THE THERAPEUTIC IMPLICATIONS OF MUSCULAR DYSTROPHY GENOMICS

The transcript of a Witness Seminar held by the History of Modern Biomedicine Research Group, Queen Mary University of London, on 27 October 2015

Edited by A Zarros, C Overy, K Mikami, S Sturdy, and E M Tansey

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This volume is dedicated to the memory of Lord Walton (1922–2016),
pioneering neurologist, co-founder of the Muscular Dystrophy Group,
and a good friend and supporter of the
History of Modern Biomedicine Research Group

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WHAT IS A WITNESS SEMINAR?

The Witness Seminar is a specialized form of oral history, where several individuals associated with a particular set of circumstances or events are invited to meet together to discuss, debate, and agree or disagree about their memories. The meeting is recorded, transcribed, and edited for publication.

This format was first devised and used by the Wellcome Trust's History of Twentieth Century Medicine Group in 1993 to address issues associated with the discovery of monoclonal antibodies. We developed this approach after holding a conventional seminar, given by a medical historian, on the discovery of interferon. Many members of the invited audience were scientists or others involved in that work, and the detailed and revealing discussion session afterwards alerted us to the importance of recording 'communal' eyewitness testimonies. We learned that the Institute for Contemporary British History held meetings to examine modern political, diplomatic, and economic history, which they called Witness Seminars, and this seemed a suitable title for us to use also.

The unexpected success of our first Witness Seminar, as assessed by the willingness of the participants to attend, speak frankly, agree and disagree, and also by many requests for its transcript, encouraged us to develop the Witness Seminar model into a full programme, and since then more than 65 meetings have been held and published on a wide array of biomedical topics.[1] These seminars have proved an ideal way to bring together clinicians, scientists, and others interested in contemporary medical history to share their memories. We are not seeking a consensus, but are providing the opportunity to hear an array of voices, many little known, of individuals who were 'there at the time' and thus able to question, ratify, or disagree with others' accounts – a form of open peer-review. The material records of the meeting also create archival sources for present and future use.

The History of Twentieth Century Medicine Group became a part of the Wellcome Trust's Centre for the History of Medicine at UCL in October 2000 and remained so until September 2010. It has been part of the School of History, Queen Mary University of London, since October 2010, as the History of Modern Biomedicine Research Group, which the Wellcome Trust

[1] See pages 113–19 for a full list of Witness Seminars held, details of the published volumes and other related publications.

funds principally under a Strategic Award entitled 'The Makers of Modern Biomedicine'. The Witness Seminar format continues to be a major part of that programme, although now the subjects are largely focused on areas of strategic importance to the Wellcome Trust, including the neurosciences, clinical genetics, and medical technology.[2]

Once an appropriate topic has been agreed, usually after discussion with a specialist adviser, suitable participants are identified and invited. As the organization of the Seminar progresses and the participants' list is compiled, a flexible outline plan for the meeting is devised, with assistance from the meeting's designated chairman/moderator. Each participant is sent an attendance list and a copy of this programme before the meeting. Seminars last for about four hours; occasionally full-day meetings have been held. After each meeting the raw transcript is sent to every participant, each of whom is asked to check his or her own contribution and to provide brief biographical details for an appendix. The editors incorporate participants' minor corrections and turn the transcript into readable text, with footnotes, appendices, and a bibliography. Extensive research and liaison with the participants is conducted to produce the final script, which is then sent to every contributor for approval and to assign copyright to the Wellcome Trust. Copies of the original, and edited, transcripts and additional correspondence generated by the editorial process are all deposited with the records of each meeting in the Wellcome Library, London (archival reference GC/253) and are available for study.

For all our volumes, we hope that, even if the precise details of the more technical sections are not clear to the non-specialist, the sense and significance of the events will be understandable to all readers. Our aim is that the volumes inform those with a general interest in the history of modern medicine and medical science; provide historians with new insights, fresh material for study, and further themes for research; and emphasize to the participants that their own working lives are of proper and necessary concern to historians.

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[2] See our group's website at www.histmodbiomed.org

ACKNOWLEDGEMENTS

A Witness Seminar on muscular dystrophy genomics was suggested by Professor Steve Sturdy, Principal Investigator of the Making Genomic Medicine Project at the University of Edinburgh, and Research Fellow, Dr Koichi Mikami, and we are very grateful for their help in planning this meeting and in editing the transcript. We thank Professor Jan Witkowski for writing a comprehensive introduction and providing photographs. We also thank the Wellcome Library, London, for permission to use photographs taken at the meeting.

As with all our meetings, we depend a great deal on Wellcome Trust staff to ensure their smooth running: Audiovisual, Catering, Reception, Security, and Wellcome Images. We are also grateful to Mr Akio Morishima for the design and production of this volume; the indexer Ms Cath Topliff; Mrs Sarah Beanland and Ms Fiona Plowman for proofreading; Mrs Debra Gee for transcribing the Seminar; Ms Emma Jones and Dr Farah Huzair for assisting with running the seminar; and Mr Adam Wilkinson, who assisted in the organization and running of the meeting. Finally, we thank the Wellcome Trust for supporting this programme.

Tilli Tansey

Apostolos Zarros

Caroline Overy

School of History, Queen Mary University of London

ILLUSTRATIONS AND CREDITS*

* Unless otherwise stated, all photographs were taken by Thomas Farnetti, Wellcome Trust, and reproduced courtesy of the Wellcome Library, London.

ABBREVIATIONS

AAV	adeno-associated virus
cDNA	complementary DNA
CINRG	Cooperative International Neuromuscular Research Group
CK	creatine kinase
CMSB	Centre for Medical Systems Biology
CSHL	Cold Spring Harbor Laboratory
EMA	European Medicine Agency
ENMC	European Neuromuscular Centre
ESTs	expressed sequence tags
FDA	Food and Drug Administration
hDMD	'humanized' Duchenne muscular dystrophy (mouse)
HUGO	Human Genome Organisation
IP	intellectual property
kb	kilobase
Mb	megabase
MDA	Muscular Dystrophy Association of America
MRC	Medical Research Council
MRI	magnetic resonance imaging
NHS	National Health Service
NIH	National Institutes of Health
PCR	polymerase chain reaction
PERT	phenol-emulsion reassociation technique
QMUL	Queen Mary University of London

RFLPs	restriction fragment length polymorphisms
SNPs	single-nucleotide polymorphisms
UCL	University College London
WIHM	Wellcome Institute for the History of Medicine

INTRODUCTION

That the phrases 'Therapeutic Implications' and 'Muscular Dystrophy' can occur in the title of this Witness Seminar should amaze those of us who began research on Duchenne muscular dystrophy in the pre-genomics world of the 1960s and 1970s. It is worth recalling what research on Duchenne muscular dystrophy was like in that period, to provide context for the topic of this Witness Seminar.

Then, Duchenne muscular dystrophy seemed one of the most intractable of the inherited disorders. The natural history of boys with Duchenne muscular dystrophy was well defined, as was the X-linked pattern of inheritance. The pathological changes seen in muscle biopsies – muscle fibre degeneration, central nuclei, increased connective tissue – should have provided clues, but the biochemical processes underlying the condition were quite unknown. As A T Milhorat put it in the introduction to the 1973 International Symposium of the Muscular Dystrophy Association of America (MDA), '…we are quite unable to explain the mechanism of the underlying morbid process [in Duchenne muscular dystrophy], and we are impotent in our effort to treat the patient in any effective manner.'[1]

Figure A: Professor Dubowitz's laboratory members; back row from left: Brian Brown, Dorothy Ackroyd, Victor Dubowitz, Jan Witkowski, Joe Neerunjen; middle: Chris Webster, Allie Moosa, Belinda Gallup; front: Christine Heinzmann, Elaine Woodcock, Bridget Lunn (Sheffield, 1972)

[1] Milhorat (ed.) (1974).

Figure B: Left to right: Dr Jan Witkowski, Professor Victor Dubowitz, and Mr Jerry Lewis (1975)

I began research on Duchenne muscular dystrophy just one year before the 1973 MDA conference. I had completed my PhD at the National Institute for Medical Research and for reasons no longer clear to me, I decided that I wanted to do research on Duchenne muscular dystrophy. There were three possibilities: Alan Emery in Edinburgh, Jack Sloper at Charing Cross and Victor Dubowitz at Sheffield. Edinburgh was a long way away, and as I didn't want to stay in London, Sheffield it was. Moreover, I was an enthusiastic rock climber and living in Sheffield, with the Peak District at my doorstep, was irresistible. And Victor had a project that involved my specialty, cell culture. However, shortly after I accepted Victor's offer, he wrote to tell me that he was coming south to take the Chair of Paediatrics at the Institute of Child Health at Hammersmith Hospital. Nevertheless, I spent the summer of 1972 in Sheffield learning about Duchenne muscular dystrophy and projects in the Dubowitz laboratory (Figure A), and in the evenings, climbing on a small crag on the bus route home.

At Hammersmith, Victor found temporary room for me in one of the buildings in the Labour Yard, an original part of the hospital dating from the time the hospital was a poor law institute. It was not until 1975 that the MDA funded the construction of a laboratory on the roof of one of the Hammersmith's buildings. It was named after Jerry Lewis, chairman of the MDA and, appropriately, he came to open it (Figure B).

The profusion of hypotheses in the early 1970s regarding the pathogenesis of Duchenne muscular dystrophy underscores Milhorat's remarks. As the Emerys describe in their book, *The History of a Genetic Disease: Duchenne Muscular Dystrophy or Meryon's Disease*, these included the highly controversial neurogenic

Figure C: Dr Jan Witkowski (1975)

hypothesis (largely espoused by Alan McComas, who claimed to find reduced numbers of motor units in a muscle of the foot), the vascular hypothesis (that was proposed to account for the clustering of degenerating fibres), and the myopathic hypothesis (that the defect was intrinsic to the muscle).[2]

The myopathic hypothesis had several lines of investigation, including biochemical and structural, and especially studies of the cell membrane. Examples from my box of reprints include: 'Effects of Duchenne muscular dystrophy on muscle protein synthesis', 'Analysis of skin fibroblast proteins in Duchenne muscular dystrophy', 'Muscle fibre size and shape in Duchenne muscular dystrophy', 'A quantitative comparison of satellite cell ultrastructure in Duchenne muscular dystrophy, polymyositis, and normal controls', 'Distribution of freeze-fracture particle sizes in the Duchenne muscle plasma membrane', and 'Human lymphocyte capping in Duchenne muscular dystrophy'.[3]

These indicate the wide range of investigations characteristic of that period and research in Victor's laboratory was similar. Caroline Sewry pursued electron microscopic studies, Mike Dunn and Arthur Burghes used 2-D gel electrophoresis to analyse Duchenne muscular dystrophy cell proteins, Steve Appleyard worked on membrane freeze-fracture analysis, and Helen Statham studied calcium transport.

I was following up work that had been carried out in Victor's Sheffield laboratory (Figure C). This research had shown that Duchenne muscular dystrophy muscle

[2] Emery and Emery (2011).

[3] Burghes *et al.* (1982), Fischbeck, Bonilla, and Schotland (1984), Horenstein and Emery (1980), Rennie *et al.* (1982), Watkins and Cullen (1982, 1986).

cells in culture were indistinguishable from those derived from normal muscle. This suggested that the Duchenne muscular dystrophy defect was not cell autonomous and that, perhaps, the myotubes in culture did not differentiate sufficiently far for a Duchenne muscular dystrophy phenotype to manifest itself. So I co-cultured human muscle cell cultures with slices of mouse embryonic spinal cord, to no avail. Similar work was going on elsewhere, but this isn't the place for an exhaustive account. Also, I sent cultures (by mail!) to Alan Harvey at Strathclyde, who carried out electrophysiological measurements, but again, finding no differences between normal and Duchenne muscular dystrophy myotubes.

I then turned to the membrane hypothesis and made measurements of cells moving in cultures of human muscle but found no differences, perhaps because there was no way of distinguishing between myoblasts and fibroblasts. Gareth Jones and I carried out measurements of intercellular adhesion, using a Couette viscometer, a technique at which Gareth excelled. One drawback was that we had to use cells of uniform shape and size and this precluded using muscle cells. Instead we used skin fibroblasts on the grounds that if there was a 'generalized' muscle defect, skin fibroblasts might show some effects. In blinded experiments we did find differences, but the significance of these is obscure. Finally, Helen Statham and I carried out experiments on protein degradation, measuring the rate of protein turnover. We used skin fibroblasts to develop the technique, intending to apply it to muscle cultures, but circumstances overtook us.

Those circumstances were the development of gene mapping using restriction fragment length polymorphisms (RFLPs) in 1980 and its application to Duchenne muscular dystrophy in 1982. This has already been covered in the Witness Seminar on *Human Gene Mapping Workshops c.1973–c.1991*,[4] and the clinical significance of cloning the dystrophin gene complementary DNA (cDNA) and its application in diagnosis was emphasized by John Yates in the Witness Seminar on *Clinical Molecular Genetics in the UK c.1975–c.2000*.[5] It was not that everything was now plain sailing, as I was sharply reminded by Bud Rowland. In 1988 I wrote a short essay with the title 'The molecular genetics of Duchenne muscular dystrophy: the beginning of the end?'[6] Bud wrote a similar article for the *New England Journal of Medicine* and used '…the end of the beginning'.[7]

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[4] Jones and Tansey (eds) (2015).

[5] Jones and Tansey (eds) (2014).

[6] Witkowski (1988).

[7] Rowland (1988).

Figure D: Dr (later Professor Dame) Kay Davies at the meeting 'Dystrophin' at the Banbury Center, Cold Spring Harbor Laboratory (CSHL), in 1989.
Dr Gert-Jan van Ommen is on the left and Dr Lou Kunkel on the right

(A personal aside. RFLP mapping changed my life too. We had a collaboration with Bob Williamson and Kay Davies (Figure D) at St Mary's. One day in 1983 Kay and I were in a pub near St Mary's and Kay told me that if I wanted to stay in human genetics, I had to go molecular. So I applied for a Medical Research Council (MRC) training fellowship in recombinant DNA techniques and I spent two years with Gordon Peters at Imperial Cancer Research Fund learning the techniques (Figure E). Tom Caskey at Baylor College of Medicine invited me to run his DNA diagnostics laboratory and I can still remember the thrill of using the phenol-emulsion reassociation technique (PERT) clones from Lou Kunkel's laboratory for prenatal diagnosis and seeing deletions. Such an advance over linkage analysis! Then Jim Watson recruited me to the Banbury Center where I kept in contact with Duchenne muscular dystrophy by holding several meetings on the topic.)

Of course Bud was right. An immense amount of work remained to be done, but once the gene and protein had been identified, the intense but diffuse research spread across many approaches became focused on analysing the gene and discovering what dystrophin did in the cell. It required advances in many other areas of molecular genetics and biochemistry to develop the therapeutic strategies discussed in this Witness Seminar.

Special training fellowships: recombinant DNA technology

Special awards are made from time to time for research training in specific fields where the Council's Research Boards consider there is a need for more trained workers. One of the areas identified in this way is Recombinant DNA Technology.

This is a technique for altering in a deliberate way the genetic make up or hereditary endowment of living organisms—it is often more popularly known as genetic engineering—but is much more complex. Genetic engineering is after all far from new—all our domesticated animals and plants are the result of the deliberate manipulation of the genetic material of living organisms by crosses and hybridization. Since the early 1970s, however, molecular biologists have learned how to remove bits of genetic material (DNA) from various organisms and insert them into bacteria in such a way that the transferred DNA becomes part of the genetic material of the bacteria. As they grow, the bacterial cells then duplicate and reduplicate this transferred DNA. So researchers can now isolate specific genes and produce large quantities of them. Recombinant DNA technology, as these techniques have come to be called, provide scientists with the ability to study the basic mechanisms of genetics in all organisms and especially in the genetically complex cells of higher organisms. This new technology holds the promise of significant practical benefits in medicine, agriculture and industry. Five new Special Training Fellowships have been awarded in the past year and are featured here.

DR JAN A WITKOWSKI

(Jerry Lewis Muscle Research Centre, Royal Postgraduate Medical School)

Figure E: Announcement of Jan Witkowski's MRC special training fellowship (*Medical Research Council Newsletter*, 1984)

There was much discussion of one particular therapeutic strategy, exon skipping. Just one year after the Witness Seminar, that therapeutic strategy became a reality, when, in September 2016, the Food and Drug Administration approved Exondys 51. Although a decision that has generated great controversy, it is a testament to the extraordinary efforts of families, scientists, and physicians who have contributed to the research, that Duchenne muscular dystrophy, once the most intractable of inherited genetic disorders, now has a treatment.

Professor Jan A Witkowski
Watson School of Biological Sciences,
CSHL Graduate School
& Banbury Center, CSHL

Figure F

THE THERAPEUTIC IMPLICATIONS OF MUSCULAR DYSTROPHY GENOMICS

The transcript of a Witness Seminar held by the History of Modern Biomedicine Research Group, Queen Mary University of London, on 27 October 2015

Edited by A Zarros, C Overy, K Mikami, S Sturdy, and E M Tansey

THE THERAPEUTIC IMPLICATIONS OF MUSCULAR DYSTROPHY GENOMICS

Participants*

Professor Bert Bakker
Professor Kate Bushby
Professor George Dickson
Professor Victor Dubowitz
Dr Michael Gait
Professor Shirley Hodgson
Professor Eric Hoffman
Professor Jennifer Morgan
Professor Francesco Muntoni
Professor Terence Partridge
Dr Rosaline Quinlivan
Professor Steve Sturdy
Professor Tilli Tansey
Professor Karen Temple (Chair)
Professor Gert-Jan van Ommen

Apologies include: Professor Martin Bobrow, Dr Serge Braun, Professor Dame Kay Davies, Professor Alan Emery, Professor Peter Goodfellow, Professor Peter Harper, Professor Veronica van Heyningen, Professor Hanns Lochmüller, Dr Marita Pohlschmidt

* Biographical notes on the participants are located at the end of the volume

Figures 1 and 2: Professor Karen Temple and Professor Tilli Tansey

Professor Tilli Tansey: Welcome everyone to this Witness Seminar on the Therapeutic Implications of Muscular Dystrophy Genomics. I'm Tilli Tansey and I run these Witness Seminars where we get together a group of people who have been involved in a particular discovery or event to discuss what really happened, what went right, what went wrong, who were the movers, because history is not inevitable and some of the events actually happened because of serendipitous events or just chance encounters. I know there are some people here who have been to a number of our meetings before and it's very nice to see you back at this meeting. This is about the sixth that we've held on the topic of clinical genetics, and we've recently just published a meeting on the Human Gene Mapping Workshops.[1] Everything we do is freely available online on our website.[2] We've provided a broad outline of discussion points, but you may feel there are avenues you wish to explore that are not included.[3]

This meeting came about at the suggestion of Professor Steve Sturdy, who heads a group in the University of Edinburgh on 'Making Genomic Medicine'. He is funded by a Senior Investigator Award of the Wellcome Trust and it's

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[1] Jones and Tansey (eds) (2015).

[2] The History of Modern Biomedicine Research Group's website is: www.histmodbiomed.org (accessed 11 January 2017).

[3] A draft outline programme was circulated to seminar participants to comment on a month in advance of this meeting. Table 1 is the final version of that programme used as a framework for this seminar.

Introduction to 'Making Genomic Medicine'

History, medical, and scientific knowledge of muscular dystrophy

- Muscular dystrophy before molecular diagnostics
- Discovery of DNA markers: lab–clinic collaboration
- Clinical use of markers:
  - circulation of diagnostic probes
  - prenatal testing
  - carrier testing
- Sequencing the gene
- Commercial dimensions:
  - diagnostics
  - therapeutics
- International collaborations:
  - reference networks
  - biobank projects
- From management to therapy: development of gene-skipping therapy

Table 1: Witness Seminar outline programme

Steve's idea, and some of his team who are also here today, to have this Witness Seminar to get behind the science and the published papers. A key part of any of these seminars is identifying a suitable chairman and I'm delighted that we've been able to persuade Karen Temple to come and chair, or facilitate, and guide you all through this process. Karen is the Professor of Medical Genetics at the University of Southampton and she is the Director of the Academic Unit of Human Development and Health, heading a large group of clinicians, scientists, and students. So, without further ado, I'm going to hand over to Karen.

Professor Karen Temple: Thank you very much. I hope first of all that you can hear alright because it is rather a big room. We've got to imagine that this is a very intimate gathering where we can actually say how it was when we were young, if you like. We're going to start in the 1970s, or at least around that sort of time, to try to get an idea of how it was before we knew where this massive

gene for Duchenne muscular dystrophy lived, as it were.[4] Now I have to say that I think one of the reasons that I am the chair is that I let on during a talk at the Genetic Alliance UK[5] that I was old enough to have used the restriction fragment length polymorphisms (RFLPs) in clinical practice in the late 1980s, and I can't tell you how exciting it is for me to have you all sitting here in this room with the opportunity to tell us how it was. So that's really your job. What's likely to happen is that someone will say something and that will make you realize, 'Oh yes, I remember that.' And that's exactly what we're trying to capture because so much of what we write is somewhat sterile and we've really got to try to work out and remember how it was. So who feels brave enough to kick this off because what we want to know is, what did we do before we knew where the gene was, and how did this adventure start? Because it really was one of the first genes to be cloned. So who feels that they could start off for us? Victor.

Professor Victor Dubowitz: Hello, I'm Victor Dubowitz, pensioner. My recent memory is not very good, but my long-term memory has become sharper.

I'll go a little bit further back than the 1970s to the 1950s when I first got interested in muscular dystrophy while a short-term locum resident at Queen Mary's Hospital for Children, Carshalton, where there were two full wards with long-stay muscular dystrophy and other muscle diseases. I'd never heard of it before and so the impact, in fact, of the clinical picture is a very striking one. Now if you can imagine what it was like from a diagnostic point of view before there was any creatine kinase (CK), that's the blood enzyme that's now done routinely.[6] If suspected, they'd get transaminases done and then many of these children ended up with a liver biopsy because the doctors thought the abnormal transaminases suggested liver disease.

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[4] Duchenne muscular dystrophy is an X-linked recessive form of muscular dystrophy, resulting in muscular degeneration and premature death. The disease occurs at a frequency of about 1 in 3,500 newborn males, and is caused by a mutation in the dystrophin gene. The latter is located at locus Xp21 (on the short arm of the X chromosome). The dystrophin gene is the largest gene found in nature, measuring 2.4 megabases (Mb); see www.ncbi.nlm.nih.gov/gene/1756 (accessed 11 January 2017).

[5] The Genetic Alliance UK is an umbrella charity for over 150 voluntary patient organizations for genetic diseases in the UK, supporting patients affected by all types of genetic conditions and their families. It was founded in 1989 as the 'Genetic Interest Group' and changed to its current name in 2010; see www.geneticalliance.org.uk/index.html (accessed 11 January 2017).

[6] Creatine kinase (CK; EC 2.7.3.2) is an enzyme catalyzing the conversion of creatine to phosphocreatine, with the utilization of adenosine triphosphate and the production of adenosine diphosphate. Increased amounts of CK in the blood could arise from muscular damage, thus CK is a routinely assessed marker for myocardial infarction and myopathies, including those of muscular dystrophies.

