of Philosophy

(Chemistry)

at the

UNIVERSITY OF WISCONSIN–MADISON

2020

Date of final oral examination: 05/01/20

The dissertation is approved by the following members of the Final Oral Committee:
Joshua J. Coon, Professor, Chemistry
Lingjun Li, Professor, Chemistry
Federico E. Rey, Associate Professor, Bacteriology
Alan D. Attie, Professor, Biochemistry

Chapter 5

MASS SPEC FOR THE MASSES

or

WHAT GRANDPA KNEW.

To my grandpa, a constant source of love and inspiration.

VL wrote this chapter in collaboration with the Wisconsin Initiative for Science Literacy.
I appreciate the initiative taken by the Wisconsin Initiative for Science Literacy, which allows me and many others to explain our research to a non-scientific audience. All scientific research is done with the goal of being useful to many people. Thus, it is imperative that we get to communicate our findings with others. Thanks to all the members of WISL for providing this platform.

"Here," the old man throws a stack of papers on the table in front of me, "am I healthy or not?" "You know I'm not becoming *that* kind of Doctor, grandpa," I respond, slightly annoyed and flattered at the same time. Somehow my whole family and their circle of acquaintances seems to believe that getting any doctoral degree grants you knowledge in the medical field. My grandfather, despite never having set foot in an institution of higher education, is a very smart and curious man. He lived his life working tirelessly as a weathered farmer in our rural homeland of central Germany without ever complaining of his fate. And besides all the prejudices that this description bears, he has been one of the greatest supporters of education that I have met. He sent my mom to *Gymnasium* (the highest form of grammar school in the German three-tiered system, bound for college education) in the 1960s when this was anything but standard in a farm household, and he praised the value of a solid education to me and my cousin all throughout our childhood, much of which was spent riding on the back of his tractor.

So, despite not working towards an MD, but mostly because he still looks at me with his expression somewhere between hopeful pride and joyful anticipation, I eventually pick up the first sheet from the pile (**Figure 5.1**). The documents contain the results of his most recent blood test, issued by a lo-

Figure 5.1: A lipid panel is often part of a blood test.

cal laboratory. My grandpa regularly asks his primary care physician for blood work as to give the newest health trend he is following some validity. I vividly remember my grandma's disapproval of his week-long diets consisting of dandelion salads, bread drink (a fermented beverage made from bread), and loess (also known as medicinal clay, my grandmother exclaiming: 'this gnashing sound it makes!').

As an analytical chemist, I agree with his belief in the power of data. When we look at the results of such a test given in **Figure 5.1**, what do we see? Besides the regular and very telling measures of glucose levels and blood cell counts telling us whether we have diabetes or our immune system is on alert, we often get back a lipid panel. Now what is in this lipid panel? Usually cholesterol and triglycerides, maybe the cholesterol measure is split into good HDL and bad LDL, but that's usually it. My grandpa has been looking at these results for decades, so he knows all that. He in particular ignores his high cholesterol values as he does not believe in the negative effects of a diet high in cholesterol - as a lifelong farmer, chicken were the last animals he kept and their eggs were a daily delicacy and source of nutritious protein and fat alike for both my grandparents.

He interrupts my thought: "So if you're not that kind of doctor, what is it you're doing at University?" I look around and see him cleaning up the leftover fish bones from our lunch. "You like to eat fish, grandpa, right? Why is that?" He is quick to respond, "Oh, it's good for your heart! I'm even gonna take some fish-oil capsules after lunch!" I smile, "That's right and the reason for that is because it contains omega-3 fatty acids. Have you heard of those?" He looks confused, "Yes, it says that on the package, but what does all that have to do with your work?" "Well, omega-3 fatty acids are, as their name suggests, fats, or what we like to call them: 'lipids'." I pull out my textbook, "look at this **Figure 5.2** here. You can imagine a lipid like this little stick figure here, with a water-loving head, and these fatty tails. A lot of lipids have two of these fatty acid tails attached to a head group. Remember the omega-3? This term describes a particular kind of fatty acid tail, where the third from the last bond is a double bond. 'The last' here is indicated in fancy-science language as omega, the last letter of the greek alphabet.

