Hans Eiberg

Personal Details

Name	Hans Eiberg
Dates	Born 1945
Place of Birth	Copenhagen
Main work places	Copenhagen
Principal field of work	Human gene mapping

Interview

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INTERVIEW WITH PROFESSOR HANS EIBERG, 7 MARCH 2013

PH = Interviewer (Peter Harper)

HE = Hans Eiberg

PH It's Thursday, 7th March 2013 and I'm talking with Professor Hans Eiberg in the Department of Molecular and Cell Medicine in Panum Institute, Copenhagen. Hans, can I start by going back to the beginning and ask: when were you born and where?

HE I was born close to Copenhagen in 1945 in April. I've been here in Copenhagen most of my life.

PH May I start very early and ask: do you come from a family with scientific or medical background at all?

HE No, no, no. My father was a carpenter and my mother took care of the family. I have 2 sisters; 2 older sisters, they are still living. My parents are dead now. I had a problem in my school because I have dyslexia so it was very difficult for me to read and write and also to talk Danish, of course also English. But I was very interested in plants and science so I succeeded to finish my school and go to university. First I wanted to study botany but then I better liked to study biochemistry and after I spent some years, I was only a technician. I started here as a technician at the Institute of Genetics and I talked with Professor Jan Mohr; he asked me if I was interested to do some research and I accepted. And because he had very big plans for mapping the human genome he asked me if I could do some developing of some methods and I accepted and he had many problems to collect many samples because it took a lot of time to prepare all the blood components so he wanted me to help him to do it much faster. So that was our problem. We could only collect 3 or 4 blood samples each day because we wanted to study HLA and then it was necessary to freeze down lymphocytes for HLA studies so we had a lot of problems. Also to freeze down red blood cells because he wanted to do blood types and it takes many years to do all the typing so it was necessary to conserve all the erythrocytes.

PH May I ask what year was it that you began to work with Jan Mohr?

HE It was 1971. And I was just starting to look at chromosomes for banding because there was some polymorphism in the chromosomes, satellites and secondary constrictions. At that moment, there were already about 20 markers I think.

PH So was Jan Mohr using chromosomes as polymorphisms?

HE Yes, yes, yes.

PH He was not doing diagnostic cytogenetics?

HE No, only for linkage studies.

PH Yes.

HE Because there were so few markers at that moment, there were only about 15 or 20 markers so -

PH I remember. Was the lab already established when you joined it? Professor Mohr's lab, or was it just blood groups?

HE There was only blood groups, yes. My colleague had some methods for acid phosphatase and phosphoglucomutase but in our lab or in our institute we could only type for only a few, perhaps 5 or 6, enzymes and we could only type about 10 enzymes on a genome. And it went very slowly. [laughs] So there was a problem to do many analyses.

PH When you were setting up these techniques, did you have contact with other labs doing linkage like the Galton lab or others?

HE No, no, no, because it took a long time just to collect all the samples and we had problems by freezing the erythrocytes. Professor Mohr had a method to mix the red blood cells with dextran and then drop it as a pellet down in the liquid nitrogen but it took so long a time to freeze it. Then I tried to, that was a big jump, because I tried to centrifuge, rotate the liquid nitrogen and then we could drop it much faster and then it took only 15 seconds to freeze down 15ml of blood. So that was a really good observation so we could freeze down about 50 samples each day when we just put red blood cells and we'd drop it down in that nitrogen, rotated nitrogen. And also we had to make everything, we have to make our apparatus ourselves; we couldn't buy anything. And we also made our freezing centrifuge. The centrifuge, we did it by buying some big cabinets [laughs] and putting it in here

PH Am I right that Jan Mohr, he was not keen on spending money on equipment if it could be done himself?

