Bert Bakker

Personal Details

Name	Egbert (Bert) Bakker
Dates	Born 1951
Place of Birth	The Hague, Netherlands
Main work places	Leiden
Principal field of work	Human molecular genetics

Short biography

After initial training in chemistry, he joined the Leiden laboratory of Peter Pearson, developing molecular techniques, notably the use of DNA polymorphisms, and applying these to the prediction and prenatal diagnosis of Duchenne muscular dystrophy. Subsequently he has been responsible for the wider development and coordination of molecular genetic diagnosis across Netherlands and the evolution of new molecular technologies.

Interview

Recorded interview made	Yes
Interviewer	Peter Harper
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Edited transcript available	See Below

INTERVIEW WITH PROFESSOR EGBERT (BERT) BAKKER. 10.12 2010

I = Interviewer (Peter Harper)

B = Bert Bakker

I It's Friday, December 10th and I'm speaking with Professor Bert Bakker at Leiden University Medical Centre. Bert, I'm very keen to hear you talk about the diagnostic aspects of molecular genetics, but first, can I go back to the beginning and ask when were you born, and where?

B I was born in 1951 in The Hague, and my father was a butcher; family of 7 children. I'm the middle one.

I And was there any background of science, medicine or similar things among your family?

B Not really. The father of my mother had a pet shop with birds, so he raised, he grew canaries, crossed them, and found a very nice new variant. So he was a little bit busy with genetics. His brothers were teaching mathematics and worked in schools and things like that. But further in the family there is no history of science.

I What was it that made you keen to go in that direction?

B Actually after my secondary school I was going into chemistry, analytical chemistry, working at laboratory school. As a trainee I worked with hair dyes and with shampoos, and after one year I thought, "Yeah, it's not what I want. I want to go the second part to biochemistry." So I switched to biochemistry; this was all at a higher education but not university level. So I did my training to become a kind of bachelor degree for biochemistry, and I start to do my work. My practical work, internship, I had to do in Rotterdam doing heart perfusion on mice, and I didn't like that so much. So in the summer time I went to Leiden and worked as a summer job here in the clinical chemistry lab, because I had already this analytical chemistry background. I worked in the clinical chemistry lab and from there I start asking questions whether they have positions for a trainee for biochemistry. So I came to the lab of Peter Pearson, and I talked with him. And he said, "Okay, you can start tomorrow."

I What year was this?

B That was 1974.

I Peter must have fairly recently arrived?

B Yes, he was 2 years in Leiden at that moment. And in '74, so I came in the lab and started. And the first day Peter gave me 3 articles: a French one from Dutrillaux, with the harlequin staining, and also a German and an English article all on incorporating BRDU and looking at the harlequin chromosomes. After a few days that he had given me these articles, he said, "Okay, what do you want to do?" "I can try to repeat it?" "Yes, that's fine," he said. Okay, so we ordered the BRDU and started growing some cells and trying to look at X-inactivation as well sister chromatid exchange and look at some other things. So my first year was mainly on this type of work. In the same time I also worked with one of his PhD students from that time, Joep Geraedts to look at centromere of chromosome 1 where there was a big chunk of heterochromatin, a big blob, if you stain it with Giemsa and Joep wanted to know if it's protein or DNA what you stained there. So there was a whole microscope with quartz lenses and UV light and we could scan and calculate to see if it was DNA or protein we were measuring. It turned out to be DNA that was folded back. After that year I thought I should go

and study further so I enlisted at the Leiden University to do chemistry, biochemistry, but I had to go into military service.

After a few months I had to go into the army and I went to do my military service. And one and a half year later I was finished with that and I wanted to have a job. I thought to go again for studying and then I will be 30 when I'm finished, and it doesn't work so I phoned Peter Pearson, "Can I give you as a reference for an application I want to do?" He said, "Yeah, that's fine, but why don't you come here and talk; I might have a position." So I came to his lab and I talked about possibilities, and he said, "I have actually two positions: you can either become technician; you're trained as a technician, you can work as a technician. Or you can finish a project of a PhD student who had just left because it didn't go so well and it all stopped, and we need to finish it for the grant money. And so I said, "Okay, I would like to try to finish that." And it was on the cells called 293. These are human embryo kidney cells that were transformed with adenovirus fragments by use of calcium precipitation by Graham and Van der Eb. This cell line was the first human cell line transformed with adenovirus.