Figure 3: Professor Victor Dubowitz

From a clinical point of view the parents often became worried in the first year of life. They said the child's a bit ungainly, not quite up to the other siblings, and then in the second year they had difficulty going up steps, about half of them were late walking, and so the parents were getting worried. But the doctors, as usual, reassured them: 'Don't worry, the child's lazy, will grow out of it,' and so on. So diagnosis was often delayed till the age of five or six or thereabouts, unless there was a previous affected child in the family, then very often the doctors would listen to parents and do the checks. So that's more or less a picture from a diagnostic point of view. Perhaps that's enough from me for the moment, but if you want a picture of the disease I can do that a little later.

Temple: Well, who better to do it? How did you diagnose Duchenne patients?

Dubowitz: Well, it affected them in a number of ways. Firstly there was late diagnosis so by the time they were diagnosed they were usually five or six or seven years of age, and then they realized when they went to school and were not keeping up with the other children. They could never run at all and they had frequent falls as if their legs were just pulled out from under them, and difficulty getting up, and with the usual classical Gowers',[7] which people then started to recognize. This was also an age of neglect, in which specialist doctors to which

[7] Gowers' sign indicates muscular weakness through the pattern of rising from the floor from a squatting position; typical for patients with muscular dystrophy, and named after a neurologist Sir William Gowers (1845–1915). For more details, see Chang and Mubarak (2012).

they were referred would make a diagnosis, sympathize with the family and say: 'I'm terribly sorry, there's nothing we can do, just make the child comfortable.' And then they ended up with the most grotesque deformities, which are unbelievable now. Imagine children who couldn't even sit because they had such a bad S-shaped scoliosis that they were bed-bound, and couldn't get into shoes because the feet were totally twisted round and so on. And then it was fortunate that at Queen Mary's, Carshalton, where I'd worked, there was a polio epidemic. Well, not fortunate for the children, but 1956 was the last big polio epidemic[8] and they had a very good facility for physiotherapy/rehabilitation. They were doing the Kenny techniques,[9] and they were providing these children with special appliances and jackets and things, and then it seemed obvious that the muscular dystrophy children could benefit, and so there was a lot of interest in doing that. And they were very inspired because it cost nothing. The nursing sister on the unit had realized there was a celluloid factory nearby and had got all the offcuts of celluloid for free, dissolved it in acetone, painted it on to muslin, put the muslin on to the kids and when it dried they had an absolutely firm hard, lightweight shell. Couldn't improve on that.

So that was a bit of a supportive thing preventing severe deformities and also, for paediatricians in a sense, it was such a rare disease that they never heard of it basically. The neurologists said, 'Well, it's a little bit beyond the liver system; you know, the central nervous system ended at the intramuscular junction, so muscle disease was also a bit peripheral so there was nobody who was really taking it on as a primary interest.'

Temple: Because it was so rare really for any one individual to see many cases?

Dubowitz: In a sense they were a sort of 'Cinderella' of every profession, a bit too peripheral for the neurologists and a bit too rare for the paediatricians. Now the first thing that happened actually was that there was an enzyme, aldolase, which was first found, and one of the enthusiasts at that time was Brian McArdle, who described McArdle's disease.[10] In fact he was at Guy's at the time when I linked up with Guy's because Queen Mary's, Carshalton, had a student group that

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[8] For more details, see Gould (1995), Smallman-Raynor and Cliff (2014), as well as discussion in Reynolds and Tansey (eds) (2011).

[9] Sister Elizabeth Kenny (1880–1952) was an Australian nurse who invented an innovative (yet controversial) treatment for polio; for more details, see Cohn (1976).

[10] Dr Brian McArdle (1911–2002) was a neurologist at Guy's Hospital, London, who described 'McArdle disease' (a type V glycogen storage disorder) in 1951; see McArdle (1951).

used to come out and then in no time he was doing enzymes for us. And this aldolase was a very good discriminative one. Then of course CK came in about the late 1950s – 1959, 1960 – from Ebashi,[11] who was a brilliant biochemist in Japan, and he found that it was extremely high in muscular dystrophy and that it was specific to muscles so it was influenced by liver disease. That was a major development. Then, of course, the pathology was fairly distinctive, but it was again delayed very often. But with the CK you could, and now CK has become a sort of potential marker at birth pre the genetic revolution. Now, of course, it's much simpler to do a whole genome than to do a CK.

Temple: Well, I don't know about that sometimes [laughter]. What about the fact that it was raised in women? Was that something people remember?

Dubowitz: What, about the X link? Yes, well Meryon actually. Meryon was a British physician who, in 1851, gave a very detailed description of Duchenne muscular dystrophy, some 16 years ahead of Duchenne.[12] It should really be called 'Meryon's disease'. Alan Emery did a book on Meryon[13] and we did an annotation in the *European Journal of Paediatric Neurology* on Meryon's disease just to put it into the literature.[14] He said that it is a disease that specifically affects boys, but seems to be transmitted by women.

Temple: And that was well recognized?

Dubowitz: That's been well recognized over the years, yes. When I worked in Sheffield in the 1960s, I was very sensitive to the family situations and I soon started taking blood from both the mothers and the fathers. Because if you only took it from the mother and you found she was a carrier, the next thing was the father said: 'I told you your family had bad blood.'

Temple: Yes, I used to do that as well, take it from both.

Dubowitz: I then used to tell the fathers: 'It is your fault that your daughters are carriers because you are responsible for giving her an X chromosome instead of a Y.' So there's all sorts of nuances in science, and sometimes you'll get a

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[11] Okinaka *et al.* (1964). Professor Setsuro Ebashi (1922–2006) was Professor of Pharmacology and of Biophysics at the University of Tokyo, Japan; for more details, see Otsuka (2007).

[12] Meryon's description of the disease was read at the Royal Medical & Chirurgical Society on 9 December 1851, but was reported the year after: Meryon (1852). For more details, see Emery and Emery (1993).

[13] Emery and Emery (2011).

[14] Dubowitz (1998).

surprise. We had one father who came with his classical Duchenne child to the clinic one morning and the child's CK was sky-high and the father's was also very high, around 2,000 units. So we were a bit puzzled because it was classical Duchenne. Then, taking his history, he'd actually been working on a clay mine, a clay pit, so we told him to come again after he'd had a day or two's rest and his CK was normal. So exercise pushes CK up.

Temple: Of course, that was what we were always told, that we had to take that CK at a time when someone had had a period of rest.

Dubowitz: That's right, excepting in carriers. Because if you exercise female carriers, or potential carriers, it may be normal at rest and then you get an abnormal rise during exercise.

Temple: Oh, I didn't know that. So who created those curves? The CK curves? Was that you?

Dubowitz: Well, actually the European method for CK, now that I come to think of it, was actually standardized by the Head of Biochemistry for many years at the Hammersmith, David Moss. He actually did a European standard, and he was trying to standardize the method at the time.[15]

Temple: Because all of us who were in genetics at that time would have those curves on our wall, because you had to work out what their risk factor was.

Dubowitz: There's always a difficulty, you see, of any sort of standardization because this is just the way the world is outside of muscular dystrophy. But one of the normal controls that the biochemist had done was that of a PhD student in his lab. She had a CK of about 1,500 and she had no obvious weakness, perhaps a bit of wasting in the shoulder, and so we weren't quite sure whether she was a potential Duchenne carrier or a potential limb-girdle muscular dystrophy case.[16] Eventually it turned out that she was a carrier, because she fell pregnant and, in fact, I think, it was already at the stage that you could do prenatal chorionic villus biopsy.

Temple: That's very interesting. So, can we hear from some of the other people about how we found the gene, for example?

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[15] Moss *et al.* (1981).

[16] Limb-girdle muscular dystrophy is a group of rare muscular dystrophies characterized by progressive muscular wasting that primarily affects muscles of the shoulder and the hip.

Figure 4: Professor Gert-Jan van Ommen

Professor Gert-Jan van Ommen: Well, I think that at some point DNA came in and one person that played a part there in parallel to Bob Williamson and Kay Davies here in the UK,[17] was our boss Peter Pearson, who originally was a cytogeneticist toying around with microscopes and developing staining technologies.[18] At some point in the late 1970s when the first non-causal but linked polymorphism was connected to a disease – that was the Kan and Dozy paper for sickle-cell anaemia,[19] and some papers right after that – he realized that by using segments of DNA you could actually count chromosomes, or count pieces of chromosomes. Perhaps I should give the microphone now to my colleague, Bert Bakker, because I wasn't in the laboratory then, but I heard it from what he told us: that he really then realized that by just picking out pieces of DNA and cloning them, you could actually make probes for specific parts of chromosomes, and in this way count. So he wasn't originally focused on Duchenne. He came from a cytogenetics point of view; he was looking at DNA as a way to measure chromosomes in a very refined fashion. And I think that

[17] Professor Bob Williamson was Professor of Molecular Genetics and Biochemistry at St Mary's Hospital Medical School, University of London and is Honorary Professor of Medical Genetics at the Murdoch Institute, the University of Melbourne; https://www.mcri.edu.au/users/professor-bob-williamson (accessed 10 February 2017). He wrote the introduction to Jones and Tansey (eds) (2014). Professor Dame Kay Davies is Dr Lee's Professor of Anatomy and Director of the MRC Functional Genomics Unit at the University of Oxford; for more details, see www.dpag.ox.ac.uk/team/kay-davies (accessed 11 January 2017).

[18] For more details, see Jones and Tansey (eds) (2014).

[19] Kan and Dozy (1978).

it was through a good friend and colleague, Martin Bobrow,[20] well known to you I would assume, and Ysbrand Poortman,[21] the leader of the Dutch patient organization, that he got interested in the Duchenne field.

Temple: So how did that come about?

van Ommen: Well, Bert can say that better than I can.

Professor Bert Bakker: Thank you. I'm Bert Bakker, also from Leiden. At the end of the 1970s, Peter Pearson had seen this paper of Kan and Dozy with the RFLP around the β-globin gene.[22] Using this type of RFLP, he thought if he could have more of these probes, or not the β-globin gene but other pieces of DNA, looking for restriction fragment variation, you could saturate the human map with these kinds of polymorphisms and follow diseases in families. That was the idea.

Temple: Had anyone else done this? Was that a novel idea?

Bakker: Of course it was known from the β-globin gene that it segregates and that you can use it, but then to really go and look for them randomly was maybe new. Botstein also wrote about these genetic markers around the same time,[23] but Peter Pearson came into the lab and said to me: 'Okay, let's make these probes.' There was nothing. So I had to go to the maternity ward, get a placenta, and from this human placenta, isolate the DNA, and digest it in small pieces using *Eco*RI restriction enzyme.[24] At that time we had to isolate these restriction enzymes ourselves by growing the bacteria, isolating the enzyme through columns, and things like that. That was a lot of work but anyway we had these pieces of DNA digested, visualized as a smear on an agarose gel – it looked nice, but then we needed more to clone them. So it (more digested DNA) was run on a sucrose gradient to pick out pieces of 1 kilobase (kb), 2 kb, and 3 kb, and these

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[20] Professor Martin Bobrow is a medical geneticist who has served as Professor of Medical Genetics at the University of Cambridge; for more details, see his profile at the Royal Society website: https://royalsociety.org/people/martin-bobrow-11104/ (accessed 11 January 2017).

[21] Mr Ysbrand Poortman is the founder of the Dutch Neuromuscular Diseases Association, and of the Dutch Genetic Alliance of Parent/Patient Organizations; for more information, see the respective websites: www.vsn.nl/ and www.vsop.nl/nl/ (both accessed 11 January 2017).

[22] See note 19.

[23] Botstein *et al.* (1980).

[24] *Eco*RI is a restriction endonuclease enzyme deriving from *Escherichia coli*, and an invaluable tool in a variety of molecular genetics techniques, including cloning, DNA screening, and deleting DNA sections.

Figure 5: Professor Bert Bakker

pieces were ligated into a plasmid. The ligation into a plasmid was not possible at that time in the Netherlands because the law prohibited us cloning these human fragments in bacteria; that was not allowed at that time.[25] A few years later it was, but in the meantime we could go to Mill Hill, to the lab of Dick Flavell,[26] who formerly worked in Amsterdam, and there I had learned Southern blotting before the RFLP time. So I went to Mill Hill National Institute for Medical Research to clone these fragments (random pieces) of human DNA in a plasmid (pAT153), put them on a plate, picked them out, and then sorted them to see if they were repetitive or non-repetitive.[27]

Temple: How did you make that collaboration with Mill Hill? Was everyone friends with each other?

Bakker: Yes, yes. Because we had already collaborated before that with some of the people, the whole group actually from Amsterdam moved to Mill Hill, because the cloning was not possible in the Netherlands. Dick Flavell wanted

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[25] For more details, see Professor Bert Bakker's video interview (clip 3) at the History of Modern Biomedicine Research Group's website: www.histmodbiomed.org/article/bakker-bert (accessed 11 January 2017).

[26] Professor Richard Anthony Flavell is Sterling Professor of Immunobiology at Yale University School of Medicine. He was at the University of Amsterdam from 1974 to 1979 and was then Head of the Laboratory of Gene Structure and Expression at the National Institute for Medical Research, Mill Hill (1979–1982); for more details, see http://immunobiology.yale.edu/people/richard_flavell.profile (accessed 11 January 2017).

[27] For more details on Professor Bert Bakker's work at Mill Hill, see Jones and Tansey (eds) (2014), pages 10–12.

to clone and work together with Alec Jeffreys,[28] so he moved to Mill Hill to do it there. Then I sorted these probes by hybridizing them back to human DNA to see if they were repetitive or not repetitive, and hybridizing them to different pieces, different digestions of human DNA, so PstI, TaqI, or BglII, and all the different enzymes hybridize them back to the DNA and detect polymorphisms. So I saw these RFLPs and, in this one experiment, I had 23 unique probes on the human gene map.

Temple: They were all over the place?

Bakker: All over the place, but then later we had to sort them on the chromosomes they were on, of course. One of them (L1.28) appeared to be on the X chromosome because we saw it was polymorphic: one son had one allele, the other son had the other allele, mother was heterozygous – so we did the whole series and it did fit. All boys had one allele and all females had two alleles, so we could say: 'Okay, this is probably on the X chromosome.' Later, by using somatic cell hybrids we could show that it was on the short arm of the X chromosome, and it was about the same time that Kay Davies in London, with her group and Rob Elles, had cloned this RC8 probe,[29] which was on the other side of the short arm of the X chromosomes. These two together flanked the region where Duchenne was supposed to be, because there were girls with Duchenne muscular dystrophy where there was a translocation on the X chromosomes, and we knew that the break in the X chromosomes was in the middle of the short arm.

Temple: So who found those girls? Can you remember?

Bakker: I think at that time, there were about 10 or 15 different girls known to have Duchenne muscular dystrophy, and it was known that there was a translocation on chromosome analysis, all rare cases, of course.

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[28] Professor Sir Alec Jeffreys is Professor of Genetics and Royal Society Wolfson Research Professor at the University of Leicester. For more details, see www2.le.ac.uk/departments/genetics/people/jeffreys (accessed 11 January 2017).

[29] Professor Andrew Read, who is Emeritus Professor of Human Genetics at the University of Manchester, explains that the 'R' in the 'RC8' probe came from the initial of the name of Rob Elles; see his oral history interview, available on the Genetics and Medicine Historical Network website; https://genmedhist.eshg.org/fileadmin/content/website-layout/interviewees-attachments/Read,%20Andrew.pdf (accessed 11 January 2017). Dr Rob Elles (b. 1951) was a technician in Bob Williamson's lab in St Mary's Hospital from 1977 to 1983, where Professor Dame Kay Davies undertook her early work on the X chromosome and developed a library of cloned sequences of the human X chromosome; Davies *et al.* (1981). Among the clone sequences in the library, RC8 was later discovered to be linked to Duchenne muscular dystrophy; Murray *et al.* (1982). For more details on Dr Rob Elles, see Jones and Tansey (2014), pages 102–3.

Temple: And were they cell-lines you could study?

Bakker: Yes, we had some of these cell-lines in the lab to study.[30]

Temple: And do you know who gave them to you?

Bakker: We had different collaborators on that. We can go back to that.[31]

Temple: Do you think it was easier then to collaborate?

Bakker: Yes, it was easy to collaborate.[32]

van Ommen: I think that one of these cases was notably quite famous, and that was the one that was discovered by Christine Verellen in Belgium and was studied together with Ron Worton and Peter Ray in Toronto, and that was an X:21 translocation.[33] There were indeed a number of these cases, but that was the specific one that Ron started working on, moving from the chromosome 21 area into the X chromosome area. But that was a few years later. In the beginning, when we were talking about the two probes that Bert talked about, RC8 from Kay Davies and L1.28 from Leiden, that must have been late 1982. The first paper was in 1982 by Murray with Kay Davies and Bob Williamson for RC8,[34] and 1983 was the joint paper with Peter Pearson for the carrier detection.[35]

Temple: So you had, by that stage, shown it in Duchenne families?

Bakker: No, Duchenne came in a little bit later, so the probes were there (in 1981) and the first meeting that Peter Pearson went to with these probes, to show that they existed, was in Oslo at the Human Gene Mapping conference.[36] A little later in the same year, also 1981, there was a meeting in

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[30] Professor Bert Bakker commented: 'There was an earlier project where Peter Pearson investigated the position effect of autosome translocations to the X chromosome and the spreading of gene activation/inactivation in the autosome part by using somatic cell hybrids; Pearson *et al.* (1978).' Note on draft transcript, 10 February 2016.

[31] For a list of the then available cell-lines, see: Bakker (1989).

[32] Professor Bert Bakker added: 'Peter Pearson had good contacts.' Note on draft transcript, 10 February 2016.

[33] Verellen *et al.* (1977).

[34] Murray *et al.* (1982).

[35] Davies *et al.* (1983).

[36] The Sixth International Workshop on Human Gene Mapping took place at the University of Oslo (29 June to 3 July 1981). For more details, see Berg (1982).

Israel, Tel Aviv,[37] and there he met Ysbrand Poortman. Ysbrand Poortman was from the patient organization, the muscular dystrophy organization in the Netherlands,[38] and he said to Peter: 'You should work on Duchenne muscular dystrophy. If you have a probe on the X chromosome, you should work on Duchenne muscular dystrophy.' And he convinced Peter because he came back from this meeting in 1981, and Peter said: 'We're going to work on Duchenne muscular dystrophy.'

Temple: So that was patient power?

Bakker: And then a clinical geneticist, Henk Venema,[39] was just starting in the lab – because clinical genetics was just starting in the early 1980s – and he then, early 1982, went to all the different families to collect lots of samples. With these we could start doing linkage in the families and show that we could do carrier detection. That was 1983,[40] and at the end of 1984 we did the first prenatal diagnosis.[41]

Temple: Before that, you had no idea that your RFLP was going to be important for Duchenne? It was just on the X.

Bakker: Yes, it was on the X and it was close to RC8, flanking Duchenne muscular dystrophy, that's what we knew, but that we had to work on Duchenne, that came through this meeting with the patient organization, with Ysbrand Poortman.

Temple: That's really special, isn't it? So then you started finding out how your probe would make a difference to families?

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[37] The Sixth International Congress of Human Genetics took place in Jerusalem, Israel (13–18 September 1981).

[38] See note 21.

[39] Professor Bert Bakker commented: 'Henk Venema, in 1987, was one of the first registered clinical geneticists in the Netherlands. After a successful career (1960–1980) as general practitioner in Dordrecht, Venema came to Leiden University where he started as a genetic counsellor at the Clinical Genetic Centre, and as a researcher at the Department of Human Genetics. In 1989 Venema produced his PhD Thesis entitled: *Clinical, cytogenetic and molecular aspects of the fragile-X syndrome*.' E-mail to Dr Apostolos Zarros, 19 January 2017.

[40] Wieacker *et al.* (1983).

[41] Bakker *et al.* (1985).

Figure 6: Professor Steve Sturdy

Bakker: Yes, this one probe was only informative in about 10 per cent of the families, and that was not enough, so we needed more of these probes. That's why Peter Pearson hired a new PhD student, Marten Hofker,[42] who came into the lab and who was dedicated to make more probes on the X chromosome. He made a phage λ library to produce more of these probes. From that series came 754 and 782 probes, which are also close to Duchenne muscular dystrophy.[43]

Temple: Were you working with Professor Williamson at that point, or was he doing something else?[44]

Bakker: We were working together. We exchanged the probes, so our probes went to them and they sent us their probes. Actually by giving all these 23 probes that we had, so sharing these with other laboratories, we got other probes back. And from the other probes we could fill up the map, and also in the Duchenne gene, we got enough probes to do reliable diagnosis.

Professor Steve Sturdy: Can I ask, just to get a sense of the larger picture that this was part of: you had 23 probes, I think you said – did you just home in on Duchenne or did you look for other possible diseases that you might conduct linkage studies on as well? Was it just by chance that Duchenne fell out of this?

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[42] Professor Marten Hofker (1956–2016) was Professor of Molecular Genetics at the University of Groningen; for his obituary, see de Winther, Dallinga-Thie, and Kuipers (2017).

[43] Hofker *et al.* (1985).

[44] See note 17.

Bakker: It was by chance that Duchenne fell out of this, but the other probes were used for other diseases, of course, and there were some on chromosome 16 and chromosome 2. One of the probes on chromosome 2 was at the tip of chromosome 2, and we had localized that by using *in situ* hybridization. I did one on the first *in situ* hybridization assays by using tritium on the tip of chromosome 2, but we didn't know how correct that was. I told Peter Pearson that I didn't know if it was really localized there. So we kept it until the acetate phosphatase gene was found on the tip of chromosome 2 and it was published, or it was published later,[45] and Peter said: 'Okay, let's use the probe' and we published, together with people in Oslo. So there are more probes that are used later in all kinds of diseases.

Sturdy: And were other people picking up as early as this on the opportunities that RFLP probes offered? I'd be interested to hear how other people were working?

Bakker: Other groups started, of course. Many of these probes were produced by laboratories within a few years.

Temple: Any American experience?

van Ommen: There was a probe called 'C7' and a few more. Actually, the generation of these probes was really in hot pursuit and, as Bert said, that field in those days was, I think, one of the most sharing fields that I have known, because, really, by yourself you didn't have anything, because everybody had only a pathetic amount of families.

Temple: It's just the same now!

van Ommen: Actually, by sharing these things around you found out that not only could you help other people, but yes, indeed, you sometimes became author of a paper on a disease that you hadn't heard of the day before. It was really a very funny type of field. There was a time, I think in 1984 or so, when these 'L' probes, the Leiden probes, were actually constituting 40 per cent of all the known polymorphisms on the human gene map, because of them starting so early. Later it exploded, but at the time there were like 150 probes or 200 probes that showed a polymorphism, while now it's billions. So this was really a field where the sharing was extremely important and Peter Pearson was really adamant. I came from a molecular biology lab that was much more restrictive studying a specific field, and here you had this boss who said that as soon as you

[45] Lothe *et al.* (1986).

had something that was of any utility, 'buy 20 Jiffy envelopes and fill 20 tubes and just send it around to all your, well, colleagues but also competitors.' That was sort of a mental transition.

