

Klaus Wrogemann: Reminiscing almost 50 years of medical research¹

CHOOSING MEDICINE

I always wanted to become a farmer, but had no farm. As a child and student, I spent almost all of my holidays and free time away from school on farms, particularly the small one of Wilhelm Hohls & Klaus Otto in Ebstorf, Germany, and the large ultra-modern one of Wilhelm Martens in Tosterglope, the Augustenhof. Even as a medical student, during one particular summer, I did the entire grain harvest of my grandfather in Klein Bünstorf, driving a very small self-propelled Massey Ferguson combine powered by an industrial air-cooled VW engine with just 25 hp.

One of the last major essays we had to write in high school was a kind of CV: what we had done and what we planned to do. When I prepared it, the logic of it dictated that I would chose to become a family physician in the country and take over the praxis from my father. And so, I applied for medicine. It was the beginning of restriction to medical schools. I applied at the Medizinische Akademie in Düsseldorf and at the Phillips University in Marburg, was accepted by both, and decided to start in Marburg right after the Abitur from high school. I was shocked by the overcrowded lecture halls and did not get much out of spending 5 – 8 hours a day following lecture monologues. Thus, I soon skipped almost all lectures and prepared for the exams from textbooks, for the Vorphysikum (after 2 terms, one year, in the subjects of Chemistry, Physics, Botany, and Zoology) and the Physikum (after 5 terms, 2.5 years, in the subjects of Physiology, Anatomy, and Biochemistry). I did well in these exams, which all were oral examinations in groups of 4 students lasting up to 4 hours. After the Physikum I left Marburg for a winter term at Innsbruck University and for a summer term at Freie Universität Berlin. That was during the time of the cold war, and I was not allowed to travel to Berlin by car or train, but had to fly in, because as students in Marburg we had protested against the East German Regime, and therefore my parents insisted on flying to Berlin. That must have resulted in my first flights in an airplane for the summer term in 1963.

I went back to Marburg University for the 8th term, winter 63/64. On the side (but actually more or less full-time), I started doing laboratory work with my Dr. med.² supervisors Frits and Ellen Schmidt, Germany's best-known clinical enzymologists at the time. They were gastroenterologists with a special interest in liver enzymes for clinical diagnoses. I worked on a model for clinical enzymology - the isolated perfused rat liver. The research was fun, and my supervisors thought I did well. Thus, they suggested that I really should go into academic medicine. But, if I wanted to do so, I would need a "B.i.A.", been in America, and so I came to Winnipeg for one year to the lab of Marcel Blanchaer, who was Head of Biochemistry at the time; this was the start of my real research career.

The decision to go to Winnipeg was helped by my classmate Uwe Bär, who had been with Marcel Blanchaer before me and completed a M.Sc. in just one year. However, Dr. Blanchaer's offer to come to Winnipeg came at the end of 1965, while I was still in my final MD exams. Then the offer was withdrawn because of a shortage of funds. In February of 1966, however, it was suddenly renewed again. By that time I had finished my final MD exams, but had not yet defended my Dr.med. thesis, which in fact was not yet fully written. It became a big rush, and when the thesis was completely written (with generous help from the Schmidts), I got a special thesis defense date by the then Dean of Medicine, Prof. Otto Braun-Falco, a well-known dermatologist. He conducted the

¹ Written originally as a memento to my children.

² In Germany you cannot call yourself "doctor" after finishing medical studies, the equivalent to an MD, but you have to do research and write a thesis for the "Dr. med."

defense himself and only insisted that I return to Germany after my B.i.A. stint in Winnipeg. Well, it worked out slightly differently.

WORKING ON MY B.i.A. (Been in America) and PhD in Biochemistry

I arrived in Winnipeg on May 8th, 1966 with one suitcase (weighing 20kg), to spend a year in America. The beginning was rough. They had held off the start of a graduate course in enzyme kinetics just for my arrival. It started on the next day and was given by Bill Black, himself a graduate student, but one with great knowledge in the field. He had kind of a speech defect, spoke fast, and was difficult to understand at the best of times, but all the more so with all the different biochemical terms, which often are the same in English or German but sound very different when pronounced in a different language. I was worried and thought I would never make it in this field.