We wanted to find out where, on which chromosome, the viral sequences were incorporated. So somatic cell hybrids were made, at that time also pretty new, and a whole series of somatic cell hybrids were tested first by immunoprecipitation on the cell lines to see with which chromosome the large T-antigen segregated. The lab of Meera Khan was next door and they were doing similar work on the somatic cell hybrids localising enzyme genes. However the HEK 293 hybrids were still too complex as cell lines with so many chromosomes that it was almost impossible to get out of there, so at a certain point we said, "Can't we do that with DNA?" That's when DNA came in. One floor up, that was the laboratory of Van der Eb, and they were doing some Southern blots; very beginning it was,1977.And so we did some Southern blots, and it turned out that we could follow the chromosomes and correlate these with the Immunoprecipitation bands; we had a kind of impression which chromosomes were involved. So we could finish the project and it was done. And then the DNA became prominent. At that time Kan and Dozy just had published their DNA markers (restriction site polymorphisms) in the beta globin locus. Peter said, "We should use these type of markers to do this:." And he came with an old letter of John H. Edwards] once published in the The Lancet from1956 proposing to find, use markers on the genome to enable antenatal detection of hereditary disorders.

Peter said, "These DNA markers, these RFLPs should do the job." So okay, let's find this type of DNA markers. And then I started to get a placenta from the hospital here, and from this placenta, isolated DNA; the DNA was digested with EcoRI and separated on a gel and so that we had a nice smear; then made more of it and separated on a sucrose gradient. From the sucrose gradient we made a whole list of fragments; different size fragments by taking this gradient and having a fraction collector and having all these different fragments. And we took the fragment size of 1kb, 2kb, 3kb, ligated these into a plasmid and cloned them in E.coli. Of course that later part was not possible here in Leiden because we were not allowed to clone human DNA fragments in E.coli, so I had to go to Mill Hill (London, MRC lab of Dick Flavell) and to do there the first ligations and cloning of these fragments. Actually before that, I have to say that the restriction enzymes used had to be isolated by myself from bacteria strains because they were not commercial available yet.

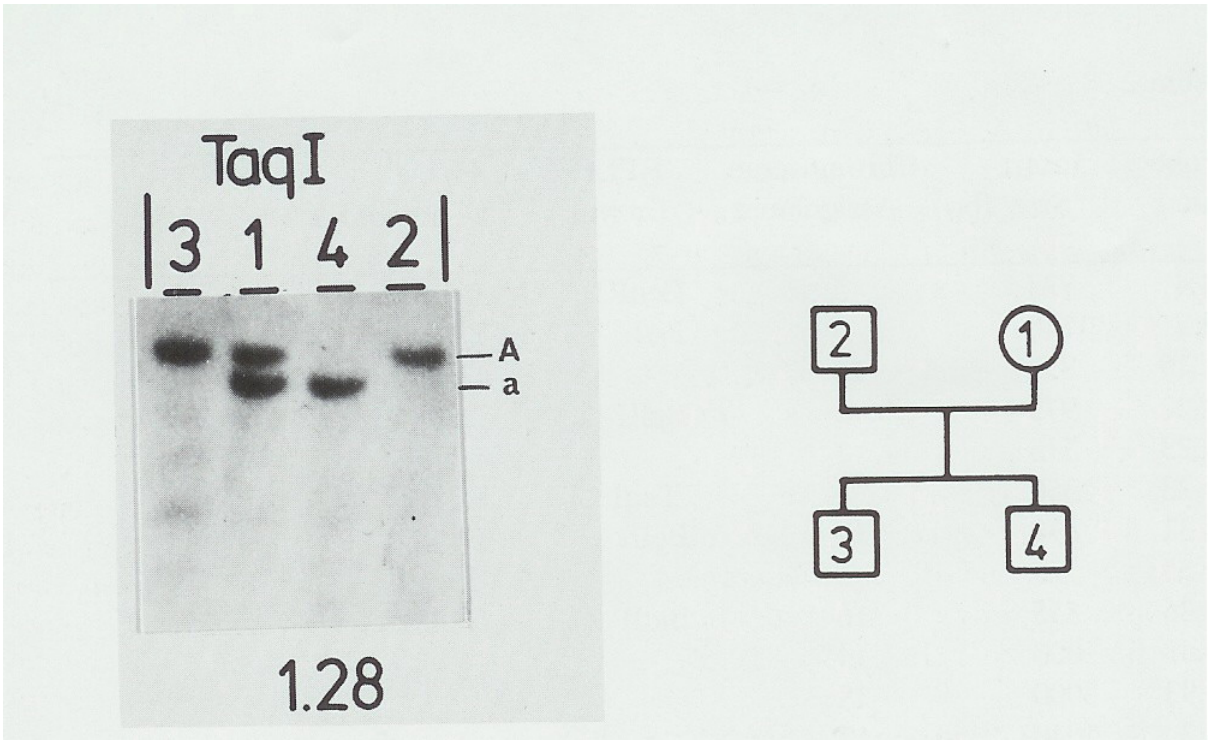
I Not commercially available.