HE Yes, yes, that's true. We had problems with money [laughter] so we needed to do it ourselves, to build it ourselves. Also because my father was a carpenter I was good with my hands. I think that could be the reason. I could also make some electronic robot also for freezing down lymphocytes. We copied our freezer to, I can't remember what, but we set the temperature and if the temperature went up we could open for liquid nitrogen after 3 or 4 seconds, I had to make some electronics for that robot. Because we had no money to buy a cryoson machine.

PH No. I mean you worked with Jan Mohr for very many years. Can you tell me a little, as a person to work with or work for, how did you find him?

HE It was easy for me to work together with him; no problems. He was very kind and helpful. But the other people, they did't want to work with him because they believed that Mohr's name would be the first on everything.

PH But this was not a problem for you?

HE No, because I have done so much, I spent so much time helping to develop all the methods so I didn't want to, I wanted to continue with the job.

PH What was the first particular project that you were especially involved with?

HE The first was the chromosome banding and then the enzyme UMPK. That was a polymorphism in red blood cells and he wanted to, he heard there was a linkage between UMPK and PGM and then we wanted to try to replicate and that succeeded very well. So we had to test about, after we collected all the samples from 850 families, we wanted to, each year we typed about 3 or 4 new, in the beginning 3 or 4 new polymorphisms and for PGM we tested both of PGM and later also we, on the other side, we tested on starch gel and we could both test for GPG and PGM and for esterase ESD on one gel. I think we tested about 20 samples on each gel. We doubled the number of samples so we could test 6 different polymorphisms a year.

PH When did you begin to make this a DNA bank as well as just a sample bank?

HE After we had tested about 65 different markers we have tested most of the markers known at that time, we also wanted to test for polymorphisms in leucocytes but it was difficult. First we had to transform our B lymphocytes with Epstein Barr. It took us a lot of time and in 1985, I think, was published some article about DNA, RFLP's, but it took so long time to test for, we could only test about 100 results each a week. So it took a lot of time but I think, I can't remember how many polymorphisms we tried, but it could be 50. But we could still type for the classical polymorphisms, you see at that time I was already interested to work with different diseases, so -

PH What was the first disease you got involved with?

HE Yeah, I wanted to type for some very common diseases and the most common disease was cystic fibrosis and I asked for samples but it was difficult getting the samples in Denmark. But suddenly I talked with a person and he could collect samples from Rigshospitalet. So we got 13 families with at least 2 affected. At that moment we could type for 65 markers and all the other labs could only type for 20 at that time. So I thought that I had a chance for finding a linkage to cystic fibrosis but the other persons, I talked with Bob Williamson on that and others and, "Okay you can have a family". And I only needed serum and erythrocytes for linkage. And at that moment I know that I have 50% chance to find a new linkage because I have 65 markers, scattered over the whole genome.

PH So how did -?

HE So it took only 3 months [laughs] before I have got a positive linkage to paraoxonase.

PH I know nothing about paraoxonase. How did you come to get that marker?

HE Because I just look for new markers! Even it was published, a marker then took 2 or 3 months before we could start, or sometimes it was easy for us. But paraoxonase, we had a very old machine, it was an auto-analyser. It was not used. It was bought in 1960 for Marchall Donations [laughs]; 20 years later I unpacked this and set it up with the coils and dialysing equipment and so on, necessary for the very poisonous substrates…

PH So did it take long to establish it was linked?

HE At first I had typed all 6,000 persons for paraoxonase but there was no linkage to any of the other 65 markers.

PH This was 6,000 normal people?

HE Yeah, yeah.

PH So when you had the cystic fibrosis families there was no existing linkage between markers to help you?

HE No.

PH That must have been a surprise.

HE Yeah. I also tested the material for 60-65 markers and suddenly got a linkage and I couldn't sleep 2 days after that. [laughs] Could it be right or wrong or right or wrong? And then we got it. I got a family from, I think 2 families from Bob Williamson and also from many from Australia and sometimes it went up and down, the LOD score; sometimes it was that they'd start at about 3 and they'd go up to 3.5 and then down to 2.8 and then up to 4 and then down to 3.5 and we succeeded to have a LOD score of 5 but paraoxonase was not linked to any other markers.