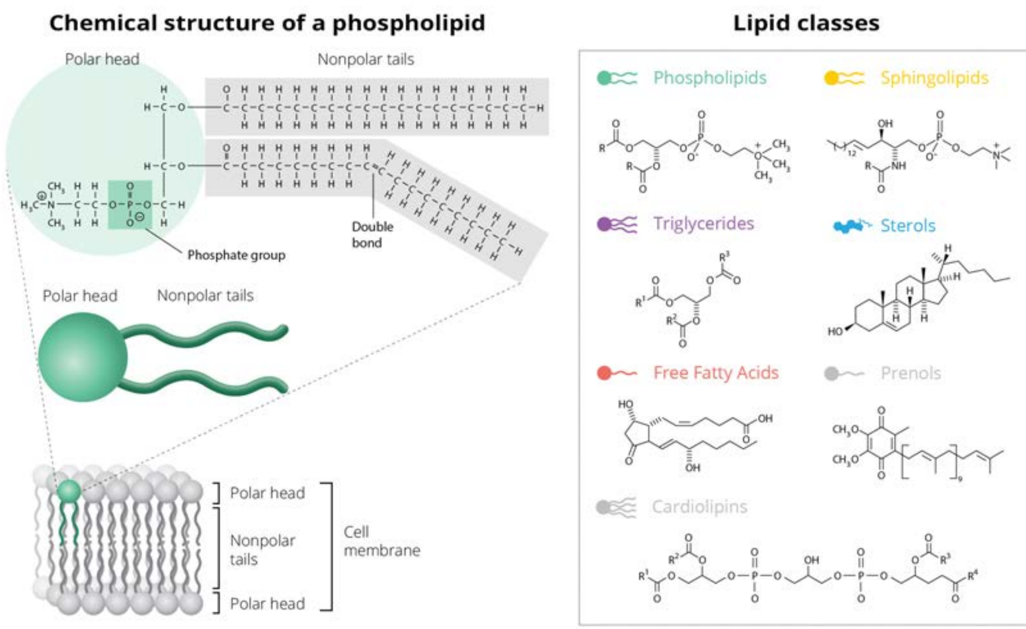

Figure 5.2: Lipids are fats. Commonly, lipids have a more polar head (also known as *hydrophilic*, or water-loving) and one, two, three, or even up to four nonpolar tails (fatty acids, also known as *hydrophobic*, or water-hating). Lipids come in various classes with a variety of functions. The members of these lipid classes have the same head group but vary in the length of their individual fatty acid tails. Phospholipids have two fatty acids and largely make up the membrane of our cells. Another lipid class are triglycerides, that have three fatty acids and are the main component in our body fat. Sterols, such as cholesterol, are also lipids. They feature a characteristic ring structure, act as messengers and are precursors to steroid hormones.

So, now why am I telling you all this? For the body, all these seemingly small differences - whether that double bond is at the third-to-last or second-from-the-front position - are actually super important. You may not be, but your body, or actually its enzymes, are very much able to distinguish between all the different lipid species. So, as scientists, if we want to understand the body, we need to understand lipids just as well!"

I know I can get him interested with this statement. My grandfather has always been especially curious about all things related to nutrition and health. For hours on end he could sit there and read about vitamins or herbal remedies and most interestingly, not shy away from conducting even the most far-flung experiments on his own. He was one of the pioneers of the smoothie cult by brewing his own so-called 'Vitamin drink' of carrots, apple juice, and whatever else nature would provide in that season, every morning, drinking sips of it throughout the afternoon. Once, after letting me try a sip, he was bewildered by my enjoyment, 'you like this?' For him, these experiments truly were about health, first and foremost. This doesn't mean they were always healthy, of course, they wouldn't have been experiments otherwise. An episode of claiming that the water from the well by the playground (with a sign 'Not drinking water!' posted by it) had healing powers ended in an episode of severe diarrhea that ended this particular belief but not his continued curiosity.