Dr. Blanchaer had an interest in muscle, had published previously key articles on red and white muscle, had grant support for muscle research and wanted me to work on muscle diseases because of my medical background. That started the work on muscular dystrophies, first on animal models and later on human muscular dystrophies. Dr. Blanchaer was interested in energy metabolism and mitochondria, and it was not a bad hypothesis that diseases of muscle weakness and wasting (muscular dystrophies) might have an underlying cause in energy production by mitochondria.

When I arrived in Winnipeg, I was immediately asked to register for a PhD program, but I said I had come only for one year and that was it. Nonetheless, they persuaded me to register for an MSc, which I did, considering Uwe Bär's success a couple of years earlier. This proved to be of significance, because 10 months later I was asked by three different people on the same day: "why not a PhD?" For the first time, I really considered this and felt that it would make sense to pursue, given my young age, but I had to consult with my parents in Germany, my Dr. med. Supervisors, and the German Army (as there was compulsory service from which I had been excused at the time to finish my studies - a good way for them to get licensed MDs cheaply). To solve all these hurdles I travelled back to Germany for one week, got an ok from all sides, and also got engaged and later that year married Dorit who then also came to Winnipeg (but this is a very long separate story).

Back to the research story: Thus, I studied oxidative phosphorylation in isolated skeletal muscle mitochondria, first from a strain of dystrophic mice and then from a strain of dystrophic hamsters. I was good in isolating mitochondria that were both functionally intact (as evidenced from high respiratory control ratios) and clean (as demonstrated by electron microscopy). The quality of these mitochondria by these criteria was better than any published in the literature, which mainly had reports from liver mitochondria because these were easy to isolate. However, we were able to isolate functionally intact mitochondria from any tissue we tried, and over the years these included organelles from skeletal muscle, heart, brain, airway smooth muscle (a very tough tissue), as well as from cultured cells, including skin fibroblasts.

In our first two studies on dystrophic mice and hamsters we could not detect any abnormalities. However, the studies were unique at the time and only years later I found out that our second paper published in 1968 on dystrophic hamsters had actually been featured and reviewed in Nature "News and Views".

These early successes with mitochondrial studies were followed by a full year of utter frustration: sometimes my mitochondria worked well and at other times they were of very poor quality and not functioning well at all, i.e. they were defective in oxidative phosphorylation. Then, one day, I noticed that the preparations showed the defect when the animals (dystrophic hamsters) had many visible white streaks. These turned out to be areas of muscle necrosis, large enough to be noticeable by the naked eye, but widely variable between individual animals. This explained why I sometimes isolated mitochondria that were functioning normally, while at other times they were defective. I had kept good records and descriptions of each animal I studied and thus I was able to

correlate the mitochondrial preparations showing a defect as coming from animals with massive muscle necrosis. The defect in oxidative phosphorylation was a combination of decreased respiration rates and diminished ADP/O ratios, resulting in much reduced rates of ATP production by these organelles. Fortuitously, I discovered that I could ameliorate this defect seen *in-vitro* by adding Magnesium to the test medium. This was towards the end of my PhD studies. Magnesium deficiency, especially in heart disease, was a popular topic at the time, and I believed that the genetically determined muscular dystrophy in the hamster led to a magnesium deficiency in muscle cells that contributed to the pathogenesis of muscle necrosis in these animals. This hypothesis was the culmination of my PhD thesis.