PH Did you know what chromosome it was on?

HE Not really but we had also a LOD score of 1.5 to Colton blood group and Colton was on chromosome 7.

PH Okay.

HE But we could exclude most of the genome, there were only 4 of the chromosomes left, I think they were 7, 8,11 and 12; all the others could be excluded. And so people asked me, "Which chromosome do you think?" "Perhaps chromosome 7 because we have a linkage to Colton." But at that time Colton wasn't on chromosome 7, it was on chromosome 18 or 7, but other resarchers had mapped it to chromosome 7; I was not sure. We had only a LOD score of 1.5.

PH What linkage methods of analysis in terms of computing did you use, or did you do it all by hand in those days?

HE In the beginning we did, we scored it by hand; it was terrible.

PH Did you use Morton's LOD score tables?

HE Dan Woien from Oslo had a semi, a computer programme we used. We have to score it as, if we have a family with 5 children we score it as 4 of the children match and one doesn't match, then we have a card with holes, you know the card?

PH A punch card?

HE A punch card, yes. So we had a lot of punch cards. Start with 2:0, 2:1, 2:2, 13, 1:4; 1:5, and so on. Then we sorted all the punch cards and put it into a computer first with Dan Woien's programme (MOSM) and then after that we used LIPED. I think we used Dan Woien's programme for the punch card but later we got computers and all the results we have done were put in the computer then we used LIPED. It was nice to use LIPED [laughs] and then we got a PDP11 but it took a lot of time sometimes to calculate because we had no data for some of the families. If there was no data it took a long time to calculate so I had to learn a little FORTRAN programming to exclude all the families with no data. And also to do it faster so I have done a lot of FORTRAN programming to be easier for me to calculate so I don't need to look at each family to make a LOD score. So at the moment I can just put in the computer, I still have the old programmes and I do it sometimes. I did it 3 days ago because then I got a LOD score of 900 [laughter]. If I calculate Rhesus blood group against Rhesus blood group I got a LOD score of 900. Of course Rhesus is linked to Rhesus so, but…

PH Did you continue working with CF after the DNA had developed or did you leave it to the DNA people?

HE I left it because I couldn't do so much. Also because people were interested to collaborate with me for other diseases and I got, I think that I have looked at about 50 different, at least 50 different markers and diseases, and I found the first linkage to about 50 genes or diseases.

PH What were some of the first, apart from cystic fibrosis, what other diseases early on were you especially interested in?

HE Different eye diseases, cataract, two forms of cataract and retinitis pigmentosa, and also Byler's disease.

PH Is this the disease in Greenland?

HE Yes, yes.

PH Tell me about it because I was interested but I know nothing about it in Greenland.

HE In Greenland were born a lot of affected children and we wanted also to map the gene for Byler's disease. And on the east coast was 1 affected out of 100 new-borns so it was a very common disease and it could be 1 out of 5 was a carrier, 1 out of 7 or something like that. Then we toured out to Greenland and collected about 100 blood samples and then I typed it for all the markers and we got 3 positive LOD scores to 3 different locations… one to the blood group JK on chromosome 18, and also on chromosome 3 and I can't remember the last, but there was association to only JK of them because all the affected had the blood group AA in JK blood group system, so if there was any founder who could be on chromosome 18. But we had really no other markers on chromosome 18 at that time.

PH Did you have good pedigree data from these families?

HE Yes, yes. They were very good. All the blood groups fitted so there was no problem to map the locus -

PH I was wondering whether there was one founder that was known that emigrated to Greenland?

HE Yeah. We believe that there was only one founder, but when we got some DNA markers it looks like there could be more than one founder because of crossing overs in the region.

PH Was this in the Inuit population?