B Not yet; not so much, or far too expensive. But there was a group also upstairs in the same building that had these bacteria strains available and with these we could isolate many different enzymes. I got the protocols and isolated most enzymes myself. So the first experiments were actually very easy to clone genomic DNA in plasmid: you plate the bacteria out on an agar plate and you have hundreds to thousands of colonies, but then which one do we need? So we took a membrane, put the membrane on the plate, marked it and took the

membrane off, lysed the sticked bacteria and hybridised with total human DNA, to see all the repeat clones. So we saw all the black clones that were carrying human repeat DNA. And the ones that were not lighting up, those ones were the interesting ones so I took these and grew these further and analysed them. And out of a couple of rounds like this we had 23 different human genetic single copy probes. And then we started to do Southern blots on restriction digests with other enzymes, hybridising the probes to identify polymorphic sites. And doing so, we had detected this whole series of polymorphic clones in one experiment. So it was quite something. [laughs]

I Now at what point then did the famous L1.28 -

B Now actually on the first plate, so all the fragments which were 1kb inserts; I had 3 plates: one with the 1kb, 2kb, and 3kb, and on this plate it was the 28th clone I picked from that set. And the L is Leiden. [laughs] That was from the first experiment at that time. And because L1.28 was polymorphic, it detected a TaqI restriction fragment length polymorphism, I had to isolate more TaqI restriction enzyme, to assess the allele frequencies on a set of DNA's from 20 individuals. Southern blot. After hybridisation the X-ray was showing two alleles in a couple of the women, and in the males it always had one allele. So we had already a suspicion that it would be on the X chromosome.



I As a matter of interest, this recognition that there was only one in males and two in females: was this something, were you recording the sex differences systematically from the beginning, or was it -?

B Yeah. From the beginning the placentas, we got them from the hospital and I got the sex, so I had the first placenta where I took the DNA for the cloning, but I had another series of 50 placentas and from all of these DNA was isolated; we all had to do that with phenol extraction, all this old stuff now. But DNAs were there and sex was known. So that was clear. But the allele frequencies… at that time Peter Pearson was very enthusiastic to see each X-ray that was developed; he almost took it out of my hands to check the bands and to see what's going on. And at a certain point there was one of the probes that gave homozygosity in all lanes, and there was one female DNA sample that was homozygous for another allele. That's of course very strange. And he said, "That can't be possible." And he started calculating this "Hardy-Weinberg formula" and showed me how you should do that, count heterozygotes; and see how many homozygotes and heterozygotes you should have. And he said, "That can't

fit. That is not in equilibrium." Later we found out from the hospital that this was a type of incest case; a rare polymorphism and the woman was homozygous for this rare variant.

But actually the X chromosome was pretty obvious as you see on this slide (see end of interview) the famous slide we have with the L1.28 on it and the two males and the parents, and you see that one male gets the one allele and the other the other; that was pretty soon we had it figured out that it was the case.

