Julian Sampson

Personal Details	
Name	Julian Sampson
Dates	Born 1959
Place of Birth	Guisborough, Yorkshire, UK
Main work places	Cardiff
Principal field of work	Tuberous sclerosis

Short biography

Julian Sampson qualified in Medicine at University of Nottingham, UK, and trained in Medical genetics in Glasgow under Malcolm Ferguson-Smith. He moved to Cardiff in 1989 and since 2000 has been head of the Wales Institute of Medical Genetics. His principal contributions include isolation of the genes for tuberous sclerosis and the discovery of a recessively inherited form of colorectal cancer with polyposis.

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INTERVIEW WITH JULIAN SAMPSON, 12/04/2013

PH = Peter Harper

JS = Julian Sampson

PH **It's Friday 12th April 2013 and I'm talking with Professor Julian Sampson at the Institute of Medical Genetics, Cardiff. Julian, may I go back and start at the beginning and ask when were you born and where?**

JS In 1959 in Guisborough, which was then North Yorkshire; it's now Teesside. And just outside Middlesbrough in the industrial north east.

PH **Did you come from a family that was either medical or scientific or anything like that?**

JS Well, my dad's a scientist and so the reason we lived in Teesside was he was a chemist, an industrial chemist and worked at ICI, which was a major employer then. In fact everyone who lived on the estate in Nunthorpe, or virtually everyone, their fathers worked in ICI; it was the huge employer at that time in that area. So he was a scientist but he was the first one really. His family were from East Anglia originally and certainly he was the first person for instance who had gone to university. I think he was probably the first person to have gone terribly far through school, past his early teens. His father had been a printer who assembled letters, you know, brass letters on big plates for printing. My mother was from a family originally from the Cornwall area and then I think to the south east of England. I think were from more business type backgrounds. So it wasn't a huge tradition, certainly nothing medical.

PH **What was it that put you in the direction of a medical/scientific career?**

JS It certainly wasn't one of these things where I was one of those born and bred people who wanted to be a doctor or a medical scientist. In fact I think when I was young I was very interested in things like architecture. I remember I'd imagined I would perhaps do something like that. But I think it was probably my late teens, when you're sort of deciding what you're actually going to do, are you going to university and what you might do. When I was a child the sorts of things that were going on were the early organ transplants and so on. And I think I got quite interested in these things that were kind of at the edge of medicine and science and so can remember looking with interest at Dr Christian Barnard, who did the first heart transplants, and thinking he's pretty amazing. And then through that getting vaguely interested in immunity and then reading about and realising that immunisation had had such a massive impact on health. So when I decided to apply to medical school I actually imagined naively that perhaps I'd become some sort of transplant surgeon and that perhaps the immune system was going to be my focus of attention. Actually, when I went to medical school, I did an intercalated year in immunology and biochemistry and I had a general interest in the scientific and experimental end of medicine really, but it came fairly late. I could easily have wandered off and become an architect or something else.

PH **Which medical school did you go to?**

JS This was Nottingham, which was a new medical school then; it hadn't been open very long. I guess I didn't know much about medicine, certainly I didn't get any career advice and my family didn't know anything about it, but I remember looking through the different sorts of medical courses and this was one that had an intercalated year, everyone did it, so that sounded like an opportunity to get a bit of research experience. I was probably quite lucky to get into medical school because I remember going to the interview, I can still

remember it actually, and they said, "So what do you want to do?" And I can remember at that point saying, "I'm interested in medical research." They sounded surprised "What? Sitting round with test tubes and things?" But I must have given some vaguely sensible answer about research with patients and got a place. So I think I was actually quite interested in the idea of research. I never thought I was going to be a family doctor or something more traditionally medical, although I didn't really know anything about the existence of academic medicine in any distinct or formal way, but I think I was always interested in the idea that I might be able to do something quite interesting, something that would actually make a difference by working at that end of the medical spectrum.

PH Once you'd qualified did you carry on and do more jobs in Nottingham or did you move elsewhere?