After I had graduated with the PhD in Biochemistry, we spent a few extra months in Canada. Dorit was expecting our first child, Jens, and we thought he should be born in Canada. During this time I got access to an atomic absorption spectrophotometer that could measure magnesium. I had saved samples from each of my experiments of the past three years and was excited to “nail” our hypothesis with solid data. To my great disappointment, the results were totally unexpected: magnesium levels in these mitochondria were entirely normal. There was no magnesium deficiency. My PhD thesis hypothesis was wrong, had to be rejected. Very disappointed, I leaned back in front of this instrument and noticed a big sign on it saying: “for calcium turn wavelength counter to 211.4”. This instrument had a lamp that allowed measuring both magnesium and calcium, each at a specific wavelength. I looked at all my test tube racks from the past three years. I had never before produced so many data in just one hour as was possible with this instrument. There were still enough samples left in each test tube. I decided to create some more data and switched to the wavelength for calcium: To my great surprise calcium levels in these mitochondria were elevated by almost an order of magnitude. This was the beginning of the calcium-overloading hypothesis, probably my biggest contribution to muscular dystrophy. Serendipity clearly played a major part in this discovery, but this was only the beginning.

It must have been November, 1975³. The Muscular Dystrophy Association of Canada was holding one of its bi-annual workshops for all its grantees to exchange the latest research results. These were nerve-wracking meetings because the entire medical advisory board would be there to observe its grantees. I had prepared a talk on altered production of Hb synthesis in dystrophic hamsters, a proteomics approach discovery, but probably unimportant for the genesis of muscular dystrophy. The speaker just before me was the well-known neuropathologist Dr. George Karpati who showed that the earliest abnormalities he could see in Duchenne muscular dystrophy by electron microscopy were wedge like lesions of the muscle cell membrane. With that observation I had a sudden idea: I knew that calcium outside the cell was 10,000 times higher than inside the cells. If you had cell membrane lesions, calcium would flood the cells, going through these lesions down the concentration gradient. Since this was a workshop, I asked whether I could leave my slides in the projector and present a new idea without slides: the calcium overloading hypothesis was born. After the talk, Dr. Alan McComas from McMaster University, a famous neurologist and author of the neurogenic hypothesis of muscular dystrophy, came to me and said, “Klaus, this is fantastic, are you going to publish it?” I hadn’t thought of it yet, but said, “Sure, I could send it to ‘Medical Hypotheses’”. “No”, he said, “send it to Lancet”. I followed his advice. This was November 1975, and in March 1976 the paper was on the shelf in our library. It was entitled: “Mitochondrial Calcium Overloading, a general mechanism for muscle cell necrosis”. This was long before the days of electronics in publishing, but it took less than 5 months from its conception to being in libraries in print. It is probably my most important paper; it had an interesting history, and it became a citation

³ Just for orientation: I received my MD and Dr. med in 1966, worked on my PhD in Winnipeg from 66 – 69, returned to Hannover, Germany in 1970 to finish my internship and to obtain my medical licence for Germany. (I never had a medical licence in Canada). I returned for good to Winnipeg in 1970 and started as an Assistant Professor on October 8, 1970.

classic. Even now, 39 years later, it is still being cited every year. It pointed to the possibility of interfering with muscle cell necrosis, one of the hallmarks of muscular dystrophies, without knowing the primary (genetic) defect in these conditions. This idea was picked up a full thirty years later by others for the treatment of Duchenne muscular dystrophy.

Something bothered me at the time about this defect in oxidative phosphorylation caused by calcium overloading. The *in vitro* observation suggested that oxidative phosphorylation in animals with massive muscle necrosis was almost totally defective. Yet these animals were running and biting as if almost not impaired. These two observations did not fit together. Eventually we found the explanation: we found several different populations of mitochondria: those from normal-appearing muscle had almost normal levels of calcium, while those from necrotic areas had up to 50 times higher levels than normal. When isolated and put into the test medium at 28C, these calcium-overloaded mitos could not retain the calcium, but would lose it into the medium to be taken up by the normal mitochondria coming from still healthy muscle fibers; the latter would then also become calcium-overloaded. Thus our observation on mitochondria was an exaggeration of what would happen *in vivo*. The earlier observation of the beneficial effect of magnesium could now be explained: magnesium in the test medium prevented the efflux of calcium from the overloaded organelles into the medium and the subsequent uptake by "healthy" organelles. In the absence of magnesium, all mitochondria in the test medium became calcium-overloaded with the resultant defect in oxidative phosphorylation. Again, luck had it that this *in vitro* exaggeration of the defect made us aware of it in a convincing manner in the first case.