Bakker: Maybe a little to add to that: by sharing these probes, we had this switch to Duchenne, but in 1981 the request for these probes was so high that we organized a meeting in Leiden. So Peter Pearson and I, and from the States, Ray White,[46] Web Cavenee,[47] David Barker,[48] and Mark Skolnick,[49] all co-organized this meeting in Leiden where we had clinical geneticists and people from laboratories in Europe on this course, a two-week course, where we demonstrated the cloning in plasmids, the Southern blotting, and showed how they worked.[50] For these two weeks we had this course and all these clinicians went home with probes that they had made themselves. So we were, at that time, starting to get more of these and, of course, we gave our probes so they could use them in Southern blotting and show that worked in their lab.

Temple: So, how did 'we' find the gene for Duchenne?

Bakker: With linkage, the gene was there. The gene was already mapped by using the girls with the translocation. The gene was located in the middle of Xp21, so in the middle of the short arm, but getting the probes closer you got linkage with the gene, with the defect in the families, and getting closer and closer, at a certain point we came at the position where we had probes on either side of the gene. But it was the same probe. In one family it was under the gene, and in the other family it was above the gene, so we couldn't localize the gene. Later, Gert-Jan van Ommen, using pulsed-field gel electrophoresis, showed that

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[46] Professor Raymond White is Rudi Schmid Distinguished Professor in Neurology at the University of California, San Francisco; see http://humangenetics.ucsf.edu/white-raymond/ (accessed 11 January 2017).

[47] Professor Webster K Cavenee is Director of Strategic Alliances in Central Nervous System Cancers at the Ludwig Institute for Cancer Research; see www.ludwigcancerresearch.org/bio/webster-cavenee-phd (accessed 11 January 2017).

[48] Dr David F Barker is a lab research manager at the University of Louisville; see http://louisville.edu/directory/index.php?record=2&query=david%20barker (accessed 11 January 2017).

[49] Dr Mark Skolnick is the founder of Myriad Genetics, Inc.; see www.dnalc.org/view/15718-Mark-Skolnick.html (accessed 11 January 2017).

[50] The postgraduate course was entitled Restriction Fragment Length Polymorphisms and Human Genetics and took place at the University of Leiden (19–30 July 1982). For more details, see Jones and Tansey (2014), page 11.

the gene was actually much larger than we ever thought.[51] It was two Mb long,[52] so it was a huge gene. So sometimes the disease defect was on one side of the gene and the probe was on the other, and sometimes it was vice versa.

Temple: And the Kunkel lab,[53] how were they involved?

Bakker: They cloned the gene.

Temple: They cloned it. What difference did that make to your…?

Bakker: Actually, one of the probes, 754, of which I talked before, was located in a deletion found by Uta Francke's group,[54] and this deletion on the X chromosome was a very tiny deletion on chromosome X [Xp21], but you could almost see it. Uta Francke said: 'There is a deletion', and this probe, 754, fitted exactly in this deletion. It was close to the Duchenne gene because this boy with the deletion had Duchenne muscular dystrophy. Later it turned out he also had chronic granulomatous disease, McLeod syndrome, and retinitis pigmentosa.[55] A set of genes were missing there, and also the Duchenne was hit. And this probe was very close. Then in the group of Lou Kunkel, Tony Monaco used this patient with the deletion, this BB deletion patient,[56] used that DNA to clone the DNA that was missing.[57] So he cloned actually the deletion by putting an overdose of normal X chromosome DNA (from a 49, XXXXY patient) to hybridize with this deletion DNA and pull it (the missing DNA) out; the BB-deletion DNA was sheared and the other was digested, and he could take out

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[51] van Ommen and Verkerk (1986).

[52] The equivalent of 2,000 kb.

[53] Professor Louis M Kunkel is Director of the Program in Genomics at Children's Hospital Boston, and Professor of Genetics and Pediatrics at the Harvard Medical School, Boston; see www.childrenshospital.org/researchers/louis-kunkel (accessed 11 January 2017).

[54] Professor Uta Francke is Professor of Genetics and Pediatrics Emerita at Stanford University; see https://med.stanford.edu/profiles/uta-francke (accessed 11 January 2017).

[55] Francke *et al.* (1985).

[56] The letters 'BB' refer to the patient's initials.

[57] Professor Anthony (Tony) Monaco was a PhD student in Kunkel's lab, and is now President of Tufts University; see http://president.tufts.edu/biography/ (accessed 11 January 2017). See, also, Kunkel *et al.* (1985) and Monaco *et al.* (1985). Professor Gert-Jan van Ommen commented: 'Tony came later, the deletion cloning really was Lou's work.' Note on draft transcript, 2 February 2016. For a useful resource on the discovery of the Duchenne muscular dystrophy gene, see Kunkel (2005).

the probes, and reach for the probes in that region. And some of the probes really showed also deletion in Duchenne patients. And then he was close, he was in the complementary DNA (cDNA), he could find the cDNA.[58]

van Ommen: Just one anecdote from 1984. In the spring of 1984, the whole of the Duchenne community was organized at a meeting by the Lions Club in the Netherlands and all the people, Ron Worton[59] and Uta Francke and Kay Davies, essentially everybody was there. That was at the time when we had these 754 and 782 probes, and we felt that it was very close. Peter had heard that Uta Francke had this patient with a deletion so he actually, at that meeting, talked Uta into testing that patient. Then the news came back, still in the early summer of 1984 and not yet known to others, that this 754 probe was absent from that patient. That was the summer when I went on holiday to the United States and I visited some of the people there, because I worked only for a year in Peter's lab. I first visited Ron Worton, then visited Kunkel's lab, and then Uta Francke at Yale. And then, we knew at the time but we hadn't written it up yet, that this probe was mapping in the BB deletion. But what we didn't know was that Lou also had the BB DNA. The person who originally discovered this BB deletion, a paediatrician in the West Coast of the United States, Hans Ochs,[60] didn't really want to part with his deletion, because Uta was still studying it. But Lou Kunkel's boss, Sam Latt is, or was – he died later – a bit of an overpowering person,[61] and he wrote to Hans Ochs himself that he had to send this DNA or he would, well, come in action to get it, because this wasn't fair. And so the Boston lab also had this deletion. And Lou, but we didn't know that at the time, already had the first probes that were isolated using this trick of getting the probes out of the deletion. So I came in this laboratory, I was sat down in the library, and when Sam Latt came in I had already told Lou that we had 754 that mapped in the deletion. I'll never forget how Lou turned around and said immediately when Sam entered: 'Sam, they have a probe in the BB deletion' and Sam, a big man, he just sort of deflated and said: 'That is *most* unfortunate.' [Laughter]. 'I mean, congratulations.' But even then we weren't told about Lou's own probes, so I went visiting Uta Francke, none the wiser. Only later, in the fall of 1984,

[58] Monaco *et al*. (1986).

[59] For Professor Ronald Worton, see www.ohri.ca/profiles/rworton.asp (accessed 11 January 2017).

[60] Professor Hans Ochs is Professor of Pediatrics and Immunology at the University of Washington; see https://depts.washington.edu/chdd/iddrc/res_aff/ochs.html (accessed 11 January 2017). See, also, Francke *et al*. (1985).

[61] For more details on Samuel (Sam) Latt (1938–1988), see his obituary: Willard (1989).

Figure 7: Professor Eric Hoffman

there was a meeting in Oxford, led by, I think, David Latchman.[62] Lou Kunkel was invited, and Kay spoke, and I spoke, and a lot more people spoke, and then Lou presented his strategy of this phenol-emulsion reassociation technique (PERT)[63] and he had a very nice model of how it would work.[64] So that was actually when it came out that he had those probes, seven different probes that all came out of that deletion, and that he was testing them and that some probes actually detected deletions that were never found before.

Professor Eric Hoffman: I think you went over linkage, it was mentioned very nicely and eloquently. The linkage data really went, later, directly to the identification of disease genes because you could narrow it to a very fine interval. But, as was commented, you ended up with these enigmatic results in Duchenne specifically where, as was mentioned by Bert Bakker, you ended up on one side or the other of your probe, and in terms of linkage that really didn't make any sense and so the linkage only got you so far. So, just to summarize what Gert-Jan said, you then needed a physical map. You needed to figure out where these different probes and deletions were, and that's what was just summarized; so

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[62] Professor David Latchman is the Master of Birkbeck College, University of London; see www.bbk.ac.uk/about-us/governance/officers/master (accessed 11 January 2017).

[63] Kunkel *et al.* (1985).

[64] Professor Gert-Jan van Ommen commented: 'And then he surprised everyone by showing that it had worked exactly as planned.' Note on draft transcript, 2 February 2016.

there were more and more people with more and more probes and deletions and it looked like it was covering a big area. Well, the next step really was to identify the gene itself, and that really became challenging, because you have a lot of intron and not much exon and, in fact, this gene – just by chance – has a much greater proportion of intron than exon. So there are about 200 base pairs of intron to every base pair of exon. So you're sort of put into the desert saying: 'Okay, find the oasis,' and all you see is sand everywhere, wondering which direction you should walk in. That was really the challenging part and sort of hit the wall as far as the field was concerned. There were different attempts at different areas of the gene to try and find that oasis, really by walking; it was called 'chromosome walking', one direction or the other.

Ron Worton's group was walking in one area, and Gert-Jan's was walking in another area, so you're all in different parts of the Sahara looking for an oasis. There's more than one exon, and it turned out there were a lot of exons, but they were really spread out and really small. They were on an average of 100–200 base pairs, where your introns could be 40,000 or 400,000; some of the introns were enormous. So it just happened that Lou Kunkel's lab ended up in the area of the desert with quite a few oases, whereas Ron Worton unfortunately was looking for one oasis really far away. That was very much a chance thing at that point. So how was that first exon discovered, which really is 'the gene' when you get a handle on the RNA as opposed to just oceans, or deserts of introns, is through what we call 'zoo blots'. You look to see which part of the DNA would cross-hybridize to the animals from the Smithsonian Zoo in Washington DC. So there was one around the PERT 87 walk that then would cross-hybridize, and then the couple that then became, because they were more highly conserved as exons, instead of introns being poorly conserved, gave you at least a hint that this might be an exon. The next step if it is an exon, it should show up in an RNA and then you're starting into these RNA libraries or cDNA libraries, which are relatively new at that time. And one of the problems was that it was starting to look like this might not be a normal gene, or a normal RNA or a normal protein.

Temple: In what context? This was one of the first genes ever to be cloned, is that right?

Hoffman: Well, there were other genes that were cloned, like haemoglobin and others, but the distinction really is that in all those other diseases you had some hint as to the biochemical defect first. Then you either used the protein to get at the gene, or had hints about what the protein dysfunction was. Why

this is called 'the first positionally cloned gene' is that you had no hints about the protein or biochemical abnormality and you really had to start from the code itself in order to try and dissect backwards – it was at one time called 'reverse genetics'; that lost favour. But 'positional cloning' just by positioning the gene. The difference is – again, to make that distinction – that in most other positionally cloned genes, the linkage was the be all and end all, whereas in this gene, the linkage only got you so far and you sort of hit a brick wall, and that brick wall ended up being this effort to find these tiny exons in these seas of introns, or deserts of introns. So one of those then ended up in cDNA, and so that's when I came to the lab to help clone what ended up being a very large RNA, so 14,000 base pairs. We estimated at 16,000.[65] With the RNA and cDNA in hand you could then sequence everything and decode it, and that became the 'dystrophin' protein, and you can make antibodies against the predicted sequence.[66]

Temple: And how quickly did you share that with everyone?

Hoffman: Very, very quickly, often before publication. And even, for example, the first piece of cDNA was published with its amino acid translation way ahead of the whole thing being known, which meant that anybody could then make peptides and make peptide antibodies against that sequence. And everybody did. So then you could make the probes to then clone the cDNA as well. But Anthony Monaco was an MD/PhD student who did a phenomenal amount of work in as far as particularly he had cloned so much real estate.[67] He had a lot of the map of the desert, and knew where different oases were and where different mutations were breaking and those different exons. So there ended up being a lot of resources that were able to be brought to bear to find these initial exons, find the cDNA, and then convert that into the protein and antibodies.

Temple: Was it difficult to get funding for Duchenne muscular dystrophy from funding bodies?

Hoffman: I think it's generally always hard to get funding for anything [laughs]. That's the nature of research but there was a lot of enthusiasm at the time and there was a lot of focus on this disease. So I think maybe, as a group, we were more successful than many others. The other thing was you had great

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[65] Koenig *et al.* (1987).

[66] Hoffman, Brown, and Kunkel (1987).

[67] For more details on Anthony Monaco, see note 57.

foundations behind the effort, like the Muscular Dystrophy Association of America (MDA) and others.[68] So I don't remember, at the time, money being limiting but, you know, those were generally good days.

Dubowitz: If I recall, the MDA offered a million dollars to somebody who discovered the gene, or cloned the gene, and I think the British followed on with a million pounds or something equivalent, and I think that's in a sense how individual people – certainly Williamson, I think – were attracted [laughter] by that.[69]

van Ommen: One thing, intermediate between that, was the mapping stage, because at the same time this was a gene, but it was also almost a genome. It had deletion hot spots and deletion cold spots and it spanned forever and ever and ever. So one of the technologies in those days was developed by the genomic community during the early days: the group of Charlie Cantor and other people had these pulsed-field gels where you could resolve pieces of DNA, of say one million base pairs or half a million base pairs, and map them on top of, or just relative to, each other.[70] So by the time that all these bits and pieces came out of the Duchenne gene, it got to be very attractive to actually make a long-range map of it. That was also around 1986, our group made a map,[71] Margit Burmeister in Hans Lehrach's group in Heidelberg also made a map,[72] and later Sue Forrest in Kay Davies' group, too.[73]

Well, not to anybody's amazement, the maps were pretty consistent and then, the next game was actually jumping across the deletions of patients. Lou Kunkel gave everybody all the probes, but there was one condition: they could do what

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[68] The Muscular Dystrophy Association (MDA; originally, Muscular Dystrophy Association of America) is a non-profit organization and major funder of research on muscular dystrophy; see the charity's history at: www.mda.org/about-mda/history (accessed 11 January 2017).

[69] For more details on Bob Williamson, see note 17.

[70] Professor Charles R Cantor (b. 1942) is Professor Emeritus of Biomedical Engineering and Professor of Pharmacology at Boston University; see www.bu.edu/bme/people/emeritus-faculty/cantor/ (accessed 11 January 2017). For more details on the pulsed-field gel electrophoresis technique, see Schwartz and Cantor (1984) and van Ommen and Verkerk (1986).

[71] van Ommen *et al.* (1986).

[72] Burmeister and Lehrach (1986).

[73] See, for example, Paulsen *et al.* (1986). Professor Gert-Jan van Ommen commented: 'The *Human Genetics* paper was just a map of the X chromosome of probes. Many groups had that. What I meant was around the Duchenne muscular dystrophy gene. I remember a paper with Sue Forrest as first author, in 1987, most likely: Forrest *et al.* (1987).' E-mail to Dr Apostolos Zarros, 24 January 2017.

they wanted and map the deletions, but they would provide the data to Lou to publish jointly. That was a paper, one of the very early multi-author papers. Kunkel *et al.* had 75 authors or so, a *Nature* paper.[74] All the different deletions were then used to just jump from the one end of a deletion to the other end of the same deletion, to actually sort of saturate the map. Most fit on the map that I made, but there was one patient in the Leiden contingency, DL66, and we made this jump from that patient, J66, and it turned out that this jump was essentially a jump across a gap of 1.2 million base pairs. We already knew that there was a sizeable portion of the gene before this 1.2 million base pairs, but then we jumped into a place where there was another Duchenne family with a deletion that actually began yet distal of that specific position. That essentially clinched the deal that the whole thing had to be more than 2 million base pairs. It was in the fall of 1987 or so that it came out that this was really so huge, and that was just by jumping around using actually the patients' deletions to get it.[75]

If you look for history filling in, there was one thing that we glossed over: in the earlier days there was heavy opposition by Peter Harper for one of the closest probes, this 754 probe, that it should be so close because he said: 'This can't be because this probe has 20 per cent recombination in our families. That's about 20 million base pairs away.'[76] So we still had this puzzle because in everybody else's, it showed 3 per cent recombination and that's sort of an unbridgeable inconsistency. The answer came at the time that we found all those probes detecting small deletions in patients, because, then, the nature of diagnosis also changed. It was no longer RFLP diagnosis, as now in 60 per cent of the cases you could actually detect deletions from the pieces that you had from the gene. That meant that you could actually see deletions happening in mothers that had *de novo* deletions. I think that one of the real discoveries that Bert made was the germinal mosaicism situation, where you found that one mother, without having any rearranged X chromosome herself, could actually give the same deletion to two subsequent sons having her same X chromosome.[77] Later, it turned out that this happened quite often when you

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[74] Kunkel *et al.* (1986).

[75] van Ommen *et al.* (1987).

[76] Harper *et al.* (1985).

[77] Bakker *et al.* (1987).

had new mutations in any chromosome, but in those days it was totally new and really complicated the diagnosis. There was even a case in a family from Christine Van Broeckhoven,[78] which seemed a clear new mutation family, but right when the germline mosaicism came about, and Bert said: 'Well, you know, you just never know. Do a prenatal diagnosis, you never know. Let's have a look.' And indeed, the same deletion!

That, two years later, retrospectively explained Peter Harper's situation because he had a mother with two Duchenne patients.[79] But then they could only use linkage so they couldn't follow any deletions. So it seemed as if there were two recombinants in one family and then you get to 20 per cent recombination.[80] So it was by the time you could actually discover all these gaps that suddenly this weird behaviour genetically became clear.

Sturdy: The story I'm picking up on is the enormous complexity of the gene and the multiplicity of possible abnormalities that there could be in the gene. I guess the story that I'm used to, as a historian, is that you discover what's wrong with the gene, you come up with a diagnostic test and then you can use that diagnostic test. But it sounds as though there are so many things you could be looking for here, that the way this impacts on diagnosis is going to be hugely complicated and the relationship between diagnosis and investigation research seems to be a very blurry one in this case.

van Ommen: It still goes on. We're trying to describe a room without the light on. Using a torch light we have some idea of the room, and then somebody flicks on the switch and you see that it looks very different to how you thought. In a way I sometimes compare it with particle physics and the waves, in that the waves only go so far to explain matters and then your resolution becomes so precise that you can no longer describe the thing in your classical concept, and there is a new concept. But, of course, the new concept, when you make everything

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[78] Professor Christine Van Broeckhoven is Director of the Department of Molecular Genetics at the University of Antwerp; see www.vib.be/en/research/scientists/Pages/Christine-van-broeckhoven-Lab.aspx (accessed 11 January 2017).

[79] Bakker *et al.* (1989). Professor Gert-Jan van Ommen added: 'and the 754 allele tracking a proven healthy X elsewhere in the family tracked the Duchenne X in her two sons.' Note on draft transcript, 2 February 2016.

[80] Professor Gert-Jan van Ommen added: 'In reality, the X chromosome with this previously "healthy allele" had undergone a deletion in a percentage of her oocytes, ending up in two sons.' Note on draft transcript, 2 February 2016.

Figure 8: Professor Kate Bushby

approach infinite in that scale, must fit in the old classical theory. And that's the same with genetics in that when we had single-nucleotide polymorphisms (SNPs) every million base pairs, we could speak about the Hardy–Weinberg equilibrium.[81] Nowadays with the 100,000 Genomes Project,[82] people being sequenced all over the place, the Hardy–Weinberg equilibrium turns out to be simply non-existent. Nobody thinks of people mating at random in populations. So that's the same thing.

Temple: Are there other memories about that time of using the gene?

Professor Kate Bushby: I started in genetics in 1989 and my first project was on muscular dystrophy so, of course, I was launched into exactly this whole environment. Newcastle had played a large role in contributing families to the big effort for identifying the gene, so Angus Clarke had spent, I think, his

[81] Hardy–Weinberg equilibrium suggests that in a very large random-mating population, the allele and genotype frequencies will remain constant from generation to generation, provided that other evolutionary influences are not present. It was developed in 1908 independently by the British mathematician Godfrey Hardy (1877–1947) and the German physician Wilhelm Weinberg (1862–1937). For more details, see Stern (1943).

[82] The 100,000 Genomes Project is a British project launched in late 2012 and aiming to sequence 100,000 whole genomes of National Health Service (NHS) patients. It is led by Genomics England, which is owned by the UK Department of Health; www.genomicsengland.co.uk/the-100000-genomes-project/ (accessed 11 January 2017).

research time in Newcastle collecting up all of the families.[83] So I inherited these fantastic sets of notes – often patients who had originally been seen by John Walton,[84] who were still being followed up by David Gardner-Medwin,[85] where Angus had taken the families, had gone out into the coal mining villages in County Durham, and had met all these mothers who had had children with Duchenne who had died, and from previous generations, and there were still quite a number of big, big families with surviving children and people who had died. Of course, this was also mixed in with the Becker population, so we had very large Becker families as well.[86] The other context with that was, I think, by that point Louise Anderson, then Louise Nicholson,[87] had gone to visit you, Eric [Hoffman], in your lab to start making the antibodies that became the diagnostic antibodies in use in many labs – Novocastra antibodies for dystrophin – which are still in widespread use.[88] So our diagnostic paradigm was very much based around the muscle biopsy, because Louise had made these antibodies and so we were revisiting all of our old muscle biopsies, looking at the dystrophin, trying to tell whether there was dystrophin present or absent, to try to distinguish between Duchenne and Becker. Then we had the paradigm, which is clearly the other way round to what we do now, of trying to get the genetics confirmed. My experience of that early NHS translation of these findings – and I'm sure it was your experience as well – was that it was really difficult to get the results.

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[83] Professor Angus Clarke is Clinical Professor at the University of Cardiff. He spent a few years at the University of Newcastle and, there, he developed the Duchenne register; for more details, see his oral history interview by Peter Harper, available at https://genmedhist.eshg.org/fileadmin/content/website-layout/interviewees-attachments/angus-clarke-interview.pdf (accessed 11 January 2017). See also Reynolds and Tansey (eds) (2010) and Jones and Tansey (eds) (2016).

[84] John Walton, Baron Walton of Detchant (1922–2016) was Professor of Neurology and Dean of Medicine at Newcastle University. He served as President of the British Medical Association (1980–1982), President of the General Medical Council (1982–1989), and President of the Royal Society of Medicine (1984–1986). He was also a founder of the Muscular Dystrophy Group (now Muscular Dystrophy UK) and its Honorary Life President; see Meadowcroft (2015).

[85] Dr David Gardner-Medwin (1936–2014) was a paediatric neurologist, a colleague of John Walton and an expert in muscular dystrophy; see Gulland (2014).

[86] Becker muscular dystrophy is a type of muscular dystrophy that refers to a group of genetic, degenerative diseases primarily affecting voluntary muscles.

[87] Nicholson *et al.* (1989a and b).

[88] Novocastra antibodies were sold by Novocastra Laboratories Ltd (founded in 1989 and based in Newcastle upon Tyne). The company is currently known as Leica Biosystems Newcastle Ltd.

Temple: It was all the maths.