"Look," I say and show him **Figure 5.3**. "Imagine these two people." "Am I the fat one?" he asks, laughing out loud. I give him a look. "Well, you are right in noticing that their *phenotype* is different. A phenotype is anything that describes that person, for example

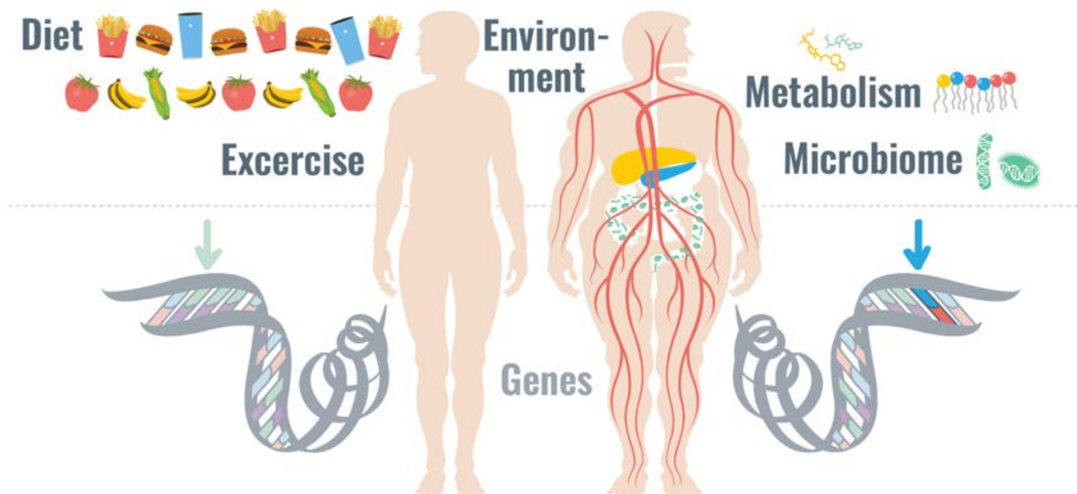

Figure 5.3: Diseases result from gene-environment interactions.

their hair color but also whether they are overweight or sick, for example with diabetes." "Despite the good portions we like to eat, diabetes does not run in our family!" he throws in happily. "Very good, grandpa," I tell him. "You just mentioned both of the important factors that determine the phenotype: your environment and your genes. Your genes are what is given to you from birth - and why some people can eat whatever they want and never gain weight." "Just like your mother and great-grandmother!" he throws in. "Yes exactly, and with environment I mean everything else: from how much you exercise to how well you eat - because, after all, it does influence your weight." He looks down on his belly and nods.

"Now," I said, "if we want to understand the body, we need to understand lipids. They are part of our metabolism and run all through our blood. Your HDL and LDL cholesterol particles also contain a lot of lipids, one of them of course cholesterol itself." "Oh wait," he responds "so this measurement on my blood test represents multiple lipids? It always has to be more complicated when you look closely, right?" "I'm afraid, yes, grandpa, HDL and LDL cholesterol particles are actually large hairballs of lipids, and in fact they also contain proteins, for example one called 'Apoa2'." I quickly show him **Figure 5.4**, a print-out of my results from the lab. "See here, that's what the whole HDL particle looks like - do you see the little green and yellow stick figures? They are the lipids I just told you about. And the blue ones represent the cholesterol molecules. And the purple ones, with the three fatty tails," I say triumphantly, happy that my example from the lab can actually explain his questions so well, "they actually are triglycerides, the last measure-" "measurement of my blood test," he completes my sentence, apparently regarding the topic as done, and turns to the kitchen.