JS I moved around quite a bit. The first job I did, because I was quite interested in things to do with cancer even at that stage, was in the breast cancer unit in Nottingham. That was with the professorial surgical unit. I'm sure they wouldn't do things like this on a surgical unit now, but actually they did a lot of chemotherapy trials. So I started with that but actually by then I'd certainly got well over the idea of being a surgeon or transplant surgeon or anything like that. After that job I really moved around a lot, without any very clear idea of what I was going to do, which I think was quite common then. I went to work in York and then in Exeter and then in Bristol and then in London, and all that was prior to taking a step into genetics by eventually moving to Glasgow to the Duncan Guthrie Institute to do the MSc in Medical Genetics that Malcolm Ferguson-Smith had established.

And that was really I think because I'd been doing medical paediatric jobs and got very interested but also realised that to be a consultant in an acute speciality was going to make it pretty difficult to get the opportunity to look at the causes of disease in the way that perhaps I was most interested in. And it seemed to me that genetics was probably going to be the next way, the next tool or approach to looking at causes of disease. But before Glasgow there wasn't a huge amount of encouragement, I have to say, for me to head off in that direction. There was a very good professor of paediatrics but his view was that there wasn't any hope of genetics becoming a proper speciality and he was worried that if genetics got going in a way that lead to a lot of prenatal diagnosis, that would be very detrimental to approaches for treatment of genetic disease. So my interest in genetics was quite actively discouraged. But I did feel that genetics was beginning to emerge as a really important area and I went to Glasgow in 1986. It was just becoming apparent that reverse genetics might give a way to get at the genes, for instance, associated with childhood cancers like Wilms tumour and retinoblastoma. So I made that step and went off to Glasgow, initially supposedly for a year but I ended up staying for several years.

PH Tell me a bit about the Glasgow MSc course because I've not interviewed anybody else who's mentioned that and yet it, you know, was a pretty important step.

JS I think it was. There were doctors, junior doctors from a number of fields who were on the course and there were quite a few people from science backgrounds as well so it was a kind of mixture of the two. And so I think some people were there because they wanted to become clinical geneticists, although there were very few openings really at that stage. And some people were there perhaps more with a view to creating laboratory diagnostics and the course accommodated both of those aspirations. And there were a number of people involved, obviously Malcolm Ferguson-Smith had been very keen to establish this opportunity for people to get into the area of medical genetics; it was an MSc in medical genetics as opposed to molecular genetics. And there were other people who played very important roles in the course: John Yates did a lot of the clinical and population genetics, and Nabil Affara, who was a non-clinical senior lecturer in Glasgow, supervised the laboratory aspects of the course. And it was very good, especially after I'd only done 4 or 5 years of junior medical jobs, it gave me the opportunity to focus on an area rather exclusively. I think, like me, most medical people paid the bills and fed themselves by doing odd nights on call and locums, but essentially it was an opportunity to really stand back from the acute medical side and to just take a year out to explore an area in a bit more depth.

So it was a very valuable thing and a lot of the people who were on that course, from the year that I was on and other years as well, are now in the field, you know, involved in running services or research groups, abroad and in the UK, so I think it did make a difference. Without that course I think a lot of people wouldn't have had a route into medical genetics. I don't think I would have made the change really, without that opportunity.

PH The MSc course was a year so how did it come about that you had the chance of staying longer in Glasgow?

JS Well, for part of the MSc you did a project of some type and I did a laboratory project. They listed a few areas they thought might be suitable and I picked one. I picked tuberous sclerosis (TS), which I've never quite managed to get over. At that stage there was a developing interest in tuberous sclerosis within the Duncan Guthrie. There was a bigger interest that Malcolm had of course in sex determination, so there was a lot of Y chromosome mapping work going on. They were also interested in X linked diseases so there was an interest in Duchenne muscular dystrophy. But I think because I'd come from a bit of an oncological and paediatric background TS seemed to sort of at least bridge tumours and neurogenetics; both areas. And it was a disorder that just about that time was mapped by protein polymorphism linkage analysis (rather miraculously) by Alan Fryer and Sue Povey working with families that John Osborne had identified.

PH Was that the ABO blood group?