Bushby: Well, there was an awful lot of maths; there was an awful lot of working out of risks of carrier status based on linkage and so forth, and CK, and building it all into your analysis. But it was also a case of our lab really struggling at a diagnostic level to produce good Southern blots, so you'd get a result maybe six months later of one probe, which, of course, just gives you a very partial picture of what's going on. We worked very closely with the Leiden group. I remember Louise just deciding to package up all the DNA samples and send them to Bert [Bakker], because that was a much better way to get a result than to try and wait for the NHS diagnostic service to produce any results. That was when we worked on the 70 or 80 families that Angus [Clarke] had put together to correlate the clinical findings with the DNA, and, of course, Louise's results on her dystrophin quantification, which she was very, very good at.

Temple: That's really interesting. Have other people got memories of that time using the probes in clinical practice?

Bakker: Of course, these families had a lot of profit from this new diagnostic work because, before that, they were relying on CK analysis for carrier detection. And, as shown in the old papers of Sarah Bundey on CK analysis for carriers, that was sometimes a disaster.[89] So you couldn't rely on that. So most of the families were told that it would be better not to have children or to have no sons, and if they did a prenatal diagnosis, to terminate all boys, even the healthy ones. Now with DNA markers you could discriminate between an affected and a non-affected boy. So from 1985 on, we could do prenatal diagnosis and, for all the families that we had collected by then, we were doing the carrier detection and prenatal diagnosis. So the diagnostic part started quite early on.

Professor Shirley Hodgson: I worked a lot with Victor Dubowitz at the Hammersmith, and we saw many hundreds of patients, because, of course, Victor accumulated them over many years. I remember being very involved with using these molecular probes in order to refine the carrier risk for these women, mothers of boys with Duchenne muscular dystrophy. It was so helpful because I remember it was so agonizing initially when they would just say they would terminate all male pregnancies, and they knew that at least half of these would be fine. So, although the linkage didn't give them an absolute guarantee that they were or weren't a carrier, pretty much 90 per cent of the time we could give them some alteration of their risk, and quite often this was

[89] Bundey (1978).

Figure 9: Professor Shirley Hodgson

sufficiently small to allow them to take a very small risk. I remember also when assessing risk, I might find myself giving a certain risk and they'd say: 'Ooh, but you mean that's one in three?' or whatever it was. The sorts of risks that people were willing to take in a pregnancy seemed to be about 5 per cent or below that the child would be affected, because, clearly, having had a son with this terrible disorder you just couldn't face it happening again. I remember going into the clinic before I knew the diagnosis sometimes, and you could tell by looking at the mother's face whether she had a child with Duchenne or with some other problem.

Bushby: I remember one family, it was a sister of a boy with Duchenne, who'd had a couple of terminations of male pregnancies before the testing was available, and then we moved into being able to use the probes for linkage and so on. We couldn't define the mutation – it turned out it wasn't a deletion – so it was very difficult because, of course, point mutation detection didn't come until later, and we worked with this family to try and define the linkages and so forth. In the end, what we did was we took the at-risk chromosome and then tested the at-risk chromosome and she decided to have a termination based on this being present in a male fetus. Then we used the dystrophin testing from the terminated fetus to show that she wasn't a carrier. It was so bittersweet, because she'd already by that stage had so many terminations and she did subsequently go on to have a couple of children who were unaffected, but it was years and

years of testing and her unwillingness to take any degree of risk. Just as Shirley said, you went down to a tiny risk really, but even at that point, she was unable to take any risk at all.

Temple: I was also practising, and I started off as a Duchenne Muscular Dystrophy Fellow in about 1987. One of the things I remember is the responsibility of using your wonderful probes, but coming out with risks like 12.25 per cent that the patient was at risk. I was really worried about using the prior probabilities and trying to get this right, let alone explaining what the risk was that I was giving to patients and what people thought it meant, because it really was quite complicated. Professor Robin Winter[90] would come and visit the Department at Great Ormond Street because he was really good at maths, and so I refused to give any of these results until I'd sat down with him and we'd gone through all the results and I got really good at doing Bayesian calculation.[91] But, on the other hand, it was a very unusual time and it was such a relief when we started to be able to detect those actual deletions and you could say: 'Yes, this boy really has got the deletion', and even more when you could tell whether a woman was, in fact, a carrier. But by that stage, I'd moved into more general clinical genetics, because that was really the early 1990s. Are there other memories of that time?

Hodgson: I remember how exciting it was when people started finding the deletions in the Duchenne gene in these boys, but it was such a long time during which we didn't know why different deletions caused different degrees of severity of disease, and there were so many Becker boys who had deletions and also those with Duchenne that had deletions, and sometimes the Duchenne deletion was smaller. I remember we had this big chart on the wall of all the different deletions, and I was looking at it saying: 'There must be some reason why some deletions give rise to Duchenne type muscular dystrophy and others give rise to Becker.' It was a long time before we realized the reason why.

Temple: Does anyone remember how that came about?

van Ommen: There was a meeting in April 1987 in Versailles where Tony Monaco presented the story, or at least his model, that depending on the specific frame consequences of the loss of the exons, you would either make an in-frame

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[90] Professor Robin Winter (1950–2004) was a consultant clinical geneticist who worked at the Great Ormond Street Hospital for Children and became Professor of Dysmorphology and Clinical Genetics in 1994; for more details, see Donnai (2004).

[91] For more details of Bayesian analysis and its application in genetic counselling, see Ogino and Wilson (2004).

or out-of-frame deletion.[92] So he presented that there. That was actually also the meeting where the germline mosaicism was presented by Bert and picked up by Marcus Pembrey.[93]

Temple: But it took a bit of time to get into clinical practice, because we didn't really necessarily know that was true at that time.

van Ommen: Well yes, I know, sometimes these things take time. But still, if you talk about the timing, I think that was the moment. Then there were two papers that actually looked at the consequences of all the deletions on the reading frame. There was one, if I'm not mistaken, by Michel Koenig from Kunkel's lab and one by Beth Gillard.[94] But there were also people who said, 'It doesn't always fit.' And then it turned out why it didn't always fit: that was that sometimes deletions would creep up so close to a specific exon that the splicing of that exon didn't go right anymore. So that the deletion at the RNA level – and that's, of course, what makes the protein – looked different from the deletion at the DNA level. At the DNA level it looked like being out-of-frame, while because there was an extra exon missing in the messenger RNA, it would be moved back into frame so that most of the exceptions to the reading frame rule, ultimately, were explainable when you looked not at the DNA level, but when you looked at the RNA level.

Hoffman: I think a key part of that was that the proteins were going in parallel with gene data, where we could show that Becker was due to a partial loss of function with those whose dystrophin was present but abnormal, whereas Duchenne was generally a loss of dystrophin. And that would correlate so well with the molecular defects, except for the exceptions you just mentioned; it made sense in the end.[95]

Professor Francesco Muntoni: I'm too young to be able to contribute a lot to this early part of the events, although I did spend a little time during my paediatric neurology training in London with Victor [Dubowitz]. Then, I went back to Italy for several years, so I was involved in developing very similar techniques to what Kate [Bushby] had been involved in using. Unfortunately,

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[92] Monaco *et al.* (1988).

[93] For a recent interview with Professor Marcus Pembrey, see http://dx.doi.org/10.17636/01012627 (accessed 11 January 2017).

[94] Gillard *et al.* (1989) and Koenig *et al.* (1989).

[95] Hoffman *et al.* (1989) and Kunkel, Beggs, and Hoffman (1989).

Figure 10: Professor Francesco Muntoni

prenatal diagnosis was very complicated in unaffected males. In those days, people did not store DNA of somebody who had died, so I think the only source of DNA was unfortunately, in many families, the fetus. I started to become more involved with the protein part, and I think I was following work done by many people in order to understand a little more about the exception to the rule, because the genetic prediction is true in approximately 89 per cent of the cases, and 89 is not 99. This is true in both directions, meaning you can have in-frame mutations that are still severe if you're missing some crucial part of the molecule, and that observation helped indirectly to identify the functional strong domain of dystrophin.[96] I'm not saying this is all my work; this is work done by many other people. And vice versa, there are the mutations that should theoretically not allow the production of any protein, but that, unexpectedly, are associated with a much milder phenotype. So I did some of the work at that time.[97]

I go back to the original question; when I think we started, the first question was: what is special about this condition? What, from a diagnostic perspective, makes you think about Duchenne? This makes me think about the central

[96] See, for example, Gilgenkrantz *et al.* (1989), Malhotra *et al.* (1988), and Nicholson *et al.* (1990).

[97] Muntoni *et al.* (1993, 1994).

nervous system manifestation of the condition. You will understand why I'm saying this because, I think, apart from the skeletal muscle problem, which these children have, the specific neuropsychological profile of 50 per cent of these children, and the mental retardation present in one third of these children, give you a good strong clue about the Duchenne muscular dystrophy diagnosis if those are present. If you're dealing with a child in whom those are not present at all, then, of course, the differential diagnosis with other conditions, muscular dystrophy and so on, is challenging clinically. Well, very challenging with some other rare forms of muscular dystrophy. However, once you have a child who fits the full neurobehavioural profile of Duchenne muscular dystrophy, then I think you are left with a much smaller differential diagnosis. Coming back to why I am saying this, firstly for the clinical perspective, but secondly, because I was interested. I come from a paediatric neurology background and my MD was on neuroscience, on aspects of dystrophin in the brain using, for example, dystrophic mice as a model to test whether their behaviour was normal or not.[98] And actually you could identify some subtle differences, so I think a very interesting part that went in parallel to the work that was done to identify what is dystrophin, what is the function of dystrophin in skeletal and cardiac muscles. But then, the very interesting aftermath of multiple, shorter isoforms, each with their promoters, have been discovered one after the other. And then, little by little, that falling into place in terms of correlation between genotype and phenotype, also for the cognitive problems in people with Duchenne muscular dystrophy; that, I think, now we understand reasonably well, not 100 per cent. But 10 years ago, we didn't really understand that very well at all.

Temple: Was the cognitive phenotype well recognized before you could diagnose Duchenne so accurately?

Muntoni: Yes, I think it was already known. Victor will remember better than me. I think this was probably already in the description from Duchenne.[99] In the very original description there was realization that many of these children had more than just muscle weakness. At that time it wasn't clear whether this was really related to, if you like, the biological basis of the disease or whether this was related to the fact that they were neglected.

Temple: Never had schooling?

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[98] Muntoni, Mateddu, and Serra (1991).

[99] For more details, see Emery and Emery (2011).

Muntoni: Not exposed to normal life, and then a number of papers came suggesting actually it's nothing to do with how weak these people are because if you take disease control – for example, spinal muscular atrophy where there is a similar level of difficulties in integrating in life – they have entirely normal cognitive behaviour, so it must be something true about Duchenne. It was then the discovery of the shorter isoforms and the early localization – and now still presume function – that allow to help, to close that circle.

Dubowitz: In the 1950s and 1960s there was a big argument going on: a lot of people said there's no mental retardation, it's simply lack of opportunity and all this. But then, as Francesco mentioned, the contrast with spinal atrophy patients, who were much more disabled but fairly bright – in fact, they had above average type of intelligence – showed that it wasn't that. I studied a group of about 65 Duchenne's in the mid-1960s, and all of them had a psychometric assessment on the usual Wechsler Scale type things;[100] and what was very interesting, one got a Gaussian distribution with a shift to the left of about one standard deviation.[101] So the medium came out somewhere around 85. And it's interesting that a lot of subsequent studies have always shown more or less the same type of distribution. So I think you've got the very bright ones, you've got the obviously below average within the retardation group, and then you've got a sort of scatter across, so there is a consistent sort of element. Then, I think Shirley did some early studies trying to link the retardation part with particular mutations or whatever in the gene, but that's still going on, I think, to an extent.[102]

Temple: It's too difficult. And the phenotype in carriers, the parents of, or the mothers of these boys? When was it appreciated that there was a real phenotype in carriers? Was that always known?

Dubowitz: The phenotype, you mean the clinical phenotype?

Temple: The clinical phenotype, yes, in carrier women.

Dubowitz: In 1963, Alan Emery and I wrote independently separate case reports at the time,[103] and we both observed something similar and that was a mother with enlarged calves and looking slightly abnormal, although with no obvious

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[100] The Wechsler Intelligence Scale for Children is an individually administered intelligence test for children between the ages of 6 and 16.

[101] Dubowitz (1965).

[102] Hodgson *et al.* (1992).

[103] Dubowitz (1963) and Emery (1963).

clinical weakness. We did biopsies at the time, which showed some pathological change. And so this would relate, of course, to CK sometimes being very high as well. So you can actually pick up a certain amount of pathology in some of the carriers, because there was a lot of contention still at that time whether carriers actually have got any manifestation. But, certainly, about 10 per cent were thought to have actual clinical weakness.

Temple: In the early 1990s, late 1980s, we didn't really worry about the mothers particularly. I don't know when that came in.

Hoffman: I think that the key thing with the carriers was some of the early cases of X chromosome translocations in girls that had severe Duchenne actually were one of the first 'events' that led us to map the gene to Xp21. And in those it's often a quite severe phenotype that you often didn't see just in typical characters, and you had a cytogenetic or molecular explanation at that point, because of what's called 'skewed exon activation', that the girls – because of dosage abnormalities – had to keep the translocated X active, which meant they preferentially shut down the normal X. Now 'preferentially shut down' really meant that those cells at the point of exon activation – at about the 100 cell stage in embryogenesis, where both Xs are active and cells should randomly decide which X is inactivated – those with the normal X active had a dosage problem. And so they then had to keep the translocated X active, because they were shutting down some autosome and didn't have the right number of X chromosomes, and it has to do with Xq13 and the X inactivation centre. So those cells had a growth disadvantage, and as a result you ended up with these X autosome translocation girls with clearly skewed X inactivation. You had a molecular and cellular mechanism for it that all made sense, and that led then to searches for 'skewed inactivation' that weren't due to translocations.

Temple: And that started in Duchenne?

Hoffman: Well, I think there are a number of examples in other genetic diseases of X autosome translocations that will give you skewed X inactivation. Skewed X inactivation as a genetic trait independent of any other cytogenetic problem was subsequently found. I think we found an extended pedigree with that. That could complicate, again, what was going on in the family. But then there are just reasons for skewed X inactivations, some of which you can explain, some you can't. One early explanation was twinning so that

when you have female monozygotic twins; the nature of when that twinning event happens, you can end up with poor sampling of the inner cell mass, so an ascertainment bias of sorts, so one girl would be highly skewed and could be severely affected and the other girl would bear normal and random X inactivation. We also found cases of it that almost seemed at the point of X inactivation – remember that's 10 per cent of your genome – all those polymorphisms are now different between those cells, there seems to be an almost repulsion of two cells into a twin. We found cases where one twin had only the normal X active and the other twin had only the abnormal X active, so one was normal and one was Duchenne. So, as all the technology kept on advancing in parallel, like molecular assays for X inactivation, and all the international network of cases and case referrals, you really spread out and found more of a development of molecular epidemiology and molecular genetics explaining these unusual cases.

Bushby: Just to come back to the dystrophin testing again as well. We'd all been looking after some cases of young girls with severe muscular dystrophy, who didn't have a translocation and who you really hadn't reached a precise diagnosis in. Then, the ability to study dystrophin in the muscle biopsy really pushed us forward on that, so the ability to detect the mosaic pattern that you typically see in manifesting carriers was a real spur to our understanding of the fact that it wasn't only these translocation cases that could be more severely affected, or more mildly affected carriers. Then we began to be able to look at them genetically and realized that it's not unheard of to have these as new cases in the family – and I suppose in our Duchenne clinic now, there are probably about five relatively severely affected girls to 100 boys, say, and none of the girls have got translocations. We were, in a way, lucky that those translocation girls were picked up, because they're not common.

Temple: And am I right in thinking there are muscular dystrophies that look very similar, but are not X-linked?

Bushby: Yes, we had two of these girls in the clinic, and I remember vividly David Gardner-Medwin would say, just as Victor was saying, 'This girl has got Duchenne, but apart from the mental profile, the learning profile and the neuropsychological profile are the same; however, this girl does not have Duchenne,' although they were indistinguishable really apart from the neuropsychological profile. It did ultimately turn out that the second girl had a sarcoglycanopathy and the first one was a manifesting carrier.

Figure 11: Dr Rosaline Quinlivan

Dr Rosaline Quinlivan: I was just going to tell a similar story; I was just starting out in clinical practice in muscular dystrophy at the time, in the early 1990s, and it took quite a long time, I think, before we could diagnose girls who were manifesting carriers, to be able to confirm the diagnosis genetically. That took a long time after being able to do it with the boys. We had exactly the same experience with muscle biopsies, when there was patchy dystrophin staining seen on muscle biopsy in some patients, we thought they were manifesting carriers, but we had one girl who had a sarcoglycanopathy. So it was a more complicated picture.

Bushby: In fact, if you look back at Louise's report on that girl, her interpretation of that finding was that either this was a primary dystrophin abnormality or it was an abnormality of a protein that interacted with dystrophin. But that was prior to the discovery of the gene, so that was really quite an insightful comment, I think, at that stage. And, of course, she was working with the people – once those subtypes were identified – to get the antibodies to them as well, and then precisely make the diagnosis.

van Ommen: One thing that's entirely at the other end of the scale, to the girls with Duchenne or Duchenne-like, is – and I think that was one of the discoveries of Kay Davies – a man with a whopping Duchenne muscular dystrophy deletion, who has just been leading a productive life as a bricklayer

and needed a walking stick when he was 61 years old.[104] Then it turned out there was a similar patient found in Canada, and there were two more found in South America. So they're extremely rare, but I think from the perspective of developing mini genes to try to just get towards the therapeutic end, these discoveries, as well as the discovery of the cognate gene, the utrophin gene, also by Kay Davies' group,[105] and also simultaneously, actually, by Lou Kunkel's group;[106] those two developments are complementing the complexity, but also the opportunities to do something about it. There was a French colleague of mine who listened to some of these things and then he said: 'Oh well, this is actually a disease that wants to be cured.' Of course, there are all sorts of things that put you on edge and also just contain the seeds of how to just think yourself out of a conundrum, into a solution.

Temple: Absolutely. But this question about how people used to communicate at that time; was there an issue with competing commercial interest and patents? Was that something that was important in this community, in all your research groups?

Hoffman: I guess I'm a co-inventor of some of those early patents.[107] I think one of the key things that is discussed extensively in medical genetics and genes, and early on, was the idea that if patent officers were going to allow patenting of genes, they should be non-exclusive licences instead of exclusive licences. And certainly, that's the path that this field took: to ensure that, if there were licences, they were not exclusive to anybody; you could buy them at a reasonable cost with reasonable royalties. That model leads to less money coming in. I can tell you as a co-inventor of the patent that maybe that meant two $100 cheques per year, when everybody was using the probes and the genes and the antibodies and everything; but it provides accessibility and at a reasonable cost. Certainly that there are other genes, like the breast cancer genes, that have gone in a different direction, and that became a very big controversy and led to an outcry to the point of where you can't – at least, I think, in the USA now and other countries – patent a gene, because it's pre-existing; it's not a composition of matter.

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[104] England *et al.* (1990).

[105] Helliwell *et al.* (1992) and Pearce *et al.* (1993).

[106] Khurana *et al.* (1991).

[107] See, for example, patent US5239060 A (entitled 'Muscular dystrophy protein, dystrophin') granted to Kunkel L M, Monaco A, Hoffman E P, and Koenig M; patent filed in 1987 and expired in 1997.

So I think this field has generally sort of taken the high road for the longest time. There were a couple of hiccups recently with the exclusive licence for the DNA tests. I know they were trying to protect some of those, but the patents are running out anyway. I don't think intellectual property (IP) and protection of IP was ever much of an issue in this field.

van Ommen: Of course, in the early days, many of the patents for testing for mutations in genes were conceived from the point of view that you would test in a focused fashion for one or more genes. There would be no way in the world that you could ever forbid someone to do a whole genome sequence and find whatever changes you will find. Perhaps it's good to say a few words about HUGO in these days, the Human Genome Organisation.[108] They had an IP Committee and an Ethics Committee, and one of the things that the IP Committee of HUGO already made clear very early on, is that you have different stages from discovery to invention: discovery is just finding something which was there and you hadn't had any major specific contribution. Like the expressed sequence tags (ESTs): snippets of gene that, in fact, were first patented by the National Institutes of Health (NIH). The NIH gave the bad example. It was Bernardine Healy who told Craig Venter to patent his first 2,000 ESTs.[109] Then the whole thing turned over and, interestingly enough, it was ten companies who actually battled, and together with two public laboratories, went against it and made all the sequences that they generated publicly accessible on a 24-hour basis after their discovery. So this was really the world in the reverse, where it was a public institution, the NIH, which started it, and several companies that actually went right through it and said: 'We're not in the business of patenting genes, we're in the business of making drugs.' So that was quite interesting, and it was then that in the early statements the HUGO IP Committee actually made very clear that when you were in the diagnostic arena and discovering mutations but had no functions attributed to the pieces of DNA that you were patenting, it was pretty pointless to patent this. By the time people had added their own intellectual contribution and found function and applicability, and had created value that somebody else might actually take on to further develop, then it became quite important to protect this IP, because it's damn difficult

[108] For more details on HUGO, see McKusick (1989), as well as the Organisation's website: www.hugo-international.org (accessed 12 January 2017).

[109] Bernardine Healy (1944–2011) served as Director of the NIH (1991–1993). Dr Craig Venter is a major contributor to the sequencing of the human genome; while an employee of the NIH, he submitted patent applications on ESTs, which caused a huge controversy; see Cook-Deegan (1994) and Roberts (1991).

to develop drugs, and if you haven't protected your IP, then the chance that something will come out of this is actually damaged if you don't protect it. You can afterwards still decide that you'll license it for a low amount, or for free, but at least you have the control of doing something about it, and negotiating with people that are interested to develop these into actual medicinal products. So that was the gradient that was identified, starting from low-IP discovery and ending in high-IP complex invention.[110]

Temple: We're going to move on to the way that we're now able to treat Duchenne muscular dystrophy, so are there others who want to comment?

Muntoni: This is a very quick one: you also mentioned the carriers, and what do we know about carriers, what do we do about carriers? One thing that is fascinating biologically – this is something that I did a little work on, but people in this room have done much more work there – it was possible to look at other ways to determine carriers on pathology and the dystrophin expression on muscle biopsies. I also did some experiments in animal models, and it was clear that what was mentioned, if you have a female with a random lyonization,[111] there will be at birth quite a significant number of dystrophin-negative fibres in the muscle biopsy, but because of the selective advantage of satellite cells that carry the dystrophin-positive but non-mutated allele, the muscle fibres will eventually lose most of the dystrophin-negative fibres to the point that it becomes quite challenging, unless you have a very skewed inactivation, to use pathology for the diagnosis of adult carriers. The drawback of this – and that's probably why I was thinking about this – is that in the heart there is no regeneration, and therefore, what we have known for a long time, despite some early recalcitrant cardiologists, is that the risk of cardiomyopathy for carriers is not that for the general population because you cannot reverse the dystrophin negativity. If you look at the heart of carriers of mice, there will often be a mosaic as at birth, and this remains unchanged throughout life. This observation, and several case reports, and then population studies in Holland, in this country (UK) and other places, clearly identified a risk of dilated cardiomyopathy in carriers.[112]

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[110] Professor Gert-Jan van Ommen added: 'Which is why many diagnostic patents in those early days were not or hardly enforced. Except for the Myriad *BRCA* patents, and look where it has brought them.' Note on draft transcript, 2 February 2016. This matter is discussed at length in Jones and Tansey (2014).