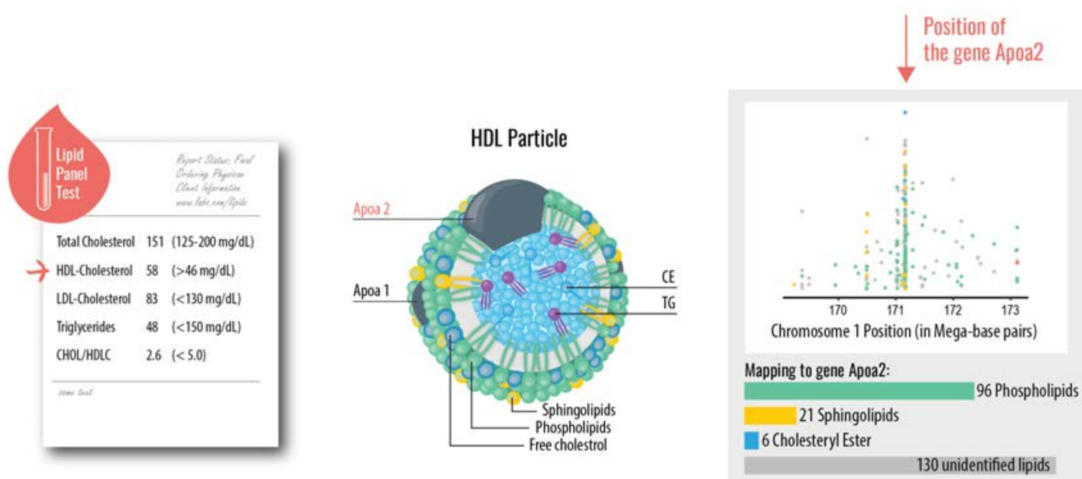

Figure 5.4: Lipids from HDL cholesterol particles link to Apoa2 gene. The HDL cholesterol measured in a blood test represents a particle containing hundreds of lipid species. In an experiment, a connection between these lipids and the protein Apoa2, that is also part of HDL particles, is observed.

"What your blood test doesn't show you," I get up and follow him into the kitchen so that he may hear me better, "are all the thousands of individual lipid molecules that are in your blood," "Wait, you're saying there are thousands of them?" he stops doing the dishes and looks at me. I laugh as I see the water dripping on his shoes and reply: "Yes, and they often take on important biological functions. And that's what I do." I now have a content smile on my face. "I like to ask, which lipids are there? And, as an analytical chemist, how could we measure them? And let me tell you, the answer is mass spec. But we're not even that good, look at that grey bar here, these are all lipids - but we don't know which ones they are - they are not identified by the mass spec." I point him to the right side of **Figure 5.4**. Then I see the confusion in his eyes. "But before I get ahead of myself, let me first help you with these dishes." "There surely is a lot of fat - sorry, lipids - on these plates," he responds, chuckling. I have to smile when I remember how he quickly learned the caloric potency of fats when stepping on the scale after adding two table spoons of ('very healthy!') olive oil to his morning oatmeal for several weeks.

Balancing two cups of coffee and a plate of cookies, we return back to the living room. Starting to chew on the first cookie, he mumbles "so you said this technique you're using is called 'massspec'?" (He pronounces it as if it was one word.) He comfortably leans back in his chair, knowing that this prompt will need a longer explanation. "That's right, it stands for mass spectrometry, but don't worry, I'll explain it with an easy example. Look at this **Figure 5.5**. Basically, a mass spectrometer functions like the scale you just used in your kitchen, just that it weighs molecules instead of vegetables. When we measure the