The observation of calcium overloading leading to muscle cell death was of significance, but it was not the cause of these diseases. These are genetic, single gene disorders, with a problem in DNA. But in those days it was not possible to study DNA: this was before the DNA revolution. It was not possible to search at the DNA level for the defective gene(s). We therefore decided to look at the gene products and searched for mutant proteins - a proteomics approach. Initially we searched for abnormal proteins by one-dimensional gel electrophoresis, SDS gel electrophoresis and isoelectric focussing. A unique aspect was the use of a dual-labeling approach: we labelled the proteins of whole newborn dystrophic hamsters with either C14 or H3 and the control animals with the other isotope and then isolated numerous tissues in addition to muscle (as the genetically defective protein might also be present in non-muscle tissues and less hidden among all kinds of secondary effects one could expect in muscle altered by the dystrophic process). We actually consistently found a protein fraction altered in dystrophic hamster livers and were even able to identify the protein: it was hemoglobin. We had detected an altered involution of embryonic hemoglobin synthesis in livers between our normal and dystrophic strains of hamsters, probably of no significance to muscular dystrophy but just a fluke difference between different strains of hamsters.

We had to do better with our proteomics approach and decided to use the much higher resolution method known as two-dimensional gel electrophoresis, and study human cells (cultured skin fibroblasts). Again we were able to use a dual-labeling approach and thus were able to overcome the notorious difficulty of running 2-D gels with hundreds of protein spots reproducibly enough to follow individual protein spots. We just labeled the proteins of normal fibroblasts with H3-amino acids and of dystrophic cells with C14 or vice versa, and then combined the labelled cells' lysates from both cell types. After 2D-electrophoresis, we were able to produce two autoradiograms: one showing only H3 protein spots and the other only C14 spots coming from the respectively labelled cells. These were thus perfectly identical and could be overlaid for analysis. To facilitate this better, we used another trick: we made a negative of the autoradiogram of one cell type (white spots on black background) and a positive autoradiogram of the other cell type (black spots on white background). When layered on top of each other, we could see whether all the white spots were covered by black spots or whether there was one missing. And we found a protein which was either missing or highly under-expressed in dystrophic cells: a 56kDa protein.

The missing protein was published in Nature in 1982, and when it appeared in print it created a storm of excitement around the world. At the time, I was in Europe attending the Vth International Congress on Neuromuscular Diseases in Marseilles, France, presenting this work in front of a packed audience. The American Muscular Dystrophy Association (MDA) had sent reprints of the paper to every grantee, and these people were all in the audience. Dr. Bud Rowland, the famous neurologist from New York, was the chairman of this session and during the discussion he asked: "Klaus, so many people are doing 2-D gels, how come nobody else has seen this?" A hand went up, saying: "we have already confirmed it". Another hand went up saying: we have tentatively confirmed it as well. In fact, the MDA had asked one of its grantees with expertise on 2-D gels - Dr. Eric Gruenstein from Cincinnati, Ohio - to check it out, and he also had confirmed the findings.

The publicity by news media around the world was unbelievable. As I was not in Winnipeg, my Department Head, Dr. Julian Kanfer, had to deal with it. An example of the coverage can be seen in the following report from the Montreal Gazette: <http://news.google.com/newspapers?nid=1946&dat=19820922&id=Pn8xAAAAIBAJ&sjid=C6UFAAAAIBAJ&pg=1092,717097>. In Europe, they tracked down my parents in Germany to inquire about my whereabouts, and I was contacted in Marseilles to be interviewed. It also resulted in an appearance on CBC's *Front Page Challenge* in December 1982 to guess who this "famous" person was. Of course, they failed to identify me in the allotted time.