[111] 'Lyonization' is another name for X-inactivation.

[112] See, for example, Hoogerwaard *et al.* (1999b). Professor Bert Bakker commented: 'First cardiac abnormalities were observed in Becker patients: Hoogerwaard *et al.* (1997); the carrier study followed in 1999: Hoogerwaard *et al.* (1999a).' E-mail to Dr Apostolos Zarros, 19 January 2017.

This had led to a programme of regular cardiac surveillance that most of the clinical centres currently implement. I think that the risk of cardiomyopathy in carriers has been established to be approximately 8 per cent, or something around that number.

Temple: Did the European networks or the world networks make a difference?

Muntoni: I think the team, for example, of Katie, myself, and John Bourke, organized one of the European Neuromuscular Centre (ENMC) Workshops perhaps 15 years ago,[113] maybe less than that. I think it was 2003. I think that this was, if you like, a network. There were no other ways than expert opinion at that time, and you had the representatives of many countries, and we started to look at the published evidence – at our own data set – and, actually, several studies were conceptualized at the time and grant applications were generated, including the one we are involved with in the UK, which is the randomized study of early intervention in Duchenne cardiomyopathy.[114] Regarding carriers, one large survey on cardiomyopathy in carriers was published in the UK,[115] and probably also one in Holland,[116] where the risk of cardiomyopathy was, you know, found to range between 10 and 18 per cent. Eventually, many studies settled at approximately the same level, and that was the basis for implementing the current standard of care.

Temple: So the networking was important?

Muntoni: Yes.

Bushby: The ENMC, which was set up through the interventions of various patients' organizations under the initial leadership of Alan Emery, was a group of patient organizations who decided that a good way to spend their money – and I'm sure Victor was involved with this as well – was to get people together and sponsor research meetings where people could focus in a small group on a really small, but focused topic.[117] I think my first one was your meeting on

[113] Bushby, Muntoni, and Bourke (2003).

[114] Professor Francesco Muntoni commented: 'This is a randomized, placebo-controlled study in which Duchenne muscular dystrophy boys with normal cardiac function are randomized to either receive placebo or cardioprotective medications (β-blockers and angiotensin-converting enzyme inhibitors).' E-mail to Dr Apostolos Zarros, 17 January 2017.

[115] Grain *et al.* (2001).

[116] Hoogerwaard *et al.* (1999b).

[117] For more details on ENMC, see Emery (1997), Rüdel, Nigro, and Poortman (2000), as well as the Centre's website: www.enmc.org/home/ (accessed 12 January 2017).

mosaicism in Duchenne in about 1991[118] or something in the snow in Groningen before they (the ENMC) started moving to Naarden. Like Francesco said, these meetings have really led to lots of collaborations, the sharing of data, the sharing of patients, the sharing of findings that have then informed practice.

Temple: Okay, I think it's been so interesting to have everyone's opinion. We are now going to jump, although I would say it has been a natural progression, to how we've used these discoveries to try and make a difference to children with Duchenne muscular dystrophy, and how people came to the idea that we might be able to treat this muscular dystrophy using the knowledge of the structure of the gene. Is there anyone who might be able to take this story forward in some way?

van Ommen: I wanted to prevent skipping over one thing, which is that the main therapeutic intervention that nowadays has really extended the life and also improved the health is actually the steroid treatment. Steroid treatment was – and Victor knows much more – really pioneered by Victor. There were countries where the steroid treatment for Duchenne was actually fiercely banned, like the Netherlands, and people would take their patients, people would come with their kids, to Victor to have the steroid treatment. So I think it's important that that's not missed and glossed over here.

Temple: Come on then, Victor, I think that's a cue for you [laughter].

Dubowitz: Well, it goes back to recent times, about 1986 I think it was, when the Americans – it was Mike Brooke, Mendell, Griggs, and a few others – they decided to do a controlled trial of steroids in Duchenne.[119] They'd already set up a group to potentially look at treatments for muscular dystrophy further back, I think in about 1986 or thereabout, testing one placebo against another, and this was the first time that there was something that they were trying. They had some natural history outline already of the course of the medication and what they did was two doses of prednisone against a placebo.

Temple: Do you know why they thought of it? Why did they think of using steroids?

Dubowitz: Now the reason for that was that some years earlier there was a short paper in *Lancet* by Drachman and a chap called Ed Myer[120] – I think I met up with him at one time in South Carolina or somewhere, but he was actually

[118] For more details on this workshop, see van Essen *et al.* (1992).

[119] Brooke *et al.* (1987).

[120] Drachman, Toyka, and Myer (1974).

the person who had done the study together with Drachman – and they gave steroids in an open study, and it looked as though it was doing something useful, and they ended up by saying that there seems to be some benefit and that further studies are needed. What then surprised me, this was in the *Lancet*, they didn't say 'we are doing further studies'. They said 'further studies are needed', and so I was sceptical; I think many people were sceptical, why aren't they doing it if it's such an important breakthrough? And then it took about five years before Mike Brooke repeated some of the studies they had done, and then found that it was actually effective.

Now to fast forward, they did a very extensive study, with about 150 patients that were recruited.[121] They weren't very clearly defined so there were a lot of what they called outliers, which were milder cases and so on, and it was just a general diagnosis of Duchenne based on the clinical details. They found benefit from two dosage levels of steroid – a 0.75 and a 1.5 mg per kilo per day – and against the placebo, but those two were more or less the same, and so they recommended that the lower one was sufficient. But they said they couldn't recommend it for use because of the side effects, which they had noted, particularly weight gaining. And there was a reluctance to use steroids in fact for quite a time and, for instance, in 1995 we had the first Workshop of the ENMC on steroids in Duchenne, and there were only seven centres in the whole of Europe that were using steroids at the time.[122] And there was a reluctance in many centres to use it. And, in fact, as Gert-Jan has said, the three senior Dutch neurologists wrote to the medical journal in Holland and said: 'There's no evidence at all that we can see that steroids are of any use in muscular dystrophy, and we would strongly oppose their use,' or something to that effect. They were very dogmatic about it.

Anyway, then it took a bit of time and people were trying alternative dosage. Henriksson, for instance, in Sweden, was giving half of the recommended lower dose and said he was still getting some benefit,[123] so that in a sense was the start of trying to adjust the dosage. We introduced an intermittent schedule because in all the original figures that Mike Brooke showed, there was an initial benefit and then a levelling off.[124] So we thought: 'If you get a benefit and levelling off,

[121] Mendell *et al.* (1989).

[122] 47th ENMC International Workshop: Treatment of Muscular Dystrophy (13–15 December 1996, Naarden, The Netherlands).

[123] Bäckman and Henriksson (1995).

[124] Sansome, Royston, and Dubowitz (1993).

why not just give it pulsed and then off?' So we gave it once a month for 10 days and then off and, by day 20, the kids were saying they were feeling weaker again, so we started 10 on, 10 off. So that's continued, and there have been benefits and disadvantages; the benefits being that they get some responses and some of the young ones – particularly under five – seem to lose almost all sign of any weakness, to go on for quite a time.[125] Then it's mainly with the growth spurt that they tend to lose ambulation, and it's also with the growth spurt that they seem to go rapidly downhill.

The long-term continuous steroid has shown a longer duration of ambulation compared to the intermittent by about two years on average, but also a stunting of growth, which is probably one factor in them not getting worse in that critical growth period. And so it's a toss-up between the quite substantial steroid side effects, and trying to avoid side effects by alternative dosage schedules. It's still an ongoing controversy, and Kate Bushby might be able to tell you about the ongoing study of the continuous versus the intermittent, which has been set up.[126] So that's the position with steroids, but there's no question that it's made a big difference.[127] Now I should also mention, when we talk about management, that the main thing with Duchenne is once they go off their feet they very rapidly get scoliosis, and that's because they have a growth spurt at the time they go off their feet, there's increased spinal growth and they're in a sitting position, and they very rapidly tend to get a progressive scoliosis. If you can keep them walking through puberty and their growth spurt, they in fact have very much less risk of scoliosis.

Temple: Did you observe that?

Dubowitz: This again has a history, because in the 1950s already and 1960s, Vignos, a neurologist in Cleveland, ran a rehabilitation centre, and there were spastic children and other children; he tried putting calipers on to children with muscular dystrophy, but they couldn't cope with them because they were too heavy.[128] It was the old leg irons. In fact we tried them in Sheffield in the 1960s, and it was the same problem because these things were too heavy. Then there was Siegel, who was an orthopaedic surgeon in the States, in Chicago, and

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[125] Kinali *et al.* (2002).

[126] Bushby *et al.* (2004).

[127] See also, Dubowitz (1991), (2013) and Ricotti, Ridout, and Muntoni (2013).

[128] Vignos and Archibald (1960) and Spencer and Vignos (1962).

Silverman, who was a very clever orthoptist; they designed a polypropylene moulded, very light-weight prosthesis with ischial hip support, and he got these kids mobile in these supports.[129] That's at the time of losing ambulation. So we introduced that as well and around about the early 1980s, I think it must have been in the 1970s. On the first consecutive series of 57 cases, we actually achieved an average of two to three years of extra ambulation, and much less scoliosis.[130] So that was a physical means of promoting ambulation beyond the usual time of loss.

The important thing there was to be able to keep going through the growth spurt, and once you got to that and they got into the sedentary position, they were much less likely to get scoliosis. And it was much easier to control. Then, of course, when steroids came, it really achieved the same, because it kept them ambulant through their period of growth spurt and then managed to continue. So it's a question always of any drug you give – there are no free lunches – there are always side effects, but you've got to weigh the potential benefits against the side effects. So, basically, and, of course, there's also the support of treatment. As I mentioned in the beginning, in the 1950s nothing was done essentially for these children in any sort of rehabilitation, so there's a lot you can prevent from an early stage with proper management and just simple things. So that's more or less an overview.

Quinlivan: I would go a step before steroids. The step before steroids was the introduction of non-invasive ventilation, and when I was at Guy's we started looking into this in about 1990/91; at the Hammersmith, Victor had already started using it on his patients.[131] But still there was a reluctance among the community to accept it as a good intervention. People were arguing about quality of life and extending life, and there were lots of discussions. And even in, I think it was 1995 or 1996, I attended a workshop that was set up by Richard Edwards,[132] who was working in Liverpool at the time, because, even at that point, there were still people reluctant to start non-invasive ventilation and, of course, now we know that that's resulting in a very marked extension in life expectancy.

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[129] Siegel (1977).

[130] Heckmatt *et al.* (1985).

[131] Heckmatt, Loh, and Dubowitz (1990).

[132] The 'Consensus Workshop on the role of non-invasive ventilation in the care of patients with muscular dystrophy' took place at the Royal Liverpool University Hospital on April 1996. It was chaired by Professor Richard Edwards, and sponsored by the Muscular Dystrophy Campaign.

Temple: What do patients think about that?

Quinlivan: Well, the patients like it as soon as they are treated with it. Because at the time we were treating people when they became symptomatic. Now we don't wait that long, but, of course, they felt much better when they started using it. It actually started to improve their quality of life.

Temple: It wasn't families that were resistant, it was health professionals?

Quinlivan: It was health professionals, and it was their opinion about quality of life. So it was their opinion about what was good quality of life.

Dubowitz: If I could just add a supplement to that actually, because there are two sides to the coin and, I think, part of the problem in the earlier days was when they got to about 16 or 17, they suddenly went into respiratory failure and very often died within three to six months. The choice, then, was do we put you on a ventilator for life with a tracheostomy or do we just let things go? And very often the families wouldn't accept this. In some countries they already had established units, where they were doing a lot of tracheostomies and the family almost had to do supportive care, nursing, and come and look after. And Rancho Los Amigos, an orthopaedic centre for rehabilitation in California, they were also doing tracheostomies as common practice.[133] What really changed the whole scene in the 1980s was the realization that it is the diaphragmatic weakness that gives you failure at that stage, and if you take a proper history, they're getting headaches, they're restless at night, they have disturbed sleep, and when non-invasive mask ventilation was introduced, I think it was in the 1980s, it made a dramatic difference, and suddenly these children were not only better sleeping at night, but also during the day, and the parents said it was a different child.[134]

Temple: Who thought of the mask, do you think?

Dubowitz: I think it was introduced by respiratory people; I don't know exactly where, but it started being applied to things like Duchenne. When John Heckmatt was with us at the Hammersmith, he got very interested and he actually started putting the Duchenne boys on to nocturnal ventilation with ventilators at home, and he would actually provide an on-call service to go and adjust the ventilators.[135]

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[133] The Rancho Los Amigos National Rehabilitation Center is a rehabilitation hospital located in Downey, California, USA.

[134] Bach *et al.* (1987).

[135] Heckmatt (1987).

We had about 25 children at one time on ventilators, and there was also another doctor who did some basic work in relation to this, so I think this became very viable. What was interesting was that the parents came back and just said, you know, the child is just so much better generally, and then they would go on till their twenties and then, of course, came a point in time where they needed the mask by day, not just by night, and then, eventually, might end up with a tracheostomy as a final stage. But certainly that transition in the late 1980s or thereabouts, I think, made a tremendous difference to many of these patients.

Then we set up a link with the Royal Brompton Hospital, and had a monthly clinic, and they gradually took over the care of the respiratory side so that it evolved as a service.

Temple: And is that how it is today?

Dubowitz: Yes, I think it still is; in most centres they'd certainly still be doing the non-invasive mask, which has been applied to many other conditions. There are some congenital myopathies that die suddenly, although they're doing quite well physically, simply because they have diaphragmatic weakness selectively – so that's important to diagnose and save them.

Bushby: I think that Victor started by talking about the era of neglect, when doctors would say to people with Duchenne: 'Just go away and love your son, and there's nothing that you can do.' I think that all of us would now be very strongly arguing for a very proactive way of treatment, thinking about the possible complications, understanding how to manage them properly, the right introduction of the right steroid at the right time, planning for the respiratory monitoring that will allow you to pick up respiratory failure before it's a problem, before it's symptomatic, allowing monitoring of the heart so that you're treating before there's any risk of symptoms. Patients are symptomatic only very late with heart problems, so you really want to pick them up very early by using increasingly sophisticated techniques, and so forth. I think that what that's boiled down to is that now, when we make a diagnosis, we actually talk about adulthood; we talk about planning for adulthood, and we talk about hope for not only, as we're going to come on to – new therapies – but also what the current therapies can do for these patients and for families. So, certainly, I can remember the days when we used to bring people in and give the diagnosis of Duchenne, and you'd have them in, do the biopsy, you'd have the dystrophin stain; you'd be giving this terrible diagnosis. I can remember one day when we had three in a row, and the third parent actually turned around to the team and said to us: 'How awful that you have to do this

three times in one day.' The parent said this to us. Of course, it was such an awfully humbling thing to happen. But now, although it's still a horrible diagnosis – a devastating diagnosis, and the family never is the same again following the diagnosis – at least you can talk about being proactive, making sure the right things are put in place so that the family can get on with their lives and you can have a proactive plan to look after the child from a medical and social perspective.

Temple: So how did it come about that we've got potential gene therapy in this disorder?

van Ommen: One thing that got it started, of course, was the difference between the Duchenne and the Becker muscular dystrophy, because that created in several places almost simultaneously – in Leiden, in London, in Australia, in Japan – the idea 'What if we could actually turn Duchenne into Becker?' So that was the idea. I remember, I just don't know precisely what year, but we were travelling through Japan for vacation and then I was invited to Kobe by Matsuo.[136] He had this peculiar patient that had a deletion of well-counted 19 base pairs inside one exon, or maybe 50 base pairs or whatever, but a small amount of base pairs within one exon, and he said: 'I'm trying like crazy to just PCR (polymerase chain reaction) this exon to show that it is shorter and I just can't get it amplified.'[137] So then I told him: 'You know what I think happens? I think that the deletion in that exon actually changes the secondary structure of that exon in such a way that the exon is not recognized as exon anymore.' That was what, on the way there and on the way back, got me thinking. Following the genomics also quite closely, I had this kind of idea, 'It can't be that the splicing machinery will read 2.5 million base pairs of RNA from beginning to end to see what splice signals it will meet; there are too many spurious pseudo-splice signals.' So there must be a two-step mechanism as it were: one that sort of identifies these exons as perhaps secondary structure or protein-containing balls or whatever, sort of too few pearls on a far too long string, and some mechanism then just sort of gets all the pearls together, and a second step would be the actual splicing that removes the excess of string between the pearls. So I reasoned that if you would make the pearls no longer look like pearls (which is what happened with this tiny deletion in my view). For example, you should take a big hammer and kill one of the pearls, and you might just get the thing back in frame.

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[136] Professor Masafumi Matsuo is a Professor in the Department of Medical Rehabilitation, Kobe Gakuin University and was Professor of Paediatrics at the School of Medicine of Kobe University (1992–2011).

[137] Matsuo *et al.* (1990, 1991).

So that was our idea, and we had this ongoing debate between different groups on: 'Do you actually target for the splice sites or do you target for the internal regions of the exons?' Our view in Leiden was: 'Let's target the inside of the exons by doing something there to sort of "unexonize" it.' Several other groups had similar schemas, but they were targeting the splice site to interfere with the actual splicing recognition. In our view that was sort of basically hitting the second part of the machinery and causing the machinery perhaps to start scratching its head – 'What was going on there?' – and skip more exons than only that specific one. So that was at the beginning, the argumentation in the field: do you target splice sites or do you target internal? But somehow you had to do something to remove exons from the RNA to actually get the gene back in frame. And so there's another person in our laboratory who is very inventive in that field, Johan den Dunnen.[138] Johan and I sat together and we just worked out schemas for many of those exons to see if we could do it this way. Then we wrote an application to the Muscular Dystrophy Campaign in England and it had two plans: one was to make a mouse with a human Duchenne gene, and the second plan was to see if this exon skipping would work. I was also on their Scientific Committee, but I had to stand in the hall, of course, for my own application. Then the verdict of the whole thing was: 'you will get your money but you shouldn't do this exon skipping because it's a nice idea, but it's not going to work. You will get your money for making the dystrophic mouse or at least the *hDMD* mouse.'[139] So then we decided to turn to the Dutch patient association, and we got money from them a year later, and at the same time had already started. And in fact both worked – the mouse with the human dystrophin gene and the skipping one[140] – but that was basically how it got started, and in 1996/1997 it materialized. And before 2000, 1999, or so, we had the first patient cells treated with antisense oligonucleotides.[141]

Judith van Deutekom[142] started on this work in 1998, and then we just got through the early stages, and we had to get in touch with the Amsterdam medical centre

[138] Professor Johan den Dunnen is Professor of Medical Genomics and Head of the Leiden Genome Technology Center at the Leiden University Medical Center; see www.nbic.nl/about-nbic/nbic-faculty/details/dunnen-den-johan-t-prof-dr/ (accessed 12 January 2017).

[139] The abbreviation *hDMD* stands for the 'humanized' Duchenne muscular dystrophy gene.

[140] Bremmer-Bout *et al.* (2004) and 't Hoen *et al.* (2008).

[141] van Deutekom *et al.* (2001).

[142] Dr Judith van Deutekom is a molecular biologist and was project leader of the Duchenne Muscular Dystrophy Genetic Therapy Group at the Leiden University Medical Center. She later became the Head of Research of Prosensa BV, and is now Vice President (Drug Discovery) of BioMarin Nederland BV (formerly Prosensa Therapeutics BV).

Figure 12: Dr Michael Gait

for cells from patients with specific correctable deletions. We looked at what exon would be the best target, and that was exon 51, because there were many deletions that ended in exon 51 and if you would remove exon 51, you would kick it back into the Becker frame.[143] So that was where we targeted. I know that in Francesco's group they were at the same time – even earlier – doing this with the *mdx* mouse.[144] So how it precisely is from day to day I only know from our study, but it was approximately 1999/2000 when we managed to get the first skip in cultured patient cells. Then we just repeated the thing with six different types of deletions in six different patient cells, and all six showed the same result, and that was in 2002.[145] So in 2000, I think in September 2000, was our first patent application before submitting the publication for the first human skip.

Dr Michael Gait: I just wanted to ask a question, Gert-Jan. Ryszard Kole from the University of North Carolina was also working in splice switching in the late 1990s and had been doing work with β-globin gene and thalassaemia

[143] Aartsma-Rus *et al.* (2003).

[144] The *mdx* mouse simulates a mild form of Duchenne muscular dystrophy, by bearing a point mutation in its dystrophin gene, and thus expressing a small, non-functional protein. For more details, see Partridge (2013).

[145] Aartsma-Rus *et al.* (2003).

Figure 13: Professor George Dickson

genes.[146] I wondered, from the historical point of view, whether your work predated that of Ryszard Kole, or whether Ryszard Kole's idea of using oligonucleotides to splice switch came first? Have you any comments on that, because I don't actually know which idea came first.

Professor George Dickson: I would say, from my opinion, that Ryszard Kole first published this observation of exon skipping using antisense oligonucleotides.[147] It was his published observations in the globin system that triggered many of the groups to think about exon skipping in the context of Duchenne, and, as far as I know, my own group and Steve Wilton's group began to look at this,[148] something we really first published back in the mid-1990s; 1995, 1996 were the first publications that I am aware of. But I have to say that I agree with Mike [Gait]: I think Ryszard's work triggered us to look at the dystrophin gene and whether we could do something similar in that model. And most of that work was performed in the *mdx* mouse model in particular and then, as Gert-Jan says, the Leiden team and our own picked up that work in the context of human Duchenne muscular dystrophy patient cells and demonstrated that the same exon

[146] See, for example, Sierakowska *et al.* (1996). Ryszard Kole is a Distinguished Scientist at Sarepta Therapeutics; he has previously served as Professor of Pharmacology at the University of North Carolina at Chapel Hill.

[147] Dominski and Kole (1993, 1994).

[148] Dunckley *et al.* (1998) and Wilton *et al.* (1999).

Figure 14: Professor Terence Partridge

skipping could be achieved in these patient cells. I agree with what Gert-Jan says: when this was first presented in the scientific community, there was significant scepticism. Certainly there is one scientist here in London, I can remember, who told me his opinion that if this ever worked in a therapeutic context that he would eat his hat [laughter]. So maybe I'll talk to him about that, maybe in six months' time, Gert-Jan. That's my view of the early historical perspective.

Temple: Who was it that said they'd eat their hat?

Dickson: I don't think I should mention that.

Temple: Well, I don't think it matters. We're among friends and it's going to be published [laughter].