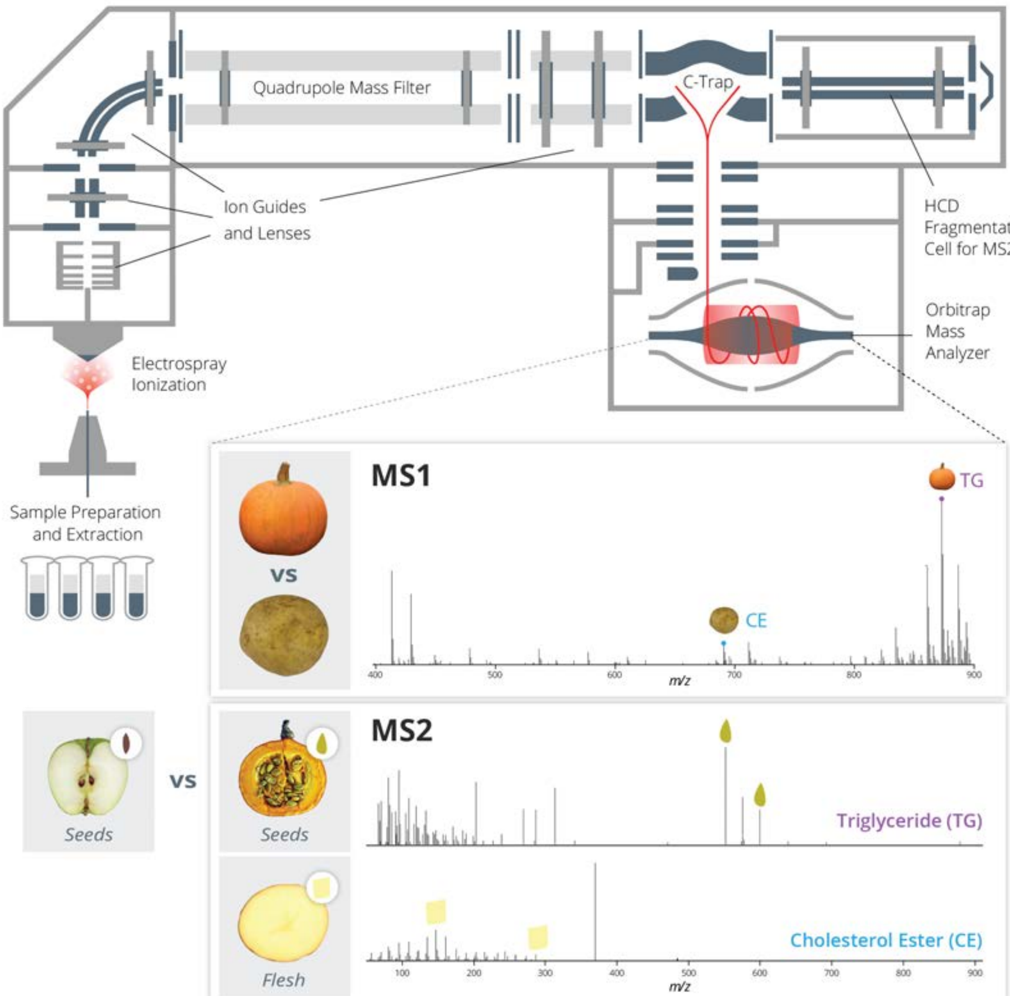

Figure 5.5: A Mass Spectrometer, or mass spec for short. A sample is first ionized, i. e. ions are formed from molecules, and sprayed into the mass spec. The ions are guided through the instrument and a quadrupole filters the ions based on their mass. The masses of whole ions can be measured by the orbitrap mass analyzer. To identify an ion, it can be fragmented in the HCD cell, followed by injection to the mass analyzer. With this technique, we can distinguish triglyceride lipids from cholesteryl esters. An analogy would be the weighing of pumpkin seeds and potato pieces on a scale to distinguish the vegetables.

mass very, very exactly, we basically know which molecule we are looking at!" My grandpa still looks a little confused, so I'm quick to add on: "To be honest, this comparison falls a little short, as your potatoes may vary in weight and still be potatoes while the weight of a molecule is exactly determined by its elemental composition. Fun fact, an element is the smallest building block of which all of our surrounding world is composed. The elements of lipid molecules usually are carbon (known in chemical formulas as C), hydrogen (H), and oxygen (O), as well as the occasional phosphorous (P)." "Water is H2O, right?" my grandfather falls in. "That's absolutely right, grandpa, you are surprising me with your Chemistry knowledge!

So, now imagine you are weighing a carrot and a potato. It could happen, that the potato and the carrot have the exact same weight, right? Yet we know, they are still different. This can happen, too, with molecules." "Yeah, but a carrot is bright orange!" he interrupts, while picking up a second cookie from the plate. "Okay, but a mass spectrometer can not *see*, it is not a 'spectroscopic' device, as we would say. A UV detector for example literally sees, i. e. it measures light waves, but a mass spec doesn't, therefore we do not get the information of the carrot being orange and the potato being yellow. In that sense, a mass spec is blind." "Well, even if it's blind, you could still tell that the carrot is long and thin compared to the potato" he falls in. "And indeed, a similar idea is used in a technique called ion mobility mass spectrometry. Anyhow, sometimes, even the shapes of two objects are very similar, so let's forget about the carrot and take the case of... let's say, a pumpkin and a potato. We can imagine a miniature pumpkin and a large round potato to have very similar weight, very similar shape and very similar color, am I right?" He nods, chewing, and I continue "It would only help us to cut them both up, because we could easily differentiate them by the pumpkin having lots of seeds and the potato being homogeneous on the inside. In mass spec, we use a similar principle when we do what's called tandem mass spec. As the name 'tandem' suggests, we execute a second measurement that gives us the weights of the pieces that result from shattering the whole molecule. We could easily distinguish light-weight (seed-like) pieces from heavier (flesh-like) parts, allowing us to make the call between pumpkin and potato. You can even imagine that, with high enough resolution, we could distinguish apple seeds from pumpkin seeds and so on."