HE Yes, it was yes. But then we did early studies with DNA markers; we got a LOD score of more than 3 and published it. Also other people have; in Umea there was I think, that we have, all 3 chromosomes was in Umea, on chromosome 18 for Byler's disease. And other people have a more benign form found to have links also to chromosome 18 but we could not; we haven't got the DNA sequence so we must collaborate with other people to find the mutation. I started to type all the pregnant women in East Greenland first and we tried to collect as many as possible samples from East Greenland to find carriers for the disease. And we have now collected about 6,000 samples from Greenland on filter paper so at the moment all women are tested. We have found some cases where both parents are carriers and the foetus was sick.

PH Is prenatal diagnosis quite acceptable to the families?

HE Yes, no problems, yes.

PH That's interesting.

HE We have found two of it only but perhaps I think we have, yes, we have typed 50 fetuses at the moment for both diseases and only two were affected so that's fine.

PH One of the diseases I noticed that you had worked a lot on earlier was enuresis. How did that start, your interest in that?

HE Yes, because one of our biggest families had enuresis segregating in the family, I sent questionnaires out to all the families which I have done several times, five times perhaps, to all the families and asked for different traits, such as eye colour, hair colour and high blood pressure. I looked for two persons affected with the same traits in a family. .

PH Was there a particular clinician who you worked with who was involved in the enuresis study who had been studying it?

HE Not at first but later I collaborate with Aarhus University, with Søren Rittig, but our problem was to find large families to show a linkage..because the families were too small . In our own families we got a high LOD score to enuresis but we have not found any mutation at the moment. In fact we have tried to do some sequencing and gene capture and so on but it's difficult because there are so many SNPs, common SNPs, so it could be a common SNP for regulation.

PH And does it segregate as a dominant in these families?

HE Yes, yes, dominant. The penetrance is about 90 or 95 %, but I think we have 3 big families; it's linked to different chromosomes so I don't know, it could be a a kind of regulation with DNA loops in combinations with transcription factors I think.

PH Tell me now, how did you first become interested in eye colour?

HE I don't know really because both my parents have blue eye colour; I have brown. [laughs] So, but my mother has brown eye colour; she has blue and some brown spots.

PH And was it also one of the markers that Jan Mohr was interested in from the beginning, or was it you who introduced it as a marker?

HE I think he was also interested in this trait in his thesis. He also asked for different traits, hair colour and what he could find out to ask. He asked about everything I think. So we were both interested in other markers also; everything.

PH And with eye colour then, did you find any linkage with protein markers before the DNA started? I should know that but I don't.

HE Absolutely not. But there was no linkage to other markers; we were unlucky. We have only 50% chance of success. Then we started just to do DNA markers, we selected some of our biggest families, about 25

of these families and we typed them for repeat-markers, I don't know how many, at least 300 repeat markers. And then we found the linkage to chromosome 15. But it was also difficult, yes there was a candidate gene OCA2 in the area; I believed it was that gene, but we could not sequence it; it was too big a gene and we had only a little information about the sequence. I was not so interested to sequence the gene at that time, also because we had no ABI at that time. So if we should sequence we must do it the old way.

PH Was there any suggestion before you found the linkage, had people suggested that OCA2 might be a possible candidate gene?

HE No, no.

PH Because to me it was quite surprising.

HE Yes, it was called P, at that time I think -

PH Yes from the animal work.

HE From the animal ideas, yes. So there were many genes for pigmentation

PH Do you remember, was it Mary Lyon or who was it that found that original gene in the mouse work? Because I seem to remember it's been around a long time that gene, but I really don't know much about it.

HE No, I think it was not for eye colour really, it was for the -

PH Coat colour.

HE Yes, coat colour.

PH Yes, yes. So tell me, that work caused a lot of interest outside specialist fields [laughter]. Tell me a little bit about some of that because it is interesting.