We didn't know on which part of the X chromosome it was. And just by using somatic cell hybrids all these probes were put on the map, on the human genome map, by using DNA isolated from the somatic cell hybrids that were also available in the laboratory in the lab of Meera Khan. So we, I did DNA isolation from the hybrid cell lines,, and Peter looked at the chromosomes and I hybridised the fragments to it, and we could localise them very nicely on different autosomes; and for the X chromosome we had some translocation breakpoint cell-lines so we could pinpoint and say, "It's on the short arm." In '81, it was all around '80, '81, Peter Pearson went to the Oslo conference where I had this first publication of these probes on a poster, and at that time we had 30% of all polymorphic DNA markers on the human map. The D1S2 on chromosome 1; the D2S1on chromosome 2; etc all the first probes. DSX7, that's the L1.28. The same year there was a meeting, a chromosome meeting, no, a human genetics meeting, International Human Genetics Meeting in Tel Aviv and Peter Pearson went there, and he was in the plane together with Ysbrand Poortman (from the Dutch patient association of muscular disorders), and they were talking about all kinds of things and Peter was telling about the new RFLP probes and one on the X chromosome.

And then Ysbrand Poortman said, "You should work on Duchenne Muscular Dystrophy. It's X linked and it's very important." So from that meeting Peter came back and he said, "We have to go and work on Duchenne Muscular Dystrophy." One of the clinical geneticists, or actually at that moment he was a physician that had almost retired and wanted to do some clinical genetics in the last part of his work, and he was in training to become clinical geneticist. That was Henk Venema. He was asked to go and collect these families. So, together with the patient organisation, families were found and, together with a genetic nurse, Henk went to these families and took blood samples. And we got these first families in early '82, and from '82 on we started to do linkage studies, using this probe L1.28, and showed that it segregated. At the same time, Kay Davies (in London) had cloned RC8 (DXS9); actually Rob Elles had done the cloning of that probe in her lab; and this probe RC8 was on the other side of the DMD locus. And then when Peter heard that he said, "Okay, if you combine them then for all families that are informative for both probes, we can do carrier detection." That was one of the first articles on that subject. And of course more probes were needed on the X chromosome and one of the first things Peter said was, "We need more probes on the whole genome and especially on the X chromosome" so he hired Martin Hofker as a PhD student who started from `82 onwards to make phage libraries and clone pieces of human DNA; and one of these probes was 754 and 782, and many others. So from that time on, with more probes; the map filled up. In `84 we had so many markers available on the short arm of the X chromosome. Others also had made new probes like C7 from Jean-Louis Mandel, and pXUT23 from Hunt Willard's lab.

So by collecting all these probes that were available in the meantime, and the ones that we had made in our own lab, we could have so much informativity on the short arm of the X chromosome that we could very well follow the segregation of the Duchenne locus in families. And we could start thinking on offering prenatal diagnosis. And the first request for prenatal diagnosis came from a Rotterdam family, in '84. And Lodewijk Sandkuijl was the clinical geneticist, he asked, "Can you do that? It's important for this family." So we tried to use the markers and it was very informative. There were two recombinants actually in this pedigree, in the same haplotype but we could show that the disease part was transferred into the fetus

and that the fetus would be affected. And in a second family we showed the fetus would not be affected. So these were the first two prenatal diagnoses. Now at the end of '84 this was written down, and in February we sent it to The Lancet, and we got almost directly, it was within a week, a letter back saying: "We hasten to accept your article. Proofs will be sent for correction." [laughs] There was no doubt about that.

I That's nice.

B So it was very quickly accepted.

I That's nice. At what point Bert, did you officially become involved in the diagnostic side because I know there was almost, not a drift, but the boundary between research and service was very fluid initially, but how did it come that you especially concentrated on the diagnostic side?