My grandfather does not seem truly impressed yet, sitting in his armchair, holding the coffee cup in one hand and a third cookie in the other, which is why I continue: "In reality, however, multiple pieces of information are needed since normally we have more than two possibilities to choose from. That is, the question is *not* 'Is it a potato or a carrot?' but rather 'Which vegetable is this?' Anyhow, when we measure lipids, it basically works the same way. A cholesterol molecule will have a different weight from a triglyceride molecule. And even if two lipids share the same weight, we can smash them in smaller pieces and tell the difference. Except for when we can't and the mass spectrometer still can't tell us what some of those lipids are" - I am trying to get to explaining my own research but he is quicker to interrupt me: "So why are we not using your technology then? Why wasn't my blood tested with a massspec?" I feel apologetic for the industry: "Well, it just takes a long time for these expensive instruments to be used widely. But actually, some hospitals already do use mass spectrometry, I remember, for example during surgeries, to distinguish cancer from healthy tissue while cutting out a tumor." "I guess you could call that 'massspec for the masses' also," he laughs. He's always had a black humour and I'm just happy he is laughing again. "And anyways, we could tell you're healthy from these blood test results perfectly fine." "I guess I just got good genes," he says while taking a big bite out of the cookie in his hand. "Well remember what I told you about genes and diseases? Those specific enzymes I was talking about? Well, sometimes they don't work properly, and then we get sick. Offers like 23andMe now allow everyone access to their personal genetic information. But in a lot of cases we don't even know what all of the enzymes are supposed to do! You know how on your results sheet from your blood test, there are healthy ranges? For most of those hundreds of lipids we don't know which levels mean that we are healthy or sick. We're still far from personalized medicine, that would mean basing treatment on this genetic knowledge. And if we truly want to understand diseases, we need to look at both genes and environment, nature and nurture. And maybe that cookie does make a difference." I smile but he looks caught. Instead of taking another cookie, he focuses all on me now and asks "Is that what your project is about? Genes and lipids?"

"Pretty much, grandpa. Remember how you used to have cats on the farm, to get rid of the mice? Well, these mice are a vital part of my research today, as we can perfectly control what they eat. Therefore, they allow us to study just genetic influences, without all the spontaneous decisions that we humans make." I show him **Figure 5.6**. "We looked at almost four hundred mice that were all genetically different from each other, just like us humans. And then, we actually fed them what we call a 'Western-style diet,' that is high in fat and high in sugar. This stresses their body out, just like it does for us. Some of the mice got

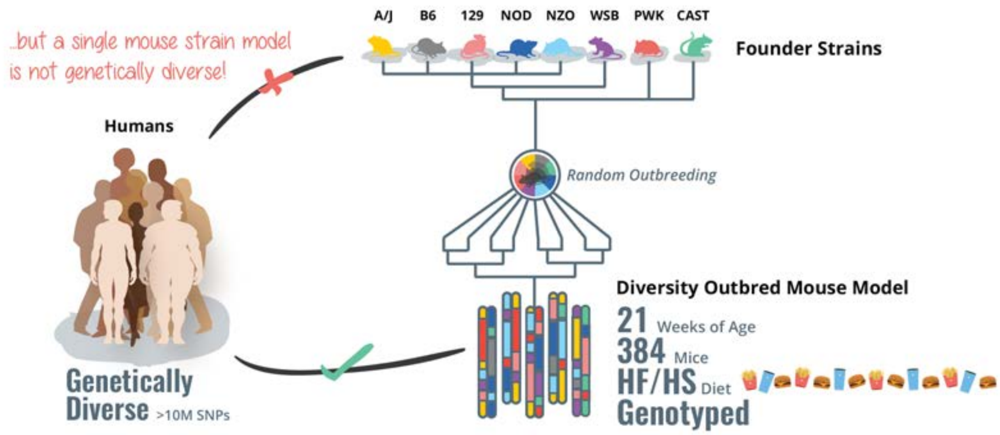

Figure 5.6: Diverse mouse models resemble the genetic diversity of humans.