JS That was the ABO blood group linkage, which I think was published in '87; somewhere around there. So that was somewhere towards the end of my MSc year in Glasgow. Another doctor in Glasgow had started to ascertain patients with tuberous sclerosis in Scotland but then I think the project had gone off the boil and gone a bit fallow and so I sort of just took the opportunity really to identify further patients and families with tuberous sclerosis for genetic investigation. And when the ABO linkage emerged I studied the families and generated new probes for the ABO linkage group. Malcolm had some great facilities, flow sorting of translocated chromosomes and so on. I remember we sorted the Philadelphia chromosome, it's very important, of course its translocation is very close to the ABO blood group. So I made some libraries and did some linkage. And the one thing that did emerge from that work apart from ascertaining a very large number of patients was that we clearly had families that weren't linked to chromosome 9. We weren't in a position to establish where they were linked to, they weren't big enough, but they clearly were not linked to chromosome 9. So the fact that there was locus heterogeneity came out of that. And I ended up staying in Glasgow really because the project had gained a bit of momentum. They were very nice to me. Malcolm did some things behind the scenes and some sort of junior clinical research fellowship emerged and this meant I could stay on and pursue the work in Glasgow as the basis an MD thesis. So that's what I did. I stayed for a couple of years and continued with that work.

PH Up in Glasgow then, were you able to get quite a bit of molecular experience in terms of using DNA probes and Southern blotting and that kind of thing?

JS Yes, absolutely. So it was very good from that point of view. Essentially it was really a lab-based project. There was some running around finding patients with tuberous sclerosis but essentially it was a project based around what would now be fairly simple stuff, generating libraries, isolating new probes and undertaking linkage analysis. It was really a linkage analysis based project but a lot of working with vectors and a lot of Southern blotting. Just towards the end of that time VNTRs emerged as well so that immediately made families much more informative than they'd previously been. So it was a stage of transition. The work that Alan Fryer had done was just protein polymorphism based and this was a transition to things being much more informative. And also new physical mapping technologies emerged during that time as well so I learnt to do pulse field electrophoresis for example which was quite new at that point, I think. Again, I think I was quite convinced that having these technologies would enable you to bridge that gap between linkage and gene level analysis. The technologies were going to be very useful. And so indeed when I came to Cardiff, you might remember, one of the first things I did, and you helped me greatly, was to get a PhD student and I went back up to Scotland to revisit all the patients with tuberous sclerosis. In those days of course a lot of these people were long-term residents in institutions so it was actually, from a researcher's point of view, incredibly efficient, although completely outwith what would be feasible now even if patients were still in institutions, in terms of access to patients and medical ethics. But you know we were able to basically go round over 100 patients and prepare high molecular weight DNA blocks. So I remember going up to Scotland with Phil Brook-Carter the student. I'd be out sampling the patients, coming back in the evening and he'd then be making pulse field blocks and then we just brought it all back to Cardiff and that really provided what turned out to be a very valuable resource for the work here.

PH Did that Glasgow work turn into any kind of thesis in the end?

JS That was my MD thesis, yes, which I think I was just finishing writing up when I came to Cardiff.

PH I'm interested that for you, and it was just the same for me, what started as a mapping project turned into a much broader clinical project. And probably for much the same reason that it was quite a tricky condition to be sure, not so much if somebody had got it but if they didn't have it. So that the unaffecteds were quite a challenge. So did the thesis end up being an all round study of tuberous sclerosis?

JS Yes it did really; it was a combination of clinical, epidemiological and molecular work and you're quite right about the critical clinical assessment of patients. That was really very important. And I'm pleased to say the critical cases we studied, you know, we got right and they were very helpful. In retrospect we did make the right decisions about clinical status. And of course, unlike conditions like Huntington's, penetrance is very full at quite an early age but to be sure you do need quite a lot of imaging studies as well as clinical assessments. So sorting out status issues was important.

PH Had germline mosaicism surfaced in TS and was it recognised as being more than something very exceptional by that point?