B It was actually at the time the prenatal diagnosis was done, there was a lot of attention towards that even from the German television because, one of the first families, was German. The German television came and made pictures in the lab, and there was a lot of requests for genetic testing. At that point in time Gert-Jan van Ommen was, in '83 he came into the lab as a post doc, mainly involved in further pindown (localise) the gene and to find the gene. And the discussion came in the lab: okay, this is now routine; that can go to the hospital and we focus on finding and clone the Duchenne gene. Then I said, "Yeah that is nice, but we need the families for doing research because you need the rare cases, just the very rare cases as a recombinant just outside of, just in the middle of, or with very strange mutations; so we need the families, and if you put this technique to the hospital it will take a couple of years before the hospital takes it up and it's really routine, and we can now do it. So I said, "I will concentrate on the diagnostics and Gert-Jan will concentrate on the research." So it was a kind of split, and fine, it worked, so the technicians who were doing the diagnostics started working with me, and the research stayed with Gert-Jan. And it worked very fine because lots of strange cases we had we could discuss directly with research.

I Do you think also that it suited your skills and your temperament to be involved with developing this diagnostic service?

B Maybe, yes. It was kind of rewarding to see that you could do something for the families. And on the other hand, there was a lot of work to do, so it had to be done. The number requests grew and the work had to be organised to keep on with it. It was a good time.

I At what point did you actually get specific funding for your DNA diagnosis? I know I was talking yesterday with Hans Galjaard and it seemed that there was some quite rapid decision about funding, but I wasn't clear -

B How that worked.

I - how quite it worked.

B In '82 when we had the first probes for Duchenne muscular dystrophy, actually the L1.28 and the RC8, which is what was known from Kay Davies, Peter had talked to Ysbrand Poortman; there was a project written by Peter for the "Prevention Fund". And the Prevention Fund aim was, to prevent genetic diseases and to offer diagnostics. So this subsidy was awarded to the laboratory and on this subsidy there was a post-doc position. Gert-Jan van Ommen was taken on this post-doc position and he came in `83. So the aim there was to develop diagnostics and of course, try to clone the gene and all of that. When this project ended 4 years later, in '86, we had already done the prenatal diagnosis, we could show that it worked, and with this, the health insurance companies were asked to fund the diagnostics, and actually they thought it was a good idea to do this.

So we got permission to do prenatal diagnosis; Peter Pearson got a letter that prenatal

diagnosis could be offered and that he was allowed to do so, but there was no money. So by talking to the health insurance companies, and that probably Peter did together with Hans Galjaard, there was a decision that okay, this should be funded from a special budget. And then I had to make a calculation, a type of calculation as you can see in there, in which we have: a list of what you all need to start a laboratory to do the diagnosis. First of all there was a discretion with the laboratories because Peter said, "Yeah, we can do Duchenne muscular dystrophy; haemophilia; Huntington" (because we were doing research at that time on these diseases) but we can't take on all diseases: CF and myotonic dystrophy and SMA; it's impossible. So we should talk to the other labs." So there was a meeting with all the human genetic labs in the Netherlands, sitting round the table playing just cards, "What kind of research you have? Do you want to put that into diagnostics: yes or no?" And at that time Groningen opted for CF and SMA and retinoblastoma; Nijmegen was in for myotonic dystrophy and Rotterdam with CF and fragile X; and so there were a couple of centres that said okay let's start the DNA diagnosis.

So, four out of the eight centres at that time were ready to start. And with that in mind we performed the calculations, what do we need? We need the space, so much square metres; we need one scientist, two technicians, half a secretary, someone to do the dishes, and things like that. Fifty square metres [laughs] for office space and eighty square metres for labs, so we need this and that and that; equipment etc. So we came to a kind of lump sum, if we would get guaranteed budget for a couple of years, four years; we could try to set it up in these four centres. So each centre started with this small amount of money; I think it was 300,000 guilders per lab at that time. Each got a lump sum funding to do diagnosis and this lump sum funding was a good start. Now you also can see that in the first few years there no funding and all was started from the research money. I think we got the first subsidy only in `88. So from '85 to `88, we had no funding. After four years we could show how much we had done.