Professor Terence Partridge: It might well have been me if I'd worn a hat.[149] At the time, I remember, I saw George's stuff and Steve Wilton's work, and I thought it was something that would interest people who were interested in PCR, because that's by and large what people were looking at. There's another

[149] Professor Terence Partridge added: 'One point that should be considered as an addendum to the Witness meeting is the fact that the discovery of the dystrophin protein set off a series of discoveries of the genetic causes of various congenital and limb-girdle dystrophies associated with defects in the genes encoding the other proteins in the complex of which dystrophin was the central organizer. This work came mainly from Kevin Campbell's laboratory, and was a very good illustrative example of how a single discovery can seed a cascade of further discoveries.' Note added with draft transcript corrections, 2 February 2016.

thing we've actually skipped a little bit, which is that the other main therapeutic push at the time was the idea of stem cells. So we got into both sides really via our collaborations with Eric Hoffman, because I worked for a long while for a man who was obsessed with the idea of circulating muscle stem cells, and we spent a lot of time showing it didn't work.[150] But whereas you could actually directly inject stem cells into muscle – Jenny Morgan and I worked for about 20 years on this – I think, altogether, that we didn't have a good model. We didn't have a mouse we could cure of anything, for a long while. We cured a mouse of phosphorylase kinase deficiency, I think, at one stage, but it has no pathology.[151]

The idea of transplanting cells to see whether it would do anything was very marginal, it must be said, and it's still a bit of a mystery there. Anyway, we didn't have a model. There was another mouse model called the *dy* mouse,[152] which we steered very clear of, because it plainly had a neuropathology as well as a muscle pathology, and we thought we would run into confusing results. The man who did cure that mouse eventually sold his stem cell therapy for $150,000 a go, and I think sued Eric and me for $11 million, at one stage, because of our comments on it [laughter].

Temple: Can we have the name of that person?

Partridge: A man called Peter Law.

Temple: How interesting.

Partridge: Anyway, I got into that because we'd been working on the *mdx* mouse[153] on the basis that it was the only possible model of Duchenne muscular dystrophy, because it was X-linked and it had a pathology that we could identify, and we did lots of experiments; we built up a whole bunch of mice with extra markers in, because we weren't quite sure what we'd be looking at, so we needed iso-enzyme markers to show that we'd done what we thought we'd done. Then Eric turned up with the antibody – the first access that we had to an antibody to dystrophin – and so we sent our stuff across the Atlantic and he found lots of results from our stem cell transplants.

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[150] Partridge *et al.* (1989).

[151] Morgan *et al.* (1988).

[152] See, for example, Meier and Southard (1970).

[153] Bulfield *et al.* (1984).

Temple: What year are we talking about?

Partridge: 1987. And at the same time we actually found – also with Eric's antibody – the 'phenomenon of reversion' as it's called.[154] So you get revertant fibres, so that even though these mice have a disabled dystrophin gene they make little patches of dystrophin-positive fibres, and that's still a fascinating area because we don't understand it – at least I don't understand it. But that tooled us up really to get into the exon skipping field because we had a very bright man – we employed him as a technician – a man called Qi Lu, who turned out to be much cleverer than the rest of us, and developed good ways of blocking.[155] When you look with monoclonal antibodies, especially on mouse tissue, you are stuck with the fact that the mouse is already full of antibodies, and he developed good ways of blocking the background for tracing monoclonals in the mouse. It turned into a commercial reagent called 'mouse on mouse', I think, which most people use for blocking. Anyway, so we were well set up to look at the exon skipping when it was being done, and I think we were the first group to show convincingly that you actually got dystrophin in the muscles when you skipped exons in the muscles.[156]

Temple: And how were you doing exon skipping?

Partridge: We were collaborating with Steve Wilton, so we were using his reagents – it was the 2'-O-methyl phosphorothioate chemical we were using at the time. And the reason we got it working and, I think, other people didn't, was that we used a carrier: so we had a carrier that would get it into the muscle cells; it gets very poorly into muscle cells *in vivo*, much better *in vitro*, which again is a mystery.

Temple: How did you design the carrier?

Partridge: We looked around for things that worked as carriers, it was a block co-polymer with hydrophilic and hydrophobic regions, and so we saw them. You can see them without the carrier, but you have to look very hard and be very convinced they are there. I think that's what you found, wasn't it? And so we actually got into both fields really via our collaboration with Eric, in the first place.

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[154] Hoffman *et al.* (1990).

[155] Lu and Partridge (1998).

[156] Lu *et al.* (2000).

Dickson: I think the other interesting point to make back in those days was that the two chemistries that were being developed for the antisense field, the 2'-O-methyl phosphorothioate and morpholino chemistries. So there were, and are, at least two different chemistries doing the same thing in an antisense context. I think one of the reasons we had a lot of difficulties in those days in the *mdx* mouse was that we were all working with the 2'-O-methyl phosphorothioate chemistry, and it turns out that in the mouse that chemistry works much less well than the morpholino chemistry. Obviously, then followed Terry's work, using a carrier to develop and improve delivery, and so eventually there were studies done with the morpholino chemistry in the *mdx* mouse. The difference between the 2'-O-methyl and the morpholino chemistries in the mouse was an unexpected situation, and perhaps in the human there may be a different scenario.

Temple: A problem always with mice, or any sort of model potentially.

Dickson: I think that's correct. So there is an issue of distribution in the animal model. Certainly those morpholino responses in the mouse were much better, and most groups including the Leiden group, I think, had the same data showing that in the animal model the morpholino chemistry is better in a dose-related sense.[157] But, obviously, the clinical trials that are running now in Duchenne muscular dystrophy patients are looking at both chemistries, almost in a close competitive manner.

Partridge: I think the dichotomy of disparity between the morpholino and the 2'-O-methyl phosphorothioate, *in vivo* and *in vitro*, is really curious, because you can't get morpholinos into cells in tissue culture, whereas the 2'-O-methyl phosphorothioate goes in fairly easily, and the converse is true – certainly with systemic delivery – in the mouse. I don't think anyone has come up with a decent explanation of that yet.

van Ommen: Well, there are at least some thoughts about it; just for the record I fully agree with Ryszard Kole working on the globin gene years earlier than this, and that was where the whole field got the idea for how actually to skip an exon using oligonucleotides. I think that's clear that they really set this whole field going. Now on the morpholinos versus the 2'-O-methyl or

[157] Professor Gert-Jan van Ommen commented: 'This is still an ongoing fight and George Dickson is misrepresenting Leiden here. We actually showed that which chemistry was better depended on the actual antisense oligonucleotide sequence and exon to be skipped.' Note on draft transcript, 2 February 2016.

later generations, if you listen to the recent talks of ISIS,[158] there are very many promising modifications in the backbone that increase the uptake or decrease the toxicity and increase the lifetime. Why are they different? Some people think morpholinos are neutrally charged, and so, *in vivo* neutrally charged molecules go more easily in and out of tissues than negatively charged molecules like 2'-O-methyl. And with regards to 2'-O-methyl, if you want to transfect cells then you can use a transfection agent to get the 2'-O-methyl properly in place. So if you do it in your model development in cell culture, then you can use all sorts of transfection agents to get negatively charged molecules in. So that part of the difference between the two has to do with the fact that they're charged or not. That, sometimes, is even being used in investor language to bias lay people by saying: 'Ours are *neutral* and the other ones are *negative*.' You know, that 'odourless liquid, dihydrogen monoxide, which is pumped by governments through underground tubes into all the houses without anyone knowing it.' Well, that's water. It's just how you phrase it.

I really think that we all have many rounds of further discovery of the technologies, and it might even be depending on the sequence; that's what comes out of the recent talks of ISIS that there are actually still not yet well-understood sequence dependencies that may make some sequences more toxic, and some sequences less toxic, and some sequences working better in this backbone, and other sequences working better in the other backbone. So I think that that's still up there, and that's probably something that you [Michael Gait] could say a lot about.

Gait: I think that the chemistry differences between the 2'-O-methyl phosphorothioates and the morpholinos are profound. The reason the 2'-O-methyl phosphorothioates are active at all, is really because of very good protein binding, particularly to serum albumin and other serum proteins of that nature, which delays their excretion into the kidneys. Particularly phosphorothioate oligonucleotides go primarily to the liver and kidneys, but they are slowed down because of the binding of the phosphorothioate, the sulphur atom, to these proteins, and that's why it gives them more time to be able to enter muscle cells and other cell types. By contrast, the morpholinos, being charged neutral, go into muscle cells, but they are excreted even faster than a phosphorothioate. And they are excreted into the kidney and end up

[158] ISIS Pharmaceuticals (widely known as ISIS), called 'Ionis Pharmaceuticals' since December 2015, is a pharmaceutical company based in California, working on antisense technology; for more details, see www.ionispharma.com (accessed 13 January 2017).

mostly there. I think that the advantage of the morpholinos is also that they are completely non-degraded, so they are excreted whole, and therefore not very toxic; in fact hardly toxic at all. Therefore they've been able to go up to very high doses in clinical trials – 30 mg/kg, 50 mg/kg – and that is the salient feature of the morpholinos, the high dosing. But it makes them very, very expensive as well, and that's the problem. In the case of 2'-O-methyl phosphorothioate, these can be mixed with other nucleotide analogues such as the C-ethyls: the various types that ISIS Pharmaceuticals have, which have a better pharmacology, higher binding to the target RNA, etc., and these will provide second generation oligos in due course. In the case of the morpholinos, work that we're doing with the University of Oxford and also separate studies being done by Sarepta,[159] is adding peptides to the morpholinos, which helps to get them even better into muscle cells, and to reduce their excretion to the kidneys. So there are many chemical changes, and you're absolutely right that these exploratory chemical changes will certainly make these oligo drugs much better in second and third generations; I think that's clear.

Temple: How did we go from making the cell-line to the clinical trials? How did that progression happen?

Partridge: We developed immortalized cell-lines of mice, including the dystrophic mouse and other mice. Jenny Morgan did all this. They were heavily used at least for defining what the best target molecules were. Steve Wilson used them – we sent them to Australia. I don't know whether you're still using them at all? The other thing was that in the mouse we were very lucky that the mutation is in exon 23, and I think there's a whole bunch of downstream exons that are in frame; so what we see under the microscope might contain a number of different skips. George [Dickson], I think, showed that there is more than one skip when you hit exon 23. Certainly the frequency of spontaneous revertants in that mouse are much higher than any other mutation we've seen. So it may be a particularly serendipitous mouse. The cell-line stuff: Jenny Morgan has done most of the more recent development I know of, looking at cell-lines; and Giulio Cossu, who is not here but lives in Manchester these days, has developed the idea of cell transplantation more fully than we did.[160]

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[159] Sarepta Therapeutics is a pharmaceutical company based in Massachusetts, working on morpholino oligomers; for more details, see www.sarepta.com (accessed 13 January 2017).

[160] Professor Giulio Cossu is Professor of Regenerative Medicine at the University of Manchester; see www.manchester.ac.uk/research/Giulio.cossu/ (accessed 13 January 2017).

Figure 15: Professor Jennifer Morgan

Professor Jennifer Morgan: I think Francesco can say more about it. We've been using human cells; I haven't been using the *mdx* cell-line to test antisense oligonucleotides *in vitro*. We have used patient fibroblasts that are converted into myogenesis, for screening antisense oligonucleotides.[161]

Muntoni: The ENMC was mentioned before, and I think it's appropriate to be mentioning it again, because at the time – I'm thinking now 2003/2004 – there had been a consolidated network in the Dutch consortium, if you like, where preparatory work for a clinical trial was clearly building with the realization of the potential that Gert-Jan has mentioned before. There was a mature effort from Steve Wilton in Australia, who had been working for quite a number of years in animal models; there was very mature work from Terry, from George, and other people in the UK. And, together with Gert-Jan, we hosted a series of ENMC Workshops in order to discuss competition and collaboration, if you like.[162] Initially, it was interesting because a lot of parents were concerned that it was a waste of time that two consortia would do some work that was relatively similar – if you look at it from a distance – because we all wanted to achieve exon skipping with antisense oligonucleotides. Actually we chose to look for the most common mutation that was exon skipping, exon 51, but at least we discussed why we would do that, what would be the type of study design we would do, to

[161] See, for example, Zhou *et al.* (2013).

[162] See, for example, Muntoni, Bushby, and van Ommen (2005).

try to at least have them reasonably aligned so that we could have some mutual understanding of what was going on. And that, I think, was certainly for me very, very useful. I was, in fact, the clinician involved in the UK study, together with Kate [Bushby], to put together a protocol for taking forward one of these two chemistries in the UK. Just to give an idea how naive we were: we decided about the chemistry – and this was the morpholino chemistry – and we decided this without asking the company whether they were interested in supporting this chemistry or not. As it turned out this company would certainly not exist today if we didn't draw them in the Duchenne field, and I don't think this is an understatement. But I think we were very, very naive. We wrote a protocol together with many people here in the room and Nic Wells,[163] who is not in the room, who also gave a lot of help with the first-in-man clinical trial using the morpholino, using a protocol that was quite similar, with some differences, to a protocol that colleagues in Holland have used. The colleagues in Holland were ahead of us, especially in the first-in-man study – you published it probably a good year and a half before our study was published, and so you clearly were ahead;[164] you especially had a very good collaboration with one particular company.[165] You may want to explain it because it was, if you like, a spin-off of an academic collaboration, while we had to find this company in the States.[166] In both consortia, we both did first-in-man by selecting a small group of children in whom we would demonstrate that whatever happened in the mice also happened in humans, in boys. After a single intramuscular injection we were concerned initially: does it work at all, does it do any harm? There was a lot of concern, theoretical concern, that you may trigger auto-immunity, for example, and therefore we wanted to make sure before thinking about a systemic delivery that we were not damaging muscle. Both consortia used a slightly different study design, but, in a sense, very similar ideas. Both consortia then moved to the next step, which was the systemic delivery, having demonstrated proof-of-concept of

[163] Professor Dominic (Nic) Wells is Professor in Translational Medicine at the Royal Veterinary College, University of London; he has previously been Professor at Imperial College, London (2005–2010); see www.rvc.ac.uk/about/our-people/dominic-wells (accessed 13 January 2017).

[164] van Deutekom *et al.* (2007) and Kinali *et al.* (2009).

[165] Five of the authors of the van Deutekom *et al.* (2007) study report being employed by or having an equity interest in Prosensa BV, which has taken PRO051 into clinical development for the treatment of patients with Duchenne's muscular dystrophy. See also note 142.

[166] Dr Ryszard Kole, co-author of Kinali *et al.* (2009), joined AVI BioPharma in 2008 as a Senior Vice President of Discovery Research; see also note 146.

a single injection that was safe and that appeared to do in the human the same that it did in the mice. The two chemistries did eventually demonstrate proof-of-concept by systemic repeated injection of the two different chemistries.[167]

Temple: So there were separate groups doing the same thing, but you'd agreed that you would both try and start with the treatment of people with the same type of mutation?

Muntoni: Yes. We had a number of workshops where we really discussed it in detail; we actually made some slightly different choices: for example, of the muscle to biopsy, and so on and so forth. However, eventually, we used the same antibodies to detect efficacy of the result, and, more than anything else, there was a lot of cross-fertilization, a lot of ideas; you know you run through ideas in a safe environment in somebody's workshop where you can think about a stupid thing, and then you probe it with colleagues and then colleagues will tell you: 'Well, actually Francesco, this is a very bad idea,' and then you wouldn't develop this further.

Temple: And you had patients who would take part in your early trials?

Muntoni: Yes. So, patients actually took part as active participants in the consortium. Our consortium, apart from academic representation, first obtained a grant from the Department of Health in 2004, and then another grant from the Medical Research Council (MRC) in 2008, for the two different studies. For the first study, the parent association was a member of the scientific consortium, the people who wrote the grant with us, so we had the patient representation all the way through from the very beginning. I know, as a fact, it's very similar to the work that Gert-Jan has done in Holland, together with a Belgian group, and so on, and so forth. But I think the ENMC Workshops allowed us to – we knew we were going to do something similar, why don't we discuss it? You know there is enough space for it; well, there will be things to be learned by doing it. I think, in a way, I'm glad that we did it in the way that we did. I think it is useful to have two chemistries moving forward; each of these chemistries will probably have their own limitations, these are first generation antisense oligonucleotides, and I think we did the right thing the way we did it.

van Ommen: I can only echo that there were patient associations, or representatives from the patient associations, from both the UK and from the Netherlands in the ENMC Workshops. And there was even quite a fiery debate between the patient

[167] Cirak *et al.* (2011) and Goemans *et al.* (2011).

associations. Some people were of the opinion that it would be a waste to just do these two trials, but others were clearly of the opinion that you would learn more, because you find that something works differently in one way and something else works differently in the other way. We had, from the early days on, a company involved, Prosensa, that was actually a Leiden spin-off company, initially broadly focused on biomolecules.[168] It was founded by somebody who synthesized many oligonucleotides in the Netherlands, called Jacques van Boom.[169] And there were two other company people involved: one was Gerard Platenburg and the other one was Herman de Boer, who made the first genetically modified bull with an extra lactoferrin gene, and caused a major debate in the Netherlands.[170] They all left the biotech company Pharming, and they had established a tiny company, end of 2002; and then I had an adviser for our genome centre, the Centre for Medical Systems Biology (CMSB),[171] Otto Postma, who also came from the same company (Pharming). It was a sort of big family from the Pharming company background. So Postma told me: 'You have to speak to Prosensa.' And they told Prosensa people: 'You have to speak to Gert-Jan van Ommen.' The patient association person from the Netherlands was Elizabeth Vroom, who founded and ran the Duchenne Parent Project, both in the Netherlands and later also the international one.[172] They had been funding us already from the beginning; they actually provided the funding when we didn't get the money from the UK. So it was really a sort of patient/company/scientists confluence. In fact, I've been sailing with Francesco in 1986 in the Bay of Cagliari, so we knew each other for a long

[168] Prosensa Therapeutics was founded in 2002 and was acquired by BioMarin Pharmaceutical Inc in 2015; see www.biomarin.com/about/history/ (accessed 13 January 2017). Interestingly, after a negative review by the Food and Drug Administration (FDA) in early 2016, BioMarin announced the withdrawal of the Market Authorization Application for Kyndrisa™ (drisapersen; PRO051) in Europe (31 May 2016); see www.ema.europa.eu/docs/en_GB/document_library/Withdrawal_letter/2016/06/WC500209201.pdf (accessed 15 March 2017).

[169] Professor Jacques H van Boom (1937–2004) was Professor Emeritus of Bioorganic Chemistry at the Leiden Institute of Chemistry; see van der Marel and Ploegh (2004).

[170] See, for example, Peerenboom (1998).

[171] The CMSB is a joint activity in genomics and bioinformatics of six institutions: Leiden University Medical Center, Leiden University and TNO in Leiden, VU University Medical Center and Free University in Amsterdam, and Erasmus MC in Rotterdam. The Centre was established in 2004 and is directed by Professor Gert-Jan van Ommen; see www.cmsb.nl (accessed 13 January 2017).

[172] Elizabeth Vroom was President (1994–2015) and she is now Director of the Duchenne Parent Project in the Netherlands; see www.duchenne.nl (accessed 13 January 2017). She is also Chair of the United Parent Projects Muscular Dystrophy; see www.uppmd.org/uppmd/board/ (accessed 13 January 2017).

Figure 16: Left to right: Dr Koichi Mikami, Professor Bert Bakker (with microphone), Dr Michael Gait, Professor Victor Dubowitz, Professor Kate Bushby, Professor Shirley Hodgson, and Dr Rosaline Quinlivan

time, and it helps that you can get along very well. So actually that helped, and there was a very open atmosphere. People agreed to disagree on several things, on the backbone, on the muscle, and on this and that, but, ultimately, I think that very often when people are doing these complex things, you find that some results are at odds with other results, but it doesn't mean that one is wrong; it only teaches you when you find the differences that there's something to be found out still. And so that was the first step.

Gait: I think that I'd like to just comment on Francesco's point about suddenly the morpholinos becoming available, because there's a history here that the company, AVI BioPharma as it was then called, was in difficulties: the morpholino chemistry had been foundering even though it is, in my opinion, wonderful chemistry. It's just because the management had not really found a good application, and it was only when they took over the small company of Ryszard Kole called Ercole,[173] which he'd founded in the University of North Carolina, that AVI BioPharma suddenly realized the potential of splice switching and exon skipping, and then could make the liaison with Francesco. But, indeed, I think that the clinical trial work that Francesco did here, on the first trials on morpholino, really did prop up that company at that time. I think

[173] Ercole Biotech (founded in 2002) was purchased by AVI BioPharma (later known as Sarepta Therapeutics) in 2008; see also note 159.

that was right. They've since got many other things and changed management several times, and changed name, and so they're all fine now. But, certainly, there was a very difficult time and it was due to the takeover of Ercole, and Ryszard Kole actually had a lot of influence on why the morpholino chemistry was going, just in the same way as Gert-Jan here had influence over starting in Leiden with the company Prosensa. So these, essentially, were very small academic start-up companies that then progressed into the two styles of chemistry, which are now prevalent today, which will clearly be taken over by newer chemistries. There are many of these now being developed, and so there will be further phases here.

Temple: So there are at least two different lines of chemistry that have now branched into two different ways of taking this forward?

Gait: I think there are two styles of chemistry, which give you different opportunities, and those are both being looked at now. However, there are further chemistries; for example, a Swiss chemistry from Christian Leumann[174] called the tricyclo-nucleotides. They look terribly promising and quite exciting too, and are being developed in France with Luis Garcia's group[175] as well, which, I think, has tremendous opportunities.[176] So, I think there are several new chemistries. The Japanese also have new chemistries that they're looking at as well,[177] so there will undoubtedly be many more opportunities for new chemistries with improved *in vivo* properties, but the two initial ones (2'-O-methyl phosphorothioate and morpholinos) were the points of entry into the field, and made clinical trials possible.

Temple: If you were a newly diagnosed child now, would most people go into clinical trials with one of these if they had the right deletion?

Gait: Bear in mind that we've only got about six or seven exons where it's commercially possible to be developing a particular oligonucleotide for patient treatment. For so many patients who have much rarer mutations, it's going to

[174] Professor Christian Leumann is Professor of Bioorganic Chemistry and Rector at the University of Bern; www.unibe.ch/university/organization/executive_board_and_central_administration/rector_s_office/prof_dr_leumann_christian/index_eng.html#pane298055 (accessed 13 January 2017).

[175] Dr Luis Garcia is Director of Research at the Université de Versailles St-Quentin-En-Yvyelines/Université Paris-Saclay.

[176] Goyenvalle *et al.* (2015).

[177] See, for example, Surono *et al.* (2004) and Yagi *et al.* (2004).

be very, very difficult for an oligo to get into clinical trials. But if you're lucky enough to be in the 60 per cent of those main groups, then, I think, you do have a good chance of being able to get into a clinical trial.

Bushby: I just wanted to comment specifically on the clinical trial side. I think that one of the things that has been amazing – and you've heard already from Gert-Jan and Francesco – was that the patient organizations have been partners in this endeavour right from the very start, and so communication hasn't always been as good as it could be with some of the bigger companies who have been involved along the way; but in essence, the communication between the scientists – and certainly with the small companies – has been excellent. So the patient organizations have really partnered with us in a very real way, not only in areas such as patient registries, which we now have under the TREAT-NMD banner as a resource,[178] but also in developing new outcome measures and understanding how the disease progression can be mapped, and so forth. To come to the access of patients to studies: this has been something which we've been able to address systematically with the patient organizations through the use of registries and so forth, so that patients are identifiable by their mutation through the registries, and you can therefore reach out directly. And then, the patients who might be eligible for a specific study, can make contact with the relevant trial sites. Certainly, up until now, the problem hasn't been the patients' willingness or ability to participate in trials; the problem is becoming now in the field that we don't have enough sites that are good enough to run all the studies that are currently on offer. So, for example, we now have, I think, 10 or 11 open studies in the UK – not all on exon skipping, of course – but this has now led to a bottleneck at the other end, which is that there just aren't the trial sites that are experienced to allow patients access to all the studies that they could have access to.