really sick while others stayed lean and healthy. So unfair, some can just eat whatever they want! Anyhow, having this really diverse set of responses to the diet was perfect for us. We measured everything we could about these mice to really understand as much as possible of what was going on. For example, I measured thousands of lipids in their blood. Just like your lipid panel from your doctor, just much more extensive. And they've basically all done a 23andMe, too. Fun fact, this would actually need to be called 20andMe for mice, as they only have 20 chromosomes. Anyhow, here's where the magic happens: Because we also know all about their genes, we can directly connect the lipids that I measured to specific locations on the genome. Basically, we can link lipids to genes. And this is so cool, because, as I said, we don't yet understand all of how lipids are metabolized or connected to diseases. We can't yet identify all of the lipids, so a particular goal of mine was to identify more of the molecules that come up in our measurements. I want to give more of them names, so that we can interpret all the measurements, and learn about diseases."

"So how do you go about that? If your massspec can't identify them, how can you?" I'm thrilled I can finally talk about my contribution in the project. "Grandpa, remember the lipids from the HDL cholesterol particles in **Figure 5.4**? We found these lipids linked to the gene making the protein in the particles - Apoa2. I told you about it earlier." "Well, yeah, but you said that's all known, right?" he asks. "Exactly!" I reply, "and because we could see this *known* link, I knew this data was worth something. Apparently the links we saw between the lipids from our mass spec measurements and the genes were correct. So, in order to find out about the nameless lipids in our data, I turned the question around. I asked which gene are they linked to?"

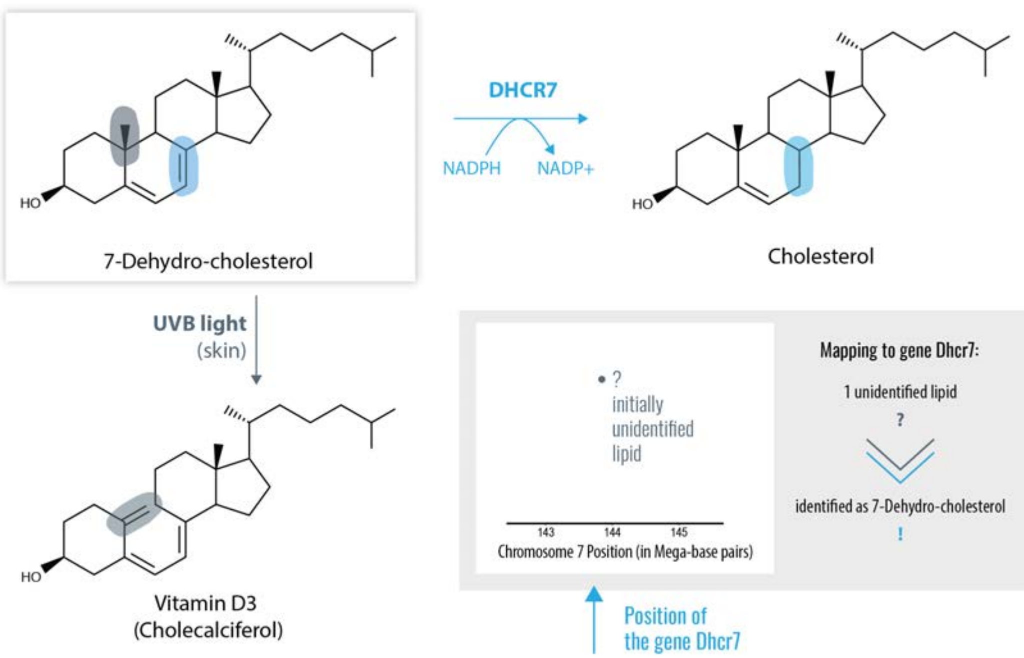

Figure 5.7: Dhcr7 is the switch between cholesterol and vitamin D production.