We were very cautious in judging the likelihood that our protein was in fact the gene product of the not-yet-identified DMD gene or just a coincidental finding, but the hype that it could be was there, as expressed (for example) by Dr. David Housmann from MIT in *Trends in Neuroscience: Volume 6, 1983, Pages 115–118 Research news: Cloning in on the gene for Duchenne's muscular dystrophy*: "An alternative possibility is presented by the results of Wrogemann and co-workers. This group, working at the University of Manitoba in Winnipeg, Canada, reports the identification of a 56 000 mol. wt polypeptide which is absent in the fibroblasts of DMD patients but present in the fibroblasts of normal individuals. If this polypeptide chain were found to be the actual gene product of the DMD gene, then the pathway to the understanding of DMD will have advanced markedly. One of the key tests to determine whether the missing polypeptide is the direct product of the DMD gene locus will be to demonstrate that the coding information for this polypeptide chain resides at Xp21⁴. Experiments are in progress in Winnipeg to explore this possibility."

Our caution about the significance of the missing protein was wise. After we had published it based on fibroblasts obtained from a cell repository we decided to collect cells ourselves from some of our patients. My family members served as controls. To our surprise, my son Mark, who definitely did not have Duchenne Muscular Dystrophy, also had the protein missing. Something had gone terribly wrong. Fortunately, we ourselves found the reason. It turned out that all the control cells from the cell repository were obtained from newborn males by circumcision, while patients' cells came generally from skin of an arm or leg. Thus, we had not only been comparing DMD vs. healthy individuals, but also genital skin fibroblasts vs. non-genital fibroblasts. Countless other investigators had used these control cells as well, and in general it was not an issue, but in our case it was the explanation for the missing protein: genital skin fibroblasts expressed this protein at very high levels while in non-genital cells the protein was missing or almost non-detectable. This was a novel observation which we published briefly in Nature as well. When we had written this manuscript, before publication, I sent it to numerous investigators who I thought might work on our original observation so as not to have them waste time on a wrong lead. Many of them commended me for this and "praised my courage", which surprised me, because I felt it was not courage, but rather just plain science, i.e., finding the truth no matter what. Interestingly, Dr. Gruenstein, who had checked

⁴ At that time the gene for DMD had not yet been identified, but it was known to reside on the X-chromosome at chromosome band Xp21.

our results for the MDA, had also used the same control cells and thus his reaffirmation of our results was also incorrect due to the same inadequate controls.

Thus, foreskin fibroblasts were not like skin fibroblasts; the biopsy site played a role. At the time, the only difference already known between genital and non-genital cells was that the former expressed higher concentrations of the human androgen receptor, which had not been isolated yet and of which the gene was still unknown. We contacted Dr. Len Pinsky of Montreal, Canada's leading expert on genetic conditions of the androgen receptor (AR)⁵. We asked him to blindly send us fibroblasts with high expression of the androgen receptor as well as cells lacking it. After we blindly analyzed 8 of his cell strains, we broke the code and correctly predicted the status of the androgen receptor (which could be determined at that time only on the basis of androgen binding assays) in all 8 samples. "My god", he said, "this protein spot must be the androgen receptor". We did not think so, because this protein was too abundant to be a steroid receptor.