HE Yes. After the genome was sequenced, most of it, and we had got an ABI sequencer and people started to look at the OCA2 gene, then I wanted to find the mutation for eye colour. I knew that we have recombinants so I tried to narrow down the region and with the recombinants, we could nearly exclude all the OCA2 gene except one exon. But the problem was at that time there were very few real markers. There were indicated 2,000 markers but none of them were real because the gene was copied, so many of the markers were false because the gene was copied in several places. So we just tried to use CA repeats to narrow down the region. we got it narrowed down to1 megabase and later to 0.2 megabase. Still it was difficult to find the location. Then I'd read about the mouse phenotypes, about 2 deletions in 2 different mice and it was something with the coat colour was black and very black for both. There were 2 deletions, they overlapped but there was no really no SNP frequency data in this region. I just started to compare.

First I bled the sequence to see if the region was duplicated and it was not duplicated, we just sequenced, just typed for polymorphism in some of our families and surprisingly we found 2 SNPs that were associated with eye colour, and we also found another SNP that was not really associated. And a common CA repeat was not associated at all, but some of the SNPs were associated. Then at last we believed that it could be in the area of the deletion in mice, where the mice had the black colour. And a half year later when frequency data were published in genome browser we found out that the frequency of blue eye colour fits with the frequency of the SNPs in the candidate region. And then I wanted to do some functional studies; it took at least a year to do that] because there were problems to cultivate the cells and so on. We submitted the article to Nature Genetics and they told me that nobody was interested in eye colour. [laughs]

PH So where did it appear finally?

HE So it appeared finally in Human Genetics. I also sent it to American Journal of Human Genetics but they were not interested in that either. But only Human Genetics was interested.

PH But the rest of the world really became very interested.

HE Yes, it was nominated as number 42 article [laughs] that year for all scientists' papers, so it was very interesting. Also National Geographic was very interested.

PH I can quite see that, because from the anthropological point of view it really has a lot of significance.

HE Yeah because Darwin's, was it Darwin's 100 year anniversary, or something like that -

PH 200 years.

HE Yes, 200 years, they published it together in that article about -

PH Oh, in National Geographic?

HE Yes.

PH That's interesting. And have you had samples from many countries sent to you as a result?

HE Not really; I asked migrant students with blue eyes from our University to help, from the Middle East, and from Turkey and India. So I got a person from Jordan and he was very black in skin but he had blue eye colour and I typed him and he had the same mutation and also people from Turkey and other persons have the same mutation. And also the same haplotype.

PH So is it one mutation and one haplotype over most of the world, or most of Europe and Middle East?

HE Europe yes, also from India perhaps you can say. It could be Alexander the Great. I don't know. [laughs]

PH But some founder mutation anyway. That is really interesting.

HE So people with blue eye colour are family together.

PH Yes.

HE I really don't know how old the mutation is but I looked at the literature and it could be very old. Six thousand years or something like that. But I really don't know how old it is and who it comes from but, I can't remember his name, Cavalli-Sforza?

PH Cavalli-Sforza.

HE Yeah, yeah. I got the results from him.

PH He must have been interested in the finding.

HE Yeah.

PH He would have been interested for sure, yes.

Now while you've been doing all this linkage work, you've been part of what started off as a very small genetic linkage community. When did you first go to any of the gene mapping meetings?

HE It was meeting number 5, I can remember.

PH Where was it? Well, don't worry, but that was an early meeting.

HE Edinburgh

HE I think that perhaps could have seen some of our results in number 4, I don't know. We could be the, it could be UMPK or PGM or something like that.

PH And was it the Paris meeting that you chaired the chromosome 17 and 19 committee with Duncan Shaw?

HE It was in Paris, yes.

PH Yes.

HE And the year before was in Helsinki.

PH In Helsinki.

HE I think we have a poster about -

PH Yes. Do you have any memories especially of those meetings, which I feel were very -

HE It was fantastic! Yes of course. Just like a kid. Cystic fibrosis, everybody was interested. I couldn't speak English so it was difficult.

PH They were very special meetings weren't they?

HE Yeah, yeah, yeah.

PH Very personal.

HE Yeah.

PH Yes.