In `92, the first four years were over and then was a discussion for the health insurance companies: is this cost efficient? And then this health technology assessment group in Rotterdam came in the picture and they made an assessment while we had another two years of the lump sum funding, The HTA on Duchenne, fragile X, myotonic dystrophy, SMA showed, it had been very cost efficient; and for Huntington not so much because there were not enough prenatal diagnosis at that time. So that came back and then they said, "Okay, now we can calculate one genetic testing tariff, so just take the average amount of money that was spent per diagnosis, so 800 diagnosis for half a million, they just divided and said, "Okay, tariff will be 600 or 700 guilders per gene tested", which is almost the same price as the chromosome analysis and that then became the standard tariff for all (genetic tests) diseases; no matter what: a gene test costs so much. And from `96 on all eight labs were offering DNA diagnosis and we still kept this division that each lab has its own set of genes. The labs would do the diseases where they have research on. On top of that there was and still is a kind of committee, the Dutch DNA Diagnostic Society actually, where we discuss on who's going to do what, and if there's a new gene e.g. for LCAM, for hydrocephalus; who's going to offer to test this huge gene, lots of work, for the same price as one Huntington's test.

Who should do that then? The clinicians were asking that we should offer it because they had to send it to Antwerp and they said, "We want to have it in the Netherlands. Which lab is going to?" So we have to discuss amongst each other, who's going to do that. And then we looked at the different packages of all the labs and decided Groningen can/must do it. [laughs] It's always been like that and it's still like that. Every few months we get a new list of genes that have been included in our diagnostics; we now have a website www.dnadiagnostics.nl and there are over 900 diseases and loci in there, and each lab has a package; we do about 100 different genes in our lab.

I Has this system continued to work while the clinical specialties have been increasingly

wanting to do things themselves? How is this worked out, this relationship between the genetics centres and the wider clinical and perhaps laboratory specialties?

B Yes, mostly because clinical genetics is different from the other specialities. The clinical geneticists see the patients, discuss with the patients the results from the lab, and of course they want to have a good connection with the lab and to discuss with the lab on what has been found, but the Netherlands is so small that by phone and by other ways, we can discuss with these clinicians easily on that. At a certain point when Huntington was found, the test for Huntington was done in our centre, and we did that for the whole of the Netherlands. The counselling on the other hand was complicated because they also wanted to include psychologists, to have special counselling and discuss how that should go. So as a kind of research it turned out that all the counselling for Huntington was also done in Leiden. And at that point, there was opposition from the clinics, from the clinicians, medical geneticists from other centres. For training they argued they also needed to do those Huntington counsellings so it should be offered in all centres, and therefore it was spread out again after the research trial setting up with the psychologists and so on. So seeing the patients that can, must be on site but the laboratory can be remote; one laboratory for Huntington in the Netherlands is fine, blood samples should travel, not the patients.

I Has there been any pressure from the overall pathology and the medical biochemistry laboratory services to say that genetics is now just part of laboratory medicine and should be incorporated? Or have you not found that?

B No, not really. We have a group of laboratory specialists where we meet every year a couple of times. We do have connection with clinical chemistry and clinical chemistry is saying that for some of the diseases they could easily do genetic tests. And of course for hemochromatosis and some of the frequent mutations that can be done in clinical chemistry laboratories, but it's also complex. If you spread the tests for a rare disorder out over the many clinical chemistry labs then they see only two or three patients a year, so it doesn't work because they don't get the throughput; they don't get the quality and just by concentrating in the Netherlands in one or two centres that offer these tests, you get a high quality. And that's the advantage of the Dutch system.

I How did you develop systems of training for the laboratory staff in molecular genetics?

B Yeah, I first started by myself, training on the job, so that's easy; that's no big deal. Then one of the technicians worked with me and then I got, let's see, in '89 my PhD and I became automatically head of the new DNA diagnostic laboratory in '90. Later in '92 Monique Losekoot was attracted as post doc to the lab to help me with all the diagnostic work and reporting and things like that, so I got a scientist next to me. And actually by working close together, Monique was trained; she worked on Huntington and on haemophilia. So she was trained, she came from thalassaemia research, she already had a background in genetics, in the group of Bernini she had been doing her PhD thesis on thalassaemias. And for the diagnostics she was trained in house. In '93 I was asked by the Ministry of Justice to start a forensic laboratory because they wanted to go into DNA testing for forensics and the police laboratory was already starting but they needed to have a second opinion laboratory. In Leiden we started the second opinion laboratory the same way as we started the DNA diagnostics: said we need one post doc, two technicians, and we got a lump sum of money and I started that. The post doc that was attracted was Peter de Knijff, he started on that part. The Ministry of Justice had decided that they wanted to have an ISO certification on the laboratory; it should all be quality controlled.