However, we pursued this possibility further and studied genital skin fibroblasts from patients with testicular feminization now called androgen resistance syndrome. These genetically male patients grow up as females with enlarged breasts and a blind vagina, but they are infertile. Some highly successful female athletes belonged to this category and for a while tests for Y-chromosomes in female athletes were performed at Olympic Games and other events. We found that genital skin fibroblasts from males with AR mutations did not have our 56K protein or had it only at very low levels. We also employed a covalent labeling technique to test androgen binding to the still elusive androgen receptor. To our surprise, we found that the only protein specifically labelled with an androgen analog was our 56K protein. Specific labeling meant that we could prevent the labeling of this radio-labelled androgen analog with excess non-labelled hormone analog, a behavior that one would expect for the still elusive AR. However, this was confusing, as we felt our protein was too abundant to be the true AR, but it got labelled with androgen and was absent from patients with mutations in the AR. We thought that we were dealing with some more abundant precursor or more stable breakdown product of the true AR. This was based on a large amount of data from numerous patients and controls, some 50 altogether. When we wrote up these data for publication I suggested sending it to JCI, the high impact Journal for Clinical Investigation. Len Pinsky was against it and warned that one of the AR experts was an editor of the journal but also a competitor of Len Pinsky. I brushed it aside, believing that the science and the data of our paper would speak for themselves. It was under review for over one year with criticisms from the journal and rebuttals from us going back and forth numerous times. We could handle all of these easily, but when their criticisms ran out, the journal resorted to one sentence: "However, we have to refer to our initial reservation that this paper is not of sufficient interest to the readers of JCI"....and rejected it finally. There was nothing we could do. This was their prerogative. We thus had to submit these data to another journal, the Canadian counterpart of JCI. It was immediately accepted, but this was a journal of much lower standing. The story does not quite end here, because about a year later we noticed a paper in JCI entitled: "High efficient covalent labeling of the human androgen receptor". We were surprised as we noticed immediately that the protein labeled was not the AR, but our 56K protein, and the first author had been a post-doc in the lab of one of the editors of JCI around the time we had our paper under review with this journal. There was nothing we could do, except contact the author of that paper to collaborate on the identity of the supposed AR with our 56K protein. This we did and published it as such together with this author.

Proteomics approaches at that time were generally not very successful; ours, on the other hand, produced highly reproducible results, albeit not on the problem we originally tried to tackle -- Duchenne muscular dystrophy -- because we had used the wrong controls. However, we had found a

⁵ Dr. Pinsky was an expert on testicular feminization, as it was called at the time. These are patients who are genetic males with an X and a Y chromosome, but they grow up and appear as females, due to mutations or absence of the androgen receptor gene.

consistent association with another condition, i.e., androgen resistance syndrome which is caused by mutations in the human androgen receptor gene. To find out the possible role for this protein in androgen resistance syndrome, we had to identify it. This we did, by cloning its gene. It was done by creating an antibody to this protein. The antibody was created by preparing several hundred 2-dimensional gel protein spots used for immunizing a rabbit. It was used to conclusively show that the 56K protein was not the human androgen receptor but an aldehyde dehydrogenase. Its natural substrate appeared to be retinaldehyde, to be converted by this enzyme to retinoic acid, a known morphogen. With this finding we proposed a sequence of events for patients with androgen resistance syndrome. The genetic cause would be mutations in the AR gene leading to many altered gene expressions of androgen dependent genes, including the aldehyde dehydrogenase which would produce retinoic acid, itself a transcription factor and morphogen contributing to a whole cascade of altered gene expressions that underlie the very different phenotypes of patients with this syndrome, i.e. genetic males appearing phenotypically as females. With this finding and this hypothesis we ended our work on androgen receptor related conditions and focused fully on muscular dystrophies. By now it was 1992. Our serendipitous side-step into the androgen receptor field had lasted for 10 years.