Temple: We need to come back to why it's difficult.

Partridge: One of the aims here was to look at the relevance of the genetic discoveries to what's happened subsequently, and two of the things that happened really is, I guess, that clinical patients, human patients, could be more closely defined in terms of actually being a Duchenne or a dystrophinopathy. And it also verified the animal models. So we worked for a long time on the

[178] TREAT-NMD is a network of neuromuscular research that maintains a Global Registries for Duchenne muscular dystrophy and spinal muscular atrophy; for more information on these registries, see www.treat-nmd.eu/resources/patient-registries/global-registries/introduction/ (accessed 13 January 2017).

mdx mouse in the hope that it was going to be a relevant model, but it wasn't until the gene was identified that we knew we were working on a model that was relevant, at least genetically, to Duchenne and Becker; and likewise a dog.[179] There's a dog that was identified and the mutation specifically described, and that's one where you've got to skip two exons, for instance, if you're going to do exon skipping, and it's been used – that dog has been used for the exon skipping studies for gene therapy. So direct delivery of mini genes has not been much talked about, but based on the Becker patients with big mutations. Also with cell therapy, the idea of cellular grafts has also been tried on the mouse and the dog, and it's a great comfort to know that we're dealing with things that are actually relevant to the human disease, and those models weren't available prior to the identification of the gene.

Dickson: I just wanted to comment on a similar point. We have focused on exon skipping to a certain extent, but the wonderful work that was described in the first half of the meeting that gave us the structure and the sequence of the gene has also led to a whole series of potential new therapies being developed: you know, exploiting utrophin, for example.

Temple: Tell us about utrophin; that's a very interesting story.

Dickson: Well, I'm sure that if Kay Davies was here she would tell you all about it. But utrophin was initially discovered based on the observation of an unexpected embryonic transcript in Northern blots that were probed with some of the probes that were developed for dystrophin.[180] Then isolating that transcript from an embryonic muscle cDNA library, it turned out not to be some alternatively spliced form of dystrophin, which is what we thought it was going to be originally, but it turned out to be a completely different gene and product, utrophin, and present in many, many tissues. In a sense, the cloning of the dystrophin gene led to the discovery of utrophin, and utrophin itself is a therapeutic target in a clinical trial for Duchenne. So that's one area. Of course, a second area is the one that Terry [Partridge] has mentioned. When you have the sequence available of a gene, we can now begin to think about what is the more classical gene therapy approach where we try to restore a whole genetic structure into the tissues of the cells of the patient. This was something which was initially attempted many years ago using a full-length dystrophin cDNA and

[179] See, for example, Valentine *et al.* (1986).

[180] Helliwell *et al.* (1992) and Pearce *et al.* (1993).

delivering a plasmid vector to skeletal muscle.[181] But inefficiency was a problem. Nowadays, there's some very good preclinical evidence in various Duchenne muscular dystrophy animal models using adeno-associated virus (AAV) vectors that perhaps will lead to an effective version of that type of classical gene therapy, potentially for Duchenne muscular dystrophy.[182] So I just wanted to make those two points. The last thing really that I find rather interesting is that, in a sense, the steroid treatment we've heard about – and I don't know, Eric [Hoffman] may have a further comment on that – but also other small molecule therapies that are being developed for Duchenne muscular dystrophy right now, and are looking very promising.[183] You have to ask yourself, apart from diagnosing the patient, would knowledge of the genetics and gene sequences in Duchenne muscular dystrophy have made any difference to the trials that have proved steroids to be particularly effective, or on the development of new steroids or anti-inflammatories and the like that are coming through the system? So, the genetics has given us some very interesting new leads into therapy, but I think many of the new therapies – small molecule drugs that are coming through – maybe don't owe anything to the actual knowledge of the genetics.

Temple: Although it always gives you diagnostic certainty, doesn't it?

Gait: I wanted to mention small molecules because, first of all, you're forgetting that there's already one small molecule that the clinic had approved: ataluren (Translarna™) for nonsense read-through, for the small number of patients with point mutations in Duchenne that go with nonsense read-through.[184] And that is currently being considered for availability as a drug in the UK right now.

Temple: Tell us more about that.

Gait: Well, it's potentially available, let's put it that way. I think others could tell you more in detail. However, the same company, which is a small company in the United States in New Jersey, PTC Therapeutics, is also developing small molecules for particular splicing events in several of these diseases, including

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[181] Acsadi *et al.* (1991).

[182] Mendell *et al.* (2010).

[183] Heier *et al.* (2013); Professor George Dickson added: 'They have come about without the use of gene sequence information.' Note on draft transcript, 2 February 2016.

[184] Ataluren (PTC124; Translarna™) is produced by PTC Therapeutics and has received market authorization from the European Commission to treat patients with nonsense mutation Duchenne muscular dystrophy; for more details, see www.ptcbio.com/en/pipeline/ataluren-translarna/ (accessed 13 January 2017).

spinal muscular atrophy and in Duchenne muscular dystrophy.[185] What they are finding, in fact, is that individual splicing events in particular systems are actually not entirely identical, and probably have some different proteins making up those splicing events, which give them a unique characteristic that may allow small molecules to actually be discovered by a discovery process, which is now being automated and robotized in order to find small molecule leads for specific splicing events. I think that's quite exciting: that you do need knowledge of the nucleotide sequence and the splicing event at that unique sequence, so that small molecules do owe a lot even so to the genetic understanding.

Temple: That's true. Tell us a little bit about how difficult it is to do these clinical trials. Why are there so few places that can do it?

Bushby: I guess the first issue is about the rarity of the conditions, and Duchenne is rare. However much it is one of the most prevalent conditions we see in our neuromuscular clinics, it is nonetheless a rare condition; therefore concentration of expertise outside very good centres is often lacking. Actually having centres where people have a good body of patients and good training of their physios, and so forth, who are going to be able to do these assessments, is a real issue. Then, of course, alongside the rarity is, can you actually identify a body of patients to participate? Even if you have a particularly good centre, have you got the pool of patients who could go to that centre in enough numbers to make it worthwhile opening it up as a trial site? Then there are sort of non-site-specific, and non-rarity-specific, issues – although they often are related to the rarity – which are that when we started, we really had no idea about what kind of outcome measures we might need for studying Duchenne. We thought we knew the condition very well, we understood the natural history, we knew that patients went off their feet by between this age and this age, but if people came to us and said: 'What about an actual measure to show progression in six months or a year?' Apart from manual muscle testing or quantitative muscle testing – which a synergy group had developed – we didn't have any really very good ways to demonstrate progression. What the regulators began to tell us was that they weren't that interested in muscle strength, because muscle strength is something which, if you talk to somebody about gaining this much in strength, what does it mean clinically, how does that translate to what a patient experiences? So we were very much pushed into the position of trying

[185] See, for example, Welch *et al.* (2007).

to develop and validate scales that had clinical meaningfulness, where you could actually say: 'Okay, this much gain in function will predict that you don't lose ambulation over this length of time.'

Temple: Again, leading the way really.

Bushby: Well, it was an interesting discussion, because you talked about ataluren and Translarna™, and that company, PTC, was the first one to set up a big, large-scale study and they did it – to a certain extent with our advice – but to some extent they also had to take the advice of the regulators, and so on. And so, we've ended up with the six-minute walk distance as a primary outcome measure for all of these studies in Duchenne. I think it's been really quite well established that, yes, you can map progression in six-minute walk distance, but there are also many, many pitfalls of potentially using this test, and whether it actually measures what we're interested in is something that is very much in question.

van Ommen: I wanted to connect this point with a point that you mentioned much earlier with the Bayesian statistics. Because the issue here was that the regulatory authorities in this type of analysis, and especially when large companies like GSK (GlaxoSmithKline) or so are involved, typically they have these hard and fast rules about *P* values for the outcome and meeting primary objectives. The *P* value should be 0.05, and if you have two trials that are almost the same, and you want to jointly analyse them then this is called a 'meta-analysis' and your *P* value has to be 0.025. But the trick is that if you have one person dropping dead in a thousand, it means that you have thousands of people dropping dead with your drug. And so, still this penny hasn't dropped. But it's also a very difficult field that is still hotly discussed – this community is prepared to take phenomenal risks. We talked about people that were considering a 10 per cent risk on a double recombinant as a takeable risk, or a 5 per cent risk and so on. Because they stare a serious disease in the face, all the time – their life is filled with it – and that's completely different from the type of $P = 0.05$ statistics, because that means that you have a chance of less than one in 20 to be wrong. I think that in this rare disease community, that type of cut-off would mean that you could give up developing any therapy for the rarer Duchenne mutations, for well, almost anything except for spinal muscular atrophy, and cystic fibrosis, and Duchenne, and a few more, and then you're done. So, really, you must project this against getting better outcomes, getting better biomarkers, also not, say, false readouts and lifestyle and so on, but to integrate that into a sort of patient-defined and

patient-centred outcome, which is meaningful to patients, and at the same time can be accepted by the regulators; but that certainly it is not going to be a 0.025 *P*.

Temple: No, with rare diseases, it is always the problem.

Muntoni: I would just second and continue this. Starting from one question to Kate of why it's difficult to do this trial. Well, firstly, because there are not too many Kate Bushbys, and Ros Quinlivans, and there is a clear issue about capacity, especially when you come to some of the early interventional trials.[186] We were actually here in this room at a meeting with EMA (European Medicine Agency) to discuss clinical trial design for Duchenne muscular dystrophy. I think it was in this room, in June 2014, and then we had a subsequent meeting hosted in London at EMA earlier on this year, so we are an active community. Again, talking with each other, with the patient community, and with the industry to understand what are the things the regulator wants. The regulator is increasingly pushing us both into what is clinically meaningful; that is absolutely right. At the same time, how can we measure things in a non-invasive way, if we can? Unfortunately, up to now, for many of the primary biochemical or secondary biochemical outcomes, we need to do a muscle biopsy in these children; ideally we'd like to do multiple muscle biopsies, because we would like to see whether dystrophin's been restored, how much there is. This is messy, unfortunately, because it's painful and also, apart from the pain, the distribution of the response is not completely uniform, therefore you cannot have a completely clear answer to your question.

So, increasingly, we have been asked to look into non-invasive ways: for example, muscle imaging as one of the outcome measures. I certainly have a European grant, together with a number of sites, including Newcastle, to look at the new antisense oligonucleotides for exon using also muscle magnetic resonance imaging (MRI) as one of the key outcome measures. But that automatically restricts the number of sites where this can be done; it's not straightforward to have muscle MRI consistency. Well, it's never been done before for muscle so it can be done in a small setting with highly trained people, it's not very easily done unless there is an academic centre behind that particular paediatric neuromuscular group. So, paradoxically, apart from Kate Bushby and Ros

[186] Professor Francesco Muntoni added: 'In addition, there is relatively little experience in this field for clinical trials, as until recently there were no drugs to be studied. In this respect the interaction with regulatory authorities has also evolved in recent years.' Note on draft transcript, 14 February 2016.

Quinlivan equivalents, I think what is behind the bottleneck, is a university, imaging, and what allows us to move further than just looking at the patient and looking how long they walk for six minutes. That, I think, is a bottleneck.

I thought I understood Duchenne reasonably well and then you start to realize, once you start to look really with very precise spectacles at the natural history study, the fact that these patients do have different trajectories, and that what it means is that it is really unsatisfactory when you can only treat a relatively small number of patients. You would like to know: 'Is the trajectory of this patient really being influenced by what I'm doing, or am I just by chance picking up five patients who would have done well anyway?' So there is also a lot of work on looking at what modifies the disease, and a lot of actually recent work, again collaborations. We are involved in a collaboration with Eric [Hoffman] on the other side of the Atlantic, and maybe you might want to say a few things?

Hoffman: I think Francesco is talking about understanding the disease trajectory and the variability between patients. We've talked about Becker versus Duchenne. Duchenne is relatively homogenous, it is out of frame missing dystrophin, but you still see variability in both onset, severity, trajectory, and the central nervous system involvement. And that complicates clinical trials, because that creates this noise in which it's more difficult to see an effect if different patients are acting different anyway. So genetic modifiers – that work is going well in multiple groups. To use the large natural history studies that Kate mentioned, where you can really look at phenotypes, have patients well phenotyped, their strength, their gross motor function, and then superimpose genetic polymorphisms that exist in everybody to see what modifies the disease progression. Again, as Francesco mentioned, that work is going reasonably well through collaborations, so you can find these individual polymorphisms that fit into our understanding of the disease pathogenesis as well. You know, what's going on in the muscle? How do these other genes influence the muscle reaction to that?

Then one other thing that is mentioned earlier that cross-references another approach to small molecule drugs, is trying to go back to glucocorticoids. Even in the original Nobel speech in 1950,[187] it was said that you've got to do something about the side effects, and you heard from Victor and others that, for many years, the delay of uptake of glucocorticoids was because of the side

[187] The 1950 Nobel Prize in Physiology or Medicine was awarded jointly to Edward C Kendall, Tadeus Reichstein, and Philip S Hench 'for their discoveries relating to the hormones of the adrenal cortex, their structure and biological effects'. See www.nobelprize.org/nobel_prizes/medicine/laureates/1950/ (accessed 13 January 2017).

effects. One of the best-studied side effects of glucocorticoids is muscle weakness, where you have what's called the FoxO pathways that are directly regulated by glucocorticoids, and directly cause muscle wasting.[188] So is what we see in the strength increases in Duchenne really a sum of maybe anti-inflammatory minus muscle weakness as a side effect? Which suggests that if you can get rid of the side effects, you might not only reduce the burden of side effects but possibly also increase efficacy.[189] So glucocorticoids are complicated. You have to peel away – chemically – the many different layers of the onion, but, I think, that's been successful, it looks like it, and that's now going into Duchenne clinical trials with Kate's help, and the European Union, and many foundations helping to move that through.

Sturdy: I just wanted to pick up on something Kate said a little while ago about registries, because it seems to me as though registries are hugely important both in terms of making clinical trials possible, and also in terms of gathering the natural history of the disease together. I just wondered: is there a history of registries that we ought to be taking into account here as well?

Bushby: Well, there are two different classes of data collection projects, which have been going on. One is the big natural history studies of which Eric [Hoffman] has led probably the biggest one in the Cooperative International Neuromuscular Research Group (CINRG) for many years;[190] very well-funded, well-supported, large cohorts of patients, followed as if they're in a trial. So that's a very valuable data set. We have a data set here in the UK, which we've been working on with Muscular Dystrophy UK for many years, the North Star database, where now there is a network of 19 centres across the UK who collect the same data.[191] We

[188] The forkhead box O (FoxO or FOXO) family of transcription factors includes atrogenes (genes involved in atrophy) that are triggered by glucocorticoids in the muscles, leading to proteolysis and atrophy; see, for example, Schakman *et al.* (2013).

[189] See note 183.

[190] The CINRG, founded in 1999 as the Clinical Research Arm of the Duchenne Muscular Dystrophy Research Center and the Research Center for Genetic Medicine at the Children's National Medical Center in Washington, DC, is now a global, state-of-the-art clinical research network that is undertaking and facilitating clinical studies in neuromuscular diseases; see www.cinrgresearch.org/cinrgnetwork/description.cfm (accessed 13 January 2017).

[191] The North Star database is a national database for Duchenne muscular dystrophy, established in 2006, and is – along with the North Star clinical network – part of the North Star Project that is run at the Dubowitz Neuromuscular Centre at Great Ormond Street Hospital; see www.gosh.nhs.uk/medical-information/clinical-specialties/neuromuscular-information-parents-and-visitors/about-us/north-star-project (accessed 13 January 2017).

did this way back with leadership from Francesco and Adnan[192] saying: 'Right if we're going to use steroids in the UK, we need to be able to monitor how it works.' So the physios went away and developed a scale that is now used in trials as well, the North Star Ambulatory Assessment. We followed something like 700 or 800 patients, but just in clinical practice, so not with the depth of data collection and monitoring the CINRG study does. There are several other natural history studies, which are really helping us to understand the history of the disease. On the other side, when we got the TREAT-NMD grant – which was a Network of Excellence development grant awarded by the European Union in 2007 – we decided that one of the biggest priorities with enabling trial readiness was patient identification through registries. We worked on a sort of federated system whereby individual countries, often with patient organization support, sometimes with academic support and/or a mixture of the two, would set up a registry in their own domain, but they would be using a minimal data set that would be in common across the different data collection methodologies, and which would then be able to be aggregated, for example, for enquiries from industry. This was a very successful model insofar as we've now got registries in about 40 different countries, all collecting that minimum core data set, and used widely by industry and academic groups, to identify patients for studies and identify, for example, areas. We've done a big health economic study through the registries, we can use it for patient preference studies, we can use it for devising new methods for outcome assessment, especially patient-reported outcomes. That's now being widely used by industry so, for example, all of the companies who want to identify patients with a specific deletion, will come and they'll say: 'How many patients are in the registries who are still ambulant, who are on steroids? And we want to know for the whole world or we want to know for these particular countries.' So that has been, I think, a very powerful initiative. It has, again, been done very much in partnership with patient organizations, and it's frequently patient organizations who run the registries and they come together on an annual basis to curate a meeting, to discuss good practice, and their good practice around data sharing, good practice around data collection, and so forth. That's now extended to other diseases as well, which is quite a powerful model.

Temple: Just to add a comment there. We have heard quite a lot of things that are really interesting about Duchenne, so I remember in the late 1980s we had a Duchenne muscular dystrophy registry, and I would say that led the way for

[192] Dr Adnan Manzur is a Consultant Paediatric Neurologist at Great Ormond Street Hospital, and the clinical leader for the North Star Project.

Figure 17: From left to right: Dr Michael Gait, Professor Gert-Jan van Ommen, Professor Karen Temple, and Professor George Dickson

lots of other diseases. There are very few that have been going for that long. We've also been hearing about how you've brought treatment into clinical trials relatively early, but what you're talking about now is personalized medicine, showing how incredibly difficult it is. Sometimes rare diseases really do show how hard it is to personalize medicine, because not only do you need to get drug company involvement, but the great variability that we've got between us all means it is so difficult, when you're down to a personal level, to tell whether your drug is any good or not. So, I think it's quite interesting that all of the things that you're describing are just what everyone's got in store for them, whatever disease they're talking about when we try and apply personal medicine.

Bushby: I think it's absolutely true, and when you think back to how long ago the dystrophin gene was identified, we have got one EMA approval, conditional approval, for one drug, but that's it. All the other benefits that have come to the patients over this same period actually have come through the good application of basic medical techniques and so forth. So I think we have seen an enormous advance in what we understand and advances in what we can offer, and advances in the trials that we can deliver, but our next challenge is now with the funding models and with the regulators and the payers because you know EMA is approving ataluren (Translarna™) conditionally in Europe and it's available in France, Italy, Germany, Greece, and Spain.[193] But in the UK, it's going through

[193] See note 184.

the NICE appraisal, which is, you know, going to be the next challenge. If you really think that Genomics England[194] is going to bring treatments for every rare disease, look at how long it's taken us, and although we've made mistakes along the way, it hasn't been totally a disaster.

Quinlivan: The other challenge we are all going to face – well, patients are going to face – is the high cost of these drugs, because for rare diseases they're not going to be high-output drugs for the company, so the costs are going to be high.

Temple: They are trying to work out a completely different model, aren't they?

van Ommen: I think there's still also one hidden message for Duchenne to the rest of the diseases, and perhaps also the common diseases, and that is, because we are mechanistically interested in the diseases, we tend to focus on the people who are sort of exemplarily ill, to find the mechanisms. But, in fact, if you think back to Duchenne and Becker, what it actually also has told us is that in other diseases we should also look at people who are unusually healthy, because that very often will show us a way towards a therapy. If you have people who should be ill but aren't, then usually they are sort of genetically compensated. Somewhere else there is a wiring cut or something else that prevents them from becoming ill, and that was the case in Duchenne; it was Becker that prevented them from being ill. Why? Because it turned out that they were fortunate as to having a proper reading frame. In many diseases there are 1, 2, 3, 5 per cent of people that should be ill, but are not. Typically, that is because somewhere else something has gone wrong, and that has actually compensated the disease process. That should be a lesson for the pharmaceutical companies, because pharmaceutical companies incidentally, in my opinion, are better at breaking things than repairing them. If we find out what is broken in those people, that prevents them from becoming ill, then it proves a way that is naturally tried, like Becker, to circumvent the disease. Now it will be different when you suddenly apply medication or when these people have it from birth, but I really think that in many of the common diseases and the rare diseases, you should actually focus more on the people that escape being ill, and that is why you need the big biobanks, because for that you need like 50 people that are similarly not ill to derive a mechanism.

Dubowitz: Just a very important point that I was also going to raise at some time and I originally was inspired into this way back in the 1950s when I used to go to lectures advertised in the *British Medical Journal* at the Royal College

[194] See note 82.

of Physicians and heard Finkel talking about childhood leukaemia, acute lymphoblastic leukaemia in childhood. He was just starting a system of cycling therapy for the first time, and he was picking out patients who seemed to be unusually responsive to an individual therapy. And this is how the whole business of cycling therapy started in a way. 'Treasure your exceptions' was the slide he had, which I have quoted ever since. Now I'm still puzzled why the *mdx* mouse is not weak. It's said to be a model for Duchenne, but it hasn't got Duchenne dystrophy. It's got no dystrophin, they say it's a small animal, but that doesn't carry any water, because the congenital dystrophy mouse is very weak. I'm also fascinated by some of the patients I've seen, and there's one I've been following now for 10 years who, at the age of four, had absent dystrophin, out-of-frame mutation, dystrophic muscle, and no clinical weakness. So no four-year old I've seen before was devoid of any signs, and had Duchenne dystrophy laboratory-wise. There's just recently been a publication of a new colony of dogs; the Golden Retriever dog is comparable to Duchenne in severity, absent dystrophin, out-of-frame mutation. There's a new colony that's got no dystrophin, they're still looking for the mutation and is clinically, practically normal.[195] So here are models, exceptions for us to look at and ask 'Why?' I mean, it may just be gene modifiers, but in some of them it's so tremendously different; it's not just a little bit better, but it seems to be almost devoid of the clinical problem. Originally, the distinction between Duchenne and Becker was really an artificial one based on the studies of the loss of ambulation, so the 95th centile of loss of ambulation was 12 years approximately – so that was the limit of Duchenne. The range of Becker was very much wider and, of course, there were cases already found with an out-of-frame mutation who were doing better and shown to have automatic splicing. So, basically, I think the whole range of Becker variation is an important one, and there's, of course, a transitional overlap between sort of intermediate cases who walked till 13 or 14. So, I think, we've got to look at all of these. But certainly, the absent dystrophin is worth looking at.