So okay, I found out what was necessary for that; got our lab accredited within half a year because it was new; everything was new. The only problem was we had all the equipment, all the tests, but we had no history. And to have a history you should build one. So I made from

the DNA samples some mock cases; some mixed samples and some pedigrees for paternity testing and put those cases through the new lab, and showed it worked as it should. As said, the lab was accredited according to ISO17025 (a standard for testing laboratories). We had calibrations on the machines, on the temperature of the water baths, on everything. And then I thought, "Yeah, for the DNA diagnostics we do prenatal diagnosis. If something goes wrong I can't say with dry eyes that I didn't know about possible risks." So I said, "Okay, now we have to change our diagnostic lab also to a quality lab" and one of the things in ISO standards is that you need training. So with the labs in the Netherlands we said, "Okay, we have to make a formal kind of training" and it took until 2000 that there was an official training introduced to the Dutch Genetics Society; it was an official training for laboratory specialists and all the elements that should go in there, were discussed amongst the 8 Dutch centres to make the programme. So that's the way it was introduced. But it was actually initiated through the introduction of the quality system. Our DNA diagnostics lab got accredited in 1998, so we were, at that time, the first DNA diagnostic lab with ISO certification.

I **How has this interacted with Europe wide systems for accredited labs? Have you been able to lead the way, really?**

B Let's see when it was. I think already a little bit longer ago, so from 1994 onwards we collaborated with the UK-Labs. First, because of the need of having proficiency testing. In the Netherlands there was no proficiency testing available, if you are the only lab that performs tests for Duchenne, proficiency testing for one lab doesn't work. So I contacted the people in the UK (Rob Elles and Simon Ramsden) and asked, “can a Dutch group of labs join the UK NEQUAS proficiency testing schemes?" And from that time the connection with Rob Elles was there. In '96 Rob Elles wrote a proposal to the European Union to get EMQN started; to get proficiency testing for Europe. And the first scheme that was plugged in of course was Huntington's Disease in 1997; with Monique Losekoot and Sue Stenhouse as organisers followed by Duchenne Muscular Dystrophy in 1998 etc.; sending samples to labs throughout Europe. Next to that proficiency testing for CF was already operational in Belgium the lab of Cassiman and Els Dequeker. They also had European funding for that. So these two projects were in parallel. And let's see, when came the European interest for quality systems? Yes, in early 2000 the idea was there to show that the quality of diagnostics is needed, and it came actually from the proficiency testing, the quality level should be raised because there were a lot of mistakes reported; 4 or 5% was not an exception; so that was terrible. Quality improvement for all laboratories was needed and to get funding for that through the European Union was very difficult. In 2002 there was a meeting organised through the patient organisations and the joint research centre in Sevilla. Dolores Ibareta, helped to get people from the diagnostic labs to meet and discuss on the problems and define how that should be tackled. Then an expression of interest was made on which, one year later, a call for proposals came out, and on this call for proposals Jean-Jacques Cassiman wrote a Eurogentest project, and this Eurogentest project was to stimulate accreditation of all molecular genetic labs for all genetics labs in Europe. So actually it was through the interaction between the different groups, I think, it spread the idea that quality was needed; was and had to be implemented everywhere.

I **How have you managed to cope with the continuing change in technology? I mean, have you found it reasonably easy to get funding for new technology and R&D to evaluate this? What's your experience with this?**