Besides cholesterol, grandpa is a big believer in the power of the sun, he likes to lie outside and enjoy the sun rays on his body. In the winter, he also takes his vitamin D supplements. What he does not know is that the two are linked, so I choose this example to tell him from my project. "So here is this example. As you know, you can get vitamin D from the sun. But in order to do that, your body actually uses 7-dehydro-cholesterol." "I only hear cholesterol" my grandpa says. I laugh "that's right, it *is* also a precursor to cholesterol. It can make both! There is an enzyme called Dhcr7 (**Figure 5.7**) that presents the switch in the body between making cholesterol and making vitamin D. If your gene making this Dhcr7 enzyme is different from most people's, then that can cause a higher risk for diseases, such as type 1 diabetes. Anyways, I found a lipid linked to Dhcr7, but I didn't know what it was. Because we know what I just told you about Dhcr7, however, and with a little bit of other knowledge about mass spec data, I could figure it out: it was 7-dehydro-cholesterol itself. Isn't that neat?" "That's quite impressive indeed," he acknowledges.

"So that was just one example, but we have literally thousands of these. And I actually realized that this could help other mass spec researchers, too. So we made our data available to everyone. And on the biology side, it is of course not just *my* project, we are collaborating with scientists in genetics, biochemistry, biostatistics and even microbiology to make sense of all this!" "Did you say 'microbes'?" It seems like he was paying close attention to my rant about my project. "Yes, we even believe that our genes determine which little microbes live in our gut. Crazy. And that they can affect how we metabolize food and whether we get sick. But we are still at the very beginning of understanding all these relationships. But knowing which lipids we're looking at helps a lot."

"So, with all this, have you found a way yet that I can eat whatever I want and not gain weight?" he concludes, half jokingly. "No, unfortunately not," I pause for a second. "Not yet, at least. I'm afraid for now you will still have to eat well and watch those sweets. But it is true that our research at the university - a few years down the road - can provide the molecular basis for biologists and ultimately physicians and pharmacists to come up with new ways of treating diseases. That's why it is so important for all of us to communicate. And why I'm so happy I could talk to you about it today." After a brief pause, all he responds is "I'm glad, too." Lastly, I want to thank you, dear reader, for following along on my journey. It has brought me all the way from my grandparents farm in rural Germany via the Wisconsin idea to you today. I want to encourage you and your children and grandchildren to never cease to ask questions. Stay curious and conduct experiments, whether they be in a lab or at our dinner table. We all are scientists of this world!

I would like to thank the Wisconsin Initiative for Science Literacy at UW-Madison for sponsoring and supporting the creation of this chapter. I commend the sentiment behind the program's mission and encourage all researchers to embrace science communication as a fundamental component of our responsibilities as scientists.

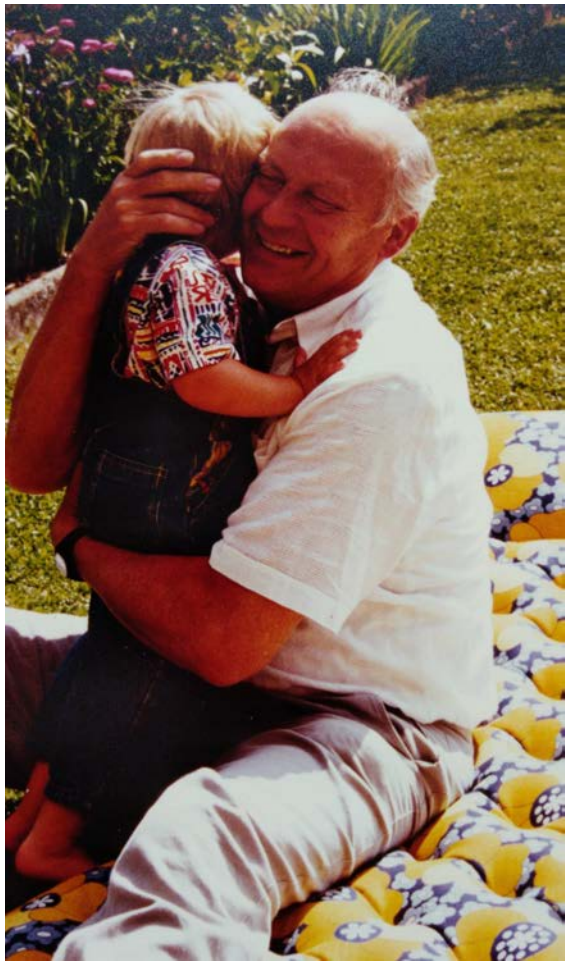

Figure 5.8: The author and her grandfather, ca. 1992.