Backstep to 1982: It was time to prepare for my second sabbatical. At our University you could go every 7 years for one year, with the University paying 80% of your salary. Good sabbaticals take a long lead time to arrange. I generally started this planning two years ahead. By now, the field of molecular biology and gene cloning had begun, and I thought the best way would be to go to a leading institute in this field. So, I applied for a sabbatical at the Institute de Chimie Biologique in Strasbourg under the direction of Pierre Chambon. I applied with the idea to clone the gene for the missing 56K protein and was accepted. For a whole year our entire family moved to Strasbourg. It was 1984. By the time we arrived the project had lost its attractiveness, because we had discovered that the missing protein had nothing to do with muscular dystrophy, my true area of interest. I therefore was assigned to the lab of Jean Louis Mandel, with the goal to clone the gene for Duchenne Muscular Dystrophy and the fragile-X syndrome⁶. I worked mainly with Michel Koenig, a graduate student at the time, from whom I learned valuable techniques, especially that of Southern Blotting. We mainly did linkage studies to zero in on the respective genes. While these goals were not fully reached during my year in Strasbourg, we made some impressive discoveries. Amongst them were two cases of prenatal diagnosis for Duchenne muscular dystrophy by linkage studies for which I had discovered the closest so-called genetic marker located on one side of the still unknown Duchenne muscular dystrophy (DMD) gene. We were able to predict the status of two male fetuses by determining which X-chromosome they had inherited from their DMD-carrier mother: the one with the DMD mutation or the normal one. We were correct in this prediction in both cases; one was correctly aborted, and the other was correctly predicted not to have DMD. And this was before the DMD gene had been identified, which was quite amazing at the time. Back in Winnipeg, we benefitted from the newly learned techniques for genetic linkage studies, along with other local investigators like Cheryl Greenberg and the lab of Dr. Hamerton. These studies contributed to the eventual cloning of the DMD gene not by us, but in the labs of Louis Kunkel at Harvard and Ron Worton in Toronto. One of our Winnipeg contributions to them was the discovery of a DMD patient with one of the largest deletions of portions of the DMD gene. The collaboration with Cheryl Greenberg proved especially fruitful, as she was a clinical geneticist with a keen interest in research. Her access to patients was perfectly complementary with our basic science expertise (updated through highly successful sabbatical leaves in leading laboratories).

While in Strasbourg, working on DMD, I had, if I remember correctly, noticed on a Southern Blot experiment a potential junction fragment of the DMD gene from a patient with an X-autosomal

⁶ The fragile X syndrome is the most common genetically determined condition leading to mental retardation, and only males are affected.

translocation that seemed to disrupt the still unknown DMD gene. Ron Worton in Toronto was using DNA from this patient to clone the DMD gene. Jean Louis Mandel and I went to Toronto to discuss this with Ron Worton as a possible collaboration, because the Strasbourg people were good in cloning and particularly in DNA sequencing at the time. This collaboration did not materialize, but if it had, one could reasonably speculate that Ron Worton - the co-discoverer of the DMD gene - might have preceded Louis Kunkel at Harvard in the race for discovering the gene. Louis Kunkel had a different patient who had a deletion of the DMD gene and started out later than Ron in the race for the gene, but he came in first by a few months. The respective publications on the DMD gene discoveries appeared in Nature (Aug 85 and Dec 85).

My two full-year sabbaticals in Strasbourg (84/85 and 92/93) equipped us well to do molecular genetic studies in Winnipeg, helped by the close links to Clinical Genetics and the local presence of unique populations with high degrees of consanguinity available for study. Thus, we identified numerous mutations in our favorite genes: the dystrophin gene and the androgen receptor, and later the discovery of two muscular dystrophy genes for two types of limb girdle muscular dystrophy found to be especially common in Hutterites, LGMD2H and LGMD2I. We also, together with Barbara Triggs-Raine and her lab, mapped and identified the gene for Bowen Conradi Syndrome, also known as the Hutterite Disease as this fatal condition occurs commonly in Hutterites.