B There are two types of changes: expansion and innovation. The expanding number of genes over the years, so expanding your portfolio of genetic tests is one. So for each gene, you have to have pre-work to get it diagnostically ready, and on the other hand you have new techniques like PCR that came along and the new applications like DGGE, SSCP, protein truncation test and pulse-field electrophoresis; all kinds of new technologies. Implementation

was usually done from the budget we got from the health insurance companies because in these budgets there was a little room for innovation, so that helped. And by using trainees/students from the technicians' school and from the University for their practical courses, they worked on these innovations and then it was implemented. So I think it's not so difficult until the sequencing came along and sequencing all the exons was a lot of work and you needed more hands. Automation by robots in the lab helped as did the quality system. We always try to find additional funding for implementation of new technologies partly through European projects. Which is difficult as shown with arrays, we started in August 2008 with the implementation the arrays in diagnostics. Before that it was all research so we had one and a half years of research before we implemented the diagnostics, and it cost a lot of money from the diagnostics. It was partly paid by the health insurance companies by writing an application, it's called a "Zorgvernieuwingsaanvraag" so you explain to the health insurance companies that you want to innovate diagnostics by doing this and this; and then getting rid of some of the karyotyping and that it will be cheaper and faster. So they invested for two years money, also for training a PhD student to work on that. So in that way we get some funds for innovation.

I **Where do you see the next big advances coming?**

B Yeah. Everybody now focuses on next gen sequencing and whole exon sequencing, or total genome sequencing in a research setting, Of course it will, become very important and it will be implemented for some of the major themes such as hearing loss or blindness, or cardiovascular diseases. You have many genes and you need to screen a lot at a time, then these techniques will work, but for routine diagnostics such as breast cancer or colon cancer or single gene genetic disorders, I think we still stick with the old fashioned Sanger sequencing. What will change is we will get more of these machines, gain efficiency and eventually we will get rid of the PCR because some of these machines such as Helicos and Pacific Bio will sequence directly from genomic DNA; so no PCR. Yeah, that might change. Maybe there will be such faster and smaller machines so that you can do a test within a day; I don't know. [laughs] But there will be some change that's for sure. But the last 25 years there have been changes all the time so I'm not afraid of that. [laughs]

I **Thank you, Bert. We've covered a lot of ground but is there anything that I haven't mentioned at all that you want to put on record before we finish?**

B Yes, maybe the germ cell mosaicism that came along over these years. If you do the first tests for Duchenne then you have families and sometimes unclear cases and we had one, actually. For that I have to go a little bit further back in time when in 86 a postdoc was in our lab as a trainee to learn about DNA diagnosis for Duchenne Muscular Dystrophy. Christine Van Broeckhoven worked in Antwerp at Born-Bunge Institute for Neurological Diseases; she started working and doing this Duchenne test. Back in her lab at a certain point she emailed me and said, "I have now a family where I've a deletion in a patient and the mother seems not to be a carrier." At that time we did Southern blotting and look at intensities of the blots and to see if the band is of lower Intensity or not. So, by eye doing quantitative assessment was a little bit difficult and Christine was not fully comfortable with this She said, the index patient has a deletion and the mother seems not to be a carrier but she's pregnant now. What should I do, can I say that she's not a carrier on based only on intensity?" I also looked at the X-ray and came to the same conclusion and said, "To be on the safe side, just ask her to give a chorion villi sample and do a prenatal test because then we will know for sure."

So the test was done and the fetus turned out to be affected. Now we had two boys affected from a mother who was obviously not a carrier; in her eyes, in my eyes. And then we confirmed again that this mother was not a carrier. And then I looked back to all our pedigrees and I found one pedigree with two mothers of patients in which the grandmother turned out to be not a carrier on dosage and by trying to clone or zoom into the deletion breakpoint, I

could show that the breakpoint was unique in the two cases, so they were not independent cases of Duchenne Muscular Dystrophy; it was familial, but the grandmother was not a carrier somatically, she must have been a carrier in part of her germ cells and that was called the germ cell mosaicism. And in 89 we had six of these cases and we could show that there really is a recurrent risk and it still holds.

I That was a real change in people's perceptions because all of us thought, before then, that these were very, very rare events, but in fact their risk ended up being 4 or 5%.

B Yeah, and still is. In that time there were clinical geneticists, especially also in our centre; Jacques van der Kamp head of our clinical genetics centre at that time; he didn't believe in it. He said, "Oh, it's so rare." [laughs] So you see it changed the scene a bit.

I Well, I'm going to finish, Bert, and thank you very much for sharing your experience.

B Okay, thank you very much.

I I'll turn off the machine now.