Partridge: So two points really. One of them is the modifying factors. If you breed the original X mutation on to other backgrounds, it changes the pathology, so we've been looking at one that two other groups have been looking at, which is breeding it on to the DBA/2J background, where it does indeed get weak, it loses muscle. It doesn't do all the things you might expect of it, because, although it's weak and loses muscle, both the amount of degeneration and the amount of regeneration go down. So it follows a quite different pathway and

[195] Ambrósio *et al.* (2008).

this is an example of the sort of modifying factor you can begin to look at in the mouse more easily than any other model system.[196] Then, the second thing is the rarity of Duchenne. From the point of view of, say, a gene replacement therapy, all Duchenne cases could be looked at as pretty much a single entity, a rare entity. But if you were looking at it from the point of exon skipping, it's a whole subset of rare entities within the original entity. So although exons 51 and 53 and 44 and 45 may well be addressed by pharmaceutical companies at some stage, all the rarer exons would never be addressed, and they won't be addressed firstly because you couldn't set up a statistically meaningful experiment around them, and secondly because there won't be the finance for them. The only way that people, families with children with those rarer skippable exons would ever get into that scheme would be if they actually raise their own funding for it.

Temple: Which is very worrying, isn't it? Now I think that this is the moment where we have a chance for people that have got something they feel has not yet come out, to just say it. This would be a perfect moment to feel you can take the microphone.

Hodgson: I just want to say a final thing: that so many times we've been talking about the patient associations and how important they are, and I just think that's something we can bring out, because clearly a lot of this work just couldn't have happened without the patient associations and how helpful they've been.

Temple: True. That's very powerful.

Dubowitz: There's one point I wanted to raise that worries me and that is when the EMA or whatever gives provisional licensing to a drug before they've completed Phase 3. How do they handle the situation if the Phase 3 study does not work or doesn't show significance? You've then got a situation where the drug is marketed, it has to be withdrawn presumably, patients are on it and they claim to be benefited by it; it seems to me a whole can of worms.

Temple: Yes, and rare diseases and individual treatments are going to be the norm in the future, and we really haven't got a plan for it. Just in the gene's defence, I do think understanding and being able to tease apart the actual pathology has made a huge difference. When I'm listening to you – I'm not saying that from a very pragmatic perspective we haven't made a big impact with regards to steroids and these other treatments, which could have come along without the knowledge of the gene – but I still maintain that the certainty of diagnosis,

[196] Coley *et al.* (2016).

the deeper understanding that we've now got, because we do understand that the gene and its function, has made a huge difference. I think we must never be tempted to think it doesn't matter; understanding the cause of diseases is such a big thing.

Hodgson: Just in the gene's defence also, there's so much genetic counselling that couldn't have been done without it.

Temple: That's very true.

Bushby: Paradoxically, even though so many of these treatments that we now apply don't need to know the gene, the fact that there is so much tension on the gene and the potential therapies that will come from knowing the gene, has actually pushed forward the whole treatment paradigm everywhere. So you get that kind of kickback effect, I suppose.

Temple: I do think that one could make a lot out of so much having been learnt from this disease that is of relevance to a lot of other diseases. So, I don't know Steve, if there's anything else you want to add to that?

Sturdy: Nothing to add of substance, but I'd really like to add a note of sentiment just to thank you all. I didn't get a chance to introduce myself at the start – I do apologize for that – but I'm running the History of Medicine project 'Making Genomic Medicine' out of which came the suggestion to organize this Witness Seminar. We put it to Tilli some while back. I've never actually attended one of these before. I've read and I've used, as an historian, the transcripts, which are absolutely fantastic resources for historians, and this kind of conversation is just wonderful for eliciting fantastic data in an historian's language. But it's even more exciting to be there in the room when the conversation is going on, so it's just been a great privilege to be here and be part of this. So thank you all very much indeed for attending and for all your input.

Tansey: If I could add to that also my thanks for you all coming and sharing your stories, trusting us, telling us all these wonderful things. And you have spoken very movingly, I think, of the ideas of collaboration and sharing things. Please join me in thanking Steve and his team for having the idea, and Karen for her excellent, engaged chairing of the meeting. Thank you very much, Karen.

Biographical notes*

Professor Bert Bakker

PhD (b. 1951) studied chemistry in Delft (BSc), and continued his studies at Leiden University (1975–1976) where he was also a technician (1977–1989). During this period he worked closely with Professor Peter L Pearson and pioneered molecular genetic techniques, which led to the first prenatal diagnosis of Duchenne muscular dystrophy in 1985. In 1989 he completed his doctoral research on Duchenne muscular dystrophy, and the same year he was awarded the Lustrum Prize by the Dutch Human Genetics Society. In 1990 he became Head of the DNA diagnostic section in Leiden University's Clinical Genetic Centre and Associate Professor at the Department of Human Genetics. In conjunction with these roles, he was Head of the Forensic DNA Laboratory at Leiden (1994–2000). In 2000 he was appointed Professor of Molecular Genetic Diagnosis at Leiden University Medical Center, where he continued as Head of the Laboratory for Diagnostic Genome Analysis, until April 2015, when he stepped down as Head of the laboratory and ceased his management duties.[197]

Professor Kate Bushby

MBChB MSc MD FRCP (b. 1962) graduated in Medicine from the University of Dundee in 1986. She moved to Newcastle upon Tyne in 1989 to participate in a newly established MSc in Genetics, where her project brought her under the mentorship of Dr David Gardner-Medwin and began a career-long interest in muscular dystrophy. As an MRC Training Fellow and Clinician Scientist in Newcastle, she developed a research programme into the genetics of rare forms of muscular dystrophy and took over the clinical management of muscular dystrophies for the north of England, as well as initiating and leading the national service for rare inherited muscular dystrophies. Alongside Professors Straub, Lochmuller, and Horvath, Kate led the Newcastle muscle team not only to further basic research and a world-leading role in clinical trials, but also to the coordination

[197] Transcript of an interview with Professor Bakker can be found at: http://dx.doi.org/10.17636/01020340.

* Contributors are asked to supply details; other entries are compiled from conventional biographical sources.

of a series of large-scale projects aimed at enhancing treatment and care opportunities for patients with muscular dystrophies, including the global translational research network TREAT-NMD. In 2015 the muscle team was awarded University Research Centre status as the John Walton Muscular Dystrophy Research Centre. She is coordinator of the first wave European Reference Network for rare neuromuscular diseases (EURO-NMD).

Professor George Dickson

BSc PhD (b. 1953) graduated in biochemistry from the University of Strathclyde in 1974, and obtained his PhD in 1979 from the Middlesex Hospital Medical School, University College London (UCL) in thyroid cell biology and nuclear medicine. He worked previously as a steroid discovery scientist for Organon Plc, and then as a Royal Society Fellow at the University of Marseilles, then returning to the UK as a Lister-Wolfson Fellow at the Institute of Neurology, UCL (studying motorneuron disease). He then moved to the Department of Experimental Pathology, Guy's Hospital, King's College London, for some eight years. In 1995 he took up the University Chair of Molecular Cell Biology at Royal Holloway College, University of London. He has spent most of his career studying neuromuscular disease, Duchenne muscular dystrophy in particular, and related gene and antisense therapies. His work includes the first cloning of a functional recombinant dystrophin gene, the discovery of the role of cell adhesion molecules in muscle stem cell fusion, contributions to the identification of utrophin, early description of exon skipping in muscular dystrophin, and development of AAV-microdystrophin gene therapies. Professor Dickson is a past President of the European Society of Gene & Cell Therapy, a past Secretary and Founder Member of the British Society for Gene Therapy, and the Muscular Dystrophy UK Scientist of the Year 2014.

Professor Victor Dubowitz

BSc MBChB MD PhD FRCP FRCPCH (b. 1931) graduated in medicine in Cape Town (1954), followed by residencies in medicine and surgery at Groote Schuur Hospital. He came to the UK in 1956 for 18 months to get broad clinical experience, exposure to culture, and planned to return to general practice in South Africa. A three-week locum at Queen Mary's Hospital for Children (Carshalton, Surrey) exposed him to two wards with muscular dystrophy patients.

Having come for three weeks he stayed for three years, initially as a Senior House Officer for a year, which he combined with doing muscle biopsies, and then got interested in doing research and contacted Professor Everson (Tony) Pearse at Hammersmith Hospital, a pathologist with a special interest in enzyme histochemistry. He embarked on a study of enzyme histochemistry of normal and dystrophic muscle, completing an MD Thesis in 1960. He realized his heart was really in clinical medicine and paediatrics, and successfully applied for a paediatric lectureship in Sheffield where he spent the next 13 years, becoming Reader in Child Health and Developmental Neurology, setting up a muscle unit and a basic research group and completing a PhD on the histochemistry of developing and diseased muscle. In 1973 he applied for the newly established Chair of Paediatrics and Neonatal Medicine at Hammersmith, and moved a large research group with him, ultimately creating the Jerry Lewis Muscle Research Labs, funded by the American MDA, on a hospital roof. He rapidly established an internationally recognized paediatric centre for Muscle Disease of clinicians and basic scientists, with a primary emphasis on the clinical management of patients and their long-term follow-up. In 1990 he established the multidisciplinary journal *Neuromuscular Disorders* of which he remains Editor-in-Chief. In 1995 he founded the World Muscle Society, which aimed primarily at providing a forum for young researchers to present their work. Elected foundation President, he was re-elected every three years until the present (2017). Professor Dubowitz published his autobiography (*Ramblings of a Peripatetic Paediatrician*) in 2005.[198]

Dr Michael Gait

BSc PhD graduated from the University of Birmingham, UK in 1970 and obtained a PhD in Chemistry at the University of Birmingham in synthesis of analogues of short oligonucleotides in 1973. After a postdoctoral fellowship at Massachusetts Institute of Technology in 1973–1975, working on gene synthesis with Har Gobind Khorana, he took up a postdoctoral position in September 1975 at the MRC Laboratory of Molecular Biology in Cambridge, working with Robert Sheppard. Apart from a brief leave of absence at Collaborative Research in Massachusetts in 1982–1983, he has remained at

[198] Transcripts of interviews with Professor Dubowitz can be found at: http://dx.doi.org/10.17636/01022366 and http://dx.doi.org/10.17636/01022367.

MRC Laboratory of Molecular Biology for the past 40 years, becoming Staff Scientist, then Senior Staff Scientist, and then MRC Programme Leader. He has now become a retired worker at MRC Laboratory of Molecular Biology, with a small research group working closely with Professor Matthew Wood at the University of Oxford. He is a Member of the Royal Society of Chemistry and the Biochemical Society, and is a recipient of a Royal Society of Chemistry award in nucleic acids chemistry, and also elected to EMBO. He has worked initially in chemical synthesis of oligonucleotides, later on oligonucleotide analogues and applications in molecular biology, and more recently on peptide conjugates of oligonucleotides and their analogues. He has worked on Duchenne muscular dystrophy with Professor Wood and other colleagues since 2007 in chemical synthesis of peptide conjugates of phosphorodiamidate morpholino oligonucleotide and electrically neutral peptide nucleic acid analogues. He edited a book with G M Blackburn, *Nucleic Acids in Chemistry and Biology* (three editions) and has some 250 publications.

Professor Shirley Hodgson

BSc BM BC DM D(Obst)RCOG FRCP DCH FRSB (b. 1945) began her career as a Paediatrician and General Practitioner. She became a Registrar in Clinical Genetics at Guy's Hospital, 1980, and worked with Professor Victor Dubowitz at the Hammersmith Hospital on muscular dystrophy while working for her DM Thesis. She became a Consultant in Clinical Genetics at Addenbrooke's Hospital in 1988, and Consultant/Reader in Clinical Genetics at Guy's in 1990. She specialized in cancer genetics from 1989, working with the Imperial Cancer Research Fund (now Cancer Research UK), developing regional cancer genetics services at Guy's, St Mark's, and St George's Hospitals in London. In 2003 she was appointed Professor of Cancer Genetics at St George's, University of London, now Emerita, and has part-time Consultant status in Leicester. Her research investigated inherited aspects of cancer predisposition. She has published widely on the subject, and co-authored several books, including *Inherited Susceptibility to Cancer* (Foulkes and Hodgson (eds), 1998), and *A Practical Guide to Human Cancer Genetics* (Hodgson

and Maher, 1993), now into its fourth edition, with W Foulkes and C Eng as co-authors (Springer).[199]

Professor Eric Hoffman

PhD (b. 1958) graduated in music and biology from Gettyburg College in 1982, and obtained his PhD in *Drosophila* molecular genetics in 1986. He worked as a Postdoctoral Fellow and Instructor at Boston Children's Hospital and Harvard Medical School as a Muscular Dystrophy Association Fellow (1986–1990), working on the identification of the Duchenne muscular dystrophy gene and dystrophin protein. He was faculty at University of Pittsburgh School of Medicine (1990–1998), then George Washington University School of Medicine (1998–2016). He directed the Center for Genetic Medicine Research at the Children's National Medical Center in Washington, DC (1998–2016). He is currently Associate Dean for Research, School of Pharmacy, Binghamton University – State University of New York, co-founder and CEO of ReveraGen BioPharma, co-founder and Vice President of AGADA BioSciences, and co-founder of TRiNDS LLC.

Professor Jennifer Morgan

BSc PhD graduated from King's College London with a BSc in zoology and did her PhD in pathology at Charing Cross and Westminster Medical School, under the supervision of Professor Terry Partridge. After postdoctoral appointments at Charing Cross and Westminster Medical School and at the MRC Clinical Sciences Centre, Imperial College London, she joined the Dubowitz Neuromuscular Group, headed by Professor Francesco Muntoni, at Imperial College, London, as a Senior Lecturer before moving to the UCL Institute of Child Health as a Reader in 2008 and Chair in 2013. She is a Member of the muscular dystrophy exon skipping (MDEX) consortium and is a PI of the MRC Centre for Neuromuscular Diseases, whose aim is to perform multidisciplinary translational research in neuromuscular diseases in order to reduce the gap between major science discoveries and patient benefit. Her major areas of research are the identification of stem cells that contribute to skeletal muscle regeneration, and the genetic and functional manipulation of these cell populations to enhance

[199] Transcripts of interviews with Professor Hodgson can be found at: http://dx.doi.org/10.17636/01012730 and http://dx.doi.org/10.17636/01012731.

muscle repair in skeletal muscle regenerative medicine and in the treatment of muscular dystrophies.

Professor Francesco Muntoni

MD FMedSci (b. 1959) graduated in Medicine from the University of Cagliari (Italy) in 1984, and obtained his Child Neurology and Psychiatry specialization at Sassari University in 1989. His MD was on neurochemical and electrophysiological aspects of ethanol addiction in rats. He originally worked as a child neurologist in Cagliari until 1993, and then moved to the Hammersmith Hospital, Royal Postgraduate Medical School, London. He became the Clinical and Research Director of the Neuromuscular Centre at the Hammersmith Hospital in 1996, following the retirement of Professor Victor Dubowitz. His research, at the time, focused on the deep phenotyping of dystrophinopathies and on the genetic basis of congenital muscular dystrophy. In 2008 he moved with the entire clinical, pathology, and research team from the Hammersmith Hospital to the UCL Institute of Child Health and Great Ormond Street Hospital for Children, in London, to pursue translational research in neuromuscular conditions. He is the Head of the Developmental Neuroscience Programme in the Institute and Novel Therapies Theme Lead of the Great Ormond Street Hospital Biomedical Research Centre. He continues to be involved in deep phenotyping of neuromuscular diseases (with the identification of more than 30 new disease genes) and the identification of novel therapeutic strategies for Duchenne muscular dystrophy and spinal muscular atrophy, from preclinical development to clinical trials.

Professor Terence Partridge

BSc PhD FMedSci (b. 1940) graduated in zoology from University of London 1962 and PhD in 1970. From 1965 to 1966, he worked in the Muséum Nationale d'Histoire Naturelle in Paris on the isolation of rodent malarias from Central African tree-rats. In 1967, he joined the newly formed Cell Biology Department in the University of Glasgow as an Assistant Lecturer. In 1970 he joined the Department of Experimental Pathology, Charing Cross Hospital Medical School, as a Research Fellow, supported by the Muscular Dystrophy Group of Great Britain. Subsequently, he become a Lecturer (1975–1978), Senior Lecturer (1978–1989), Reader (1989–1992), and Professor (1993–1994) in Experimental Pathology at the Charing Cross and

Westminster Medical School. In 1994 he was appointed Professor of Experimental Pathology at the Royal Postgraduate Medical School and Head of Muscle Cell Biology at the newly formed MRC Clinical Sciences Centre, where he remained until retirement in 2005. In his final year, he took sabbatical leave to the Genethon and the Pasteur Institutes in Paris, on an Award of 'Chaire International de Rechearche Blaise Pascal'. On retirement from the MRC in 2005, he took up a post in the Center for Genetic Medicine at the Children's National Medical Center in Washington DC, where he remains at present, continuing his research on the pathology of muscular dystrophy and on the potential of exon skipping as a therapeutic avenue.

Dr Rosaline Quinlivan

BSc(Hons) MBBS DCH FRCPCH FRCP MD (b. 1959) graduated in psychology in 1977 and medicine in 1980 from University College London, and obtained her MD from the University of London in 2010 on work she did to investigate the cardiomyopathy of Duchenne and Becker muscular dystrophy in the 1990s. Her neuromuscular training was at Guy's Hospital in London. Her first Consultant post in 1995 was in the West Midlands as a Paediatrician with an interest in neuromuscular disease. From 2002, she became a full-time Neuromuscular Consultant, working at Birmingham Children's Hospital and the Robert Jones and Agnes Hunt Hospital, where she was Director of the Wolfson Centre for Neuromuscular Disease. She currently works at the MRC Centre for Neuromuscular Disease at the National Hospital for Neurology and Neurosurgery, Queen Square, and the Dubowitz Neuromuscular Unit at Great Ormond Street Hospital, where she leads for transition and in the management of young adults with neuromuscular disease.

Professor Steve Sturdy

PhD (b. 1957) graduated in natural sciences from the University of Cambridge in 1979. He took an MA in philosophy of science from the University of Western Ontario, then a PhD in science studies from the University of Edinburgh in 1987, writing his thesis on the life and work of the physiologist J S Haldane. He then spent seven years as a Wellcome Research Fellow in the Centre for the History of Science, Technology and Medicine at the University of Manchester. In 1994 he secured a Wellcome Trust University Award in the History of Medicine at the University of Edinburgh, where he has remained ever since,

serving from 2006 to 2012 as Deputy Director of the Economic and Social Research Council Genomics Policy and Research Forum, and from 2012 to 2015 as Head of Science, Technology and Innovation Studies. In 2013 he was promoted to a personal Chair in the Sociology of Medical Knowledge. He currently holds a Wellcome Trust Senior Investigator Award in Medical Humanities, with a project entitled 'Making Genomic Medicine'.

Professor Tilli Tansey

OBE PhD PhD DSc HonMD HonFRCP FMedSci (b. 1953) graduated in zoology from the University of Sheffield in 1974, and obtained her PhD in *Octopus* neurochemistry in 1978. She worked as a neuroscientist in the Stazione Zoologica Naples, the Marine Laboratory in Plymouth, the MRC Brain Metabolism Unit, Edinburgh, and was a Multiple Sclerosis Society Research Fellow at St Thomas' Hospital, London (1983–1986). After a short sabbatical break at the Wellcome Institute for the History of Medicine (WIHM), she took a second PhD in medical history on the career of Sir Henry Dale, and became a member of the academic staff of the WIHM, later the Wellcome Trust Centre for the History of Medicine at UCL. She became Professor of the History of Modern Medical Sciences at UCL in 2007 and moved to Queen Mary University of London (QMUL), with the same title, in 2010. With the late Sir Christopher Booth she created the History of Twentieth Century Medicine Group in the early 1990s, now the History of Modern Biomedicine Research Group at QMUL.

Professor Karen Temple

MBChB MD FRCP graduated with Honours from the University of Birmingham Medical School in 1981. She trained first in paediatrics in Birmingham and London, and after a short period at the MRC in the Gambia. She became a Lecturer in Clinical Genetics at the Institute of Child Health, London, and later Senior Registrar at Great Ormond Street Hospital. In 1990, she moved to Southampton as Consultant in Clinical Genetics, where she and her colleagues, Dr Nick Dennis and Professor Pat Jacobs, developed Medical Genetics on the South Coast and established the Wessex Regional Genetics Service. She continues to work as a clinician. Her research into new genetic mechanisms of human developmental identified novel imprinting disorders changing the diagnosis and medical care of patients. She is currently Professor

of Medical Genetics at the University of Southampton (since 2006), and leads the academic unit of Human Development and Health, one of four research units in the Faculty of Medicine. She co-leads the Wessex Genome Medicine Centre with Professor Tony Williams and the Southampton Biomedical Research Centre bioinformatics group.

Professor Gert-Jan van Ommen

PhD (b. 1947) graduated in biochemistry at the University of Amsterdam, worked at the University of Amsterdam Children's Clinic, and then moved to the Department of Human Genetics of Leiden University Medical Center, which he headed from 1991 to 2012. He established the Leiden Genome Technology Center, and the CMSB. He is Editor-in-Chief of the *European Journal of Human Genetics*, past President of HUGO (1998–2000) and of the European and Dutch Societies of Human Genetics. He is National Coordinator of Orphanet, Founding Member of the European Biobanking and Biomolecular Research Infrastructure (BBMRI) and of BBMRI-NL. His department has contributed to the finding of the gene defects and disease mechanisms underlying Duchenne muscular dystrophy, Huntington disease, polycystic kidney disease, hereditary neuropathies, fragile X syndrome, Rubinstein–Taybi syndrome, familial hemiplegic migraine, and facioscapulohumeral muscular dystrophy. His group performed the first prenatal diagnosis using DNA markers of a disease (Duchenne muscular dystrophy), of which the gene was (then) still unknown, developed many gene mapping and mutation detection techniques, the first megabase map of a human gene, and pioneered the exon-skipping approach for therapy of Duchenne muscular dystrophy. Further exon skip developments are under way for Huntington disease, cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy, and limb-girdle muscular dystrophy (dysferlin).

Professor Jan Witkowski

PhD (b.1947) graduated in zoology from the University of Southampton in 1968 and obtained his PhD in biochemistry at the National Institute for Medical Research in 1974. That year Witkowski joined Victor Dubowitz at Hammersmith Hospital to carry out research using tissue culture to study the behaviour and biochemistry of human muscle cells. He spent 1976 with Andrew Engel at the Mayo Clinic, Minnesota, and

in 1984 was awarded an MRC fellowship in recombinant DNA. Witkowski took up this fellowship with Gordon Peters at the Imperial Cancer Research Fund, working on oncogenes. In 1986, Tom Caskey invited him to run the DNA diagnostics laboratory at the Institute for Molecular Genetics, Baylor College of Medicine, Houston. He became Director of the Banbury Center at CSHL in 1987, and is responsible for the topics and organization of some 20 meetings each year. He has published several books, including *Davenport's Dream: 21st Reflections on Heredity and Eugenics*, *Recombinant DNA* with Jim Watson, and *The Annotated and Illustrated Double Helix*, a new edition of Watson's classic memoir. His latest book is *The Road to Discovery: A Short History of Cold Spring Harbor Laboratory*. Witkowski is Editor-in-Chief of *Trends in Biochemical Sciences*.

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