Classical good research was generally hypothesis driven: you have an idea, formulate a hypothesis and test it. By the end of the 20th century another approach to research began to emerge: omics approaches. Basically you look at everything (genes, proteins, metabolism, bugs etc) and see what you find. In my early days these were labeled and criticized as fishing expeditions. Our early proteomics approaches faced these criticisms at times, but fortunately not by everybody. And the pendulum was swinging to favoring omics approaches, which ultimately are the key to better individualized or personalized medicine. I chose my last two sabbaticals with this in mind and spent these at the Max Planck Institute for Human Genetics in Berlin under the directorship of Hilger Ropers, a man with a brilliant mind who became a very good friend. These were easier to arrange as only Dorit and I went, and we were content with living in one of the guest apartments of the Max Planck Institute. In 2001 I worked on creating a peripheral blood expression chip, which enabled the study of the expression of any protein ever known to be expressed in peripheral blood. It covered almost 17,000 cDNA's⁷ which were printed onto a microscopic slide in an area the size of your thumb by a printing robot. It later turned out that commercial companies could do this much better and more reliably, but our chip that we produced in 2001 worked.

In 2009 Dorit and I went again to Berlin for half a year. By this time the new genomics was in DNA sequencing: to sequence the haploid genome for the first time it took 13 years and \$3Billion, comparable in effort and resources to the first manned mission to the moon. This has evolved dramatically and today (2015) it can be done in a day for \$1000. The day is not far away when it can be done in an hour for a hundred bucks. When in Berlin in 2009 we focused just on the exome (cheaper and faster), restricting the sequencing just to those sequences coding for proteins, i.e., only about 1% of the entire genome. I worked in a larger team as a group leader (would you believe it, because the previous one had just left when I arrived) on three projects. The first was a peculiar genetic condition which nobody understands properly --- and we did not succeed in it either. Number two was a big project to discover genes leading to mental retardation. This was done in collaboration with geneticists in Iran, because of the high degree of consanguinity in that country, which makes tracking autosomal recessive genes leading to intellectual disability much easier. The amazing result was the potential discovery of 50 new genes which, when mutated for both copies,

⁷ cDNAs are DNA segments coding for proteins.

lead to mental retardation. The findings were published as a full article in Nature in 2011. Here we had 50 new disease-causing genes in a single paper, whereas my entire time spent previously on single-gene disorders resulted in the discovery of just three genes by conventional positional cloning approaches. The power of the new genomics based on the new sequencing methods is just mind-boggling. The third project was a huge collaboration with a European consortium to find more X-chromosome genes leading to mental retardation. Up to that time (2009) most known mental retardation genes were on the X-chromosome. Additional ones were found by this collaboration, but the publication of the findings took more than 5 years because of the difficulty in writing the paper well and concisely, in addition to the difficulty in choosing the right journal: it was first sent to Science, then to Nature Genetics, then to Am J Hum Genet, all with lengthy periods of reviews. It was finally published in Molecular Psychiatry in 2015.

Another few words on sabbaticals: I took five of them and went to excellent institutions. The first one was at the Max Planck Institute for Immune Biology in Freiburg, Germany. I had chosen it, because there was a well-known membrane biologist, and muscular dystrophies were becoming known as membrane diseases. When I arrived there in 1977, the researcher had just left for holidays, and the director of the Institute, Prof. Herbert Fischer, snatched me and said he also wanted me to work with him a bit. I did, and never left him for that year, which became quite productive, but I had worked on immune cell activation instead of basic membrane biology. I mention this, because it turned out to be a very good year, in part, because I did not work on my own project and primary interests, but joined the host in working on his interests. That way you get much better attention. Working on your own project would generally result in being at the bottom of the priority list of the host institution. I followed the same rule for all my sabbaticals, and only this first one was a bit off my own research interests.

In hindsight I can look back on almost 50 years of medical research. There are some success stories to look back on, and these were helped by serendipity, no doubt, but also by being tenacious in periods when things did not seem to go well, by keeping good records, and, in no small measures, by choosing and engaging in high quality sabbaticals that facilitated quantum leaps in competency of new and emerging fields in genetics. I started out as a biochemist working on monogenic diseases and became a molecular geneticist as the field developed. The sabbaticals, of course, were of benefit to the entire family with our children having attended schools in Germany and France so that they all are tri-lingual today.

THE END