HE Yes, I can remember like it was as if you took pictures of everybody. [laughter]

PH Yes. Now I've been asking everybody I talk with two questions and I'll ask the same to you, and the first is: of the people who have been an influence on your career, who do you feel has been the biggest influence in your work and career developing?

HE Yes, of course Professor Mohr, of course. He helped me a lot writing articles and to correct my articles.

PH I've been going through a lot of his records today and I'm interested, for in almost everything there are comments in handwriting and things underlined; a lot of detail and this must have been actually very helpful.

HE Yeah.

PH Yeah.

HE [laughs]

PH And then the other thing I've been asking people: if you had to choose just one piece of work as your favourite or what you feel you look back on with most pleasure, which would you choose? You perhaps are allowed two if it's difficult.

HE Of course eye colour and there was also the myotonic dystrophy, which was also a very difficult work for me too; it took a lot of time because all the LOD score was calculated really by hand. [laughs]

PH Yes, of course and your work on myotonic dystrophy was mostly in the pre-DNA days.

HE I remember we found a linkage between complement C3 and myotonic dystrophy and there was also linkage between complement C3 and secretor.

PH I remember that from my own work, which goes really right back to the end of the 1960s when we used Jan Mohr's work on Lutheran and secretor and possibly myotonic dystrophy as the start point. And it was only after your C3 linkage that we began to know what chromosome it was on -

HE Yes, yes, yes.

PH Because before that we didn't know.

HE That's true.

PH Now I've got one last question for you: somebody told me that you're really interested in plants, especially rhododendrons. [laughter]

HE Yeah. That's only for, I have a meeting here today at 19 hours. But yes, I have a big garden and I had a talk and they were around in gardens and I talked with a man and he showed me his garden and he collected seeds and I got some seeds from him and I looked at all his plants and I got interested and put rhododendrons in my garden. So, I have a very big garden, three thousand square meters.

PH Fantastic. Is this in Copenhagen or near the outside?

HE It's 20km from here.

PH Yeah.

HE So I have about 1,000 rhododendrons in my garden. [laughs]

PH And do you think you'll do some serious rhododendron genetics? You have a good precedent because Mendel was always interested in fuchsias.

HE I can remember that I downloaded some DNA sequence from a biobank of rhododendron, done by people in the Edinburgh Botanical Garden -

PH In Edinburgh?

HE In Edinburgh, yeah. David Chamberlain, who was a professor in Edinburgh who was interested in rhododendron, he tried to make a system of classification of rhododendrons but he couldn't understand the DNA and it doesn't fit with the classification and he didn't want let anyone write anything down about it at first [laughs], but I contacted him and said, "Could I publish what I have found?" and they were not very interested in it so I could say it doesn't fit with his classification. But it was only some teaching for me because I was interested in how to do that, because I've never, usually I don't make any classification of species.

PH But you have plenty of rhododendrons in your garden anyway.

HE But I published it in a Danish publication.

PH Alright. Botanical?

HE Just in our society.

PH Oh, you have a Danish Rhododendron Society?

HE Yeah, I published it in RhodoNyt and later it was printed in a book so it's, perhaps it's good enough, 600 copies. [laughs] But it fits. I did it by hand because that was before I knew the program Phylip. I didn't know about alignment programs such as clustal-W at that moment, I just put it in a kind of a word perfect file and looked at a different, and tried to align the sequence. And then I could draw a tree and so on.

PH So is there anything else, Hans, that I've not asked you about that you'd like to tell me or do you think we've covered some of the main things?

HE I still work with linkage and I can use SNP6 results from Affymetrix for doing linkage studies with my own SNP6-LINK program. I use my programme for calculating lod scores for 1 million SNPs and that's very good.

Now we have got a lot of money for genome sequencing of 150 persons, from 50 families, 3 years. So we'll find out the polymorphism in Denmark.

PH Well thank you Hans very much for sparing the time. I'll finish there and switch off the machine.