JS Yes. There was a little bit, I remember there was a bit in my thesis about germline mosaicism and in some of the publications that were produced from that work. We recognised there were several instances of clear germline mosaicism within that cohort of patients that we worked on in Glasgow from the west of Scotland. Alec Jeffries' fingerprinting had emerged and I remember doing some petty poor fingerprinting to show that a pair of twins were non identical but both had TS, and that was one of the germline mosaic families. So yes it was recognised, but within the context of the linkage studies it wasn't going to be a major issue.

PH Had a tuberous sclerosis research community evolved by that time or was it just one or two people dotted around in isolation?

JS There was a very good and fairly new Tuberous Sclerosis Association, which I certainly want to say a bit more about because it was a fantastic boost to research. But what there was, from the genetics side, of course was the focus on different chromosomes through the human gene mapping efforts. I guess because perhaps of the ABO linkage group on chromosome 9q and the fact that there were a number of markers there and disease genes like TS was and torsion dystonia, there was quite an interest in this chromosomal region. Within the individual chromosome groups there were subgroups that were very interested in particular little bits of chromosomes as I'm sure you will remember. And because TS was clearly an interesting disorder at 9q34 there was an overlap between those interests purely in mapping and those interested in the diseases and that was certainly true of TS. So, as you know Sue Povey was highly involved; she'd been involved in the original linkage mapping of TS and her input really kept TS quite high up the agenda. So we'd go along to these gene mapping workshops and meeting there. I remember going off to the chromosome 9 specific meetings which tended one year to be over in the US and perhaps the next year they'd drift over to Europe. And they always gave a real opportunity for all the people working on molecular genetics and TS who were trying to identify the gene. We'd all be there as a funny little subgroup within the bigger group. That's how I remember it. And there was quite a lot of excitement. I think perhaps even the people who weren't initially so interested in the disease kind of got interested because there was quite a focus on it.

PH One thing which I find it quite hard now to remember is when you came to Cardiff; I think I'm right that this was a general training post initially.

JS It was, yes.

PH Not research funded specifically.

JS No, no, it wasn't. Well of course it was a senior registrar post in those days. Because I'd been in Glasgow and spent a few years there and done my MD, that bit of the requirement for medical training was done; what was needed was the rest. But I do remember things were very much more flexible in those days so you encouraged me to write some grants when I arrived, which I did and, remarkably, they were successful. And so what happened very quickly was that I got a little group going with a couple of PhD students, Phil Carter and Mark Nellist. And what was very nice at that time of course was that the bigger teams in Cardiff were following parallel positional cloning approaches on Huntington's and myotonic dystrophy, so it was very easy to slip another group into that environment and that context. And it was great. Clearly there hadn't been a prime interest in tuberous sclerosis but there was no problem in bringing it in, which was fantastic because obviously it could have been seen as a bit of defocusing or, people might have asked - why doesn't he come and work on Huntington's or something proper? But there was none of that. I do remember all the samples that I had assembled from the TS patients. I'm sure you can't do this nowadays, but I remember going to

Glasgow and carefully packaging them all up into a big polystyrene box with a load of ice and driving them down. And so for the TS project, I literally took it with me and brought it to Cardiff. At that stage Malcolm had recently gone to Cambridge and Mike Connor had taken over and it was very much with Mike's blessing; it wasn't a kind of illicit movement of the project away from Glasgow.

PH Because one of the things which again it's difficult to recognise now is the fact that the mapping involved very much a common technology across diseases which meant that it was possible to accommodate a completely different disease.

JS Yes.

PH But I'm always struck by how suddenly that changed. Once you knew what the basic defect was then you really had to get back to the drawing board and everything went in different directions.

JS Yes, in a very unpredictable way. And that was I guess, a very particular period in positional cloning where you really had no earthly idea where it was going to land you in terms of the biology. You just had no idea what you were going to find. And I suppose for you it was a huge surprise of course when both diseases you worked on were triplet repeat disorders. It became slowly apparent that one might be able to guess what was going on, but it was pretty last minute, wasn't it, when it was really clear that they were so closely related in that way.

PH Yes.

JS And we had no idea where the TS positional cloning was going to land us.

PH Just going back a tiny bit, at what point did the two loci really become clear? I mean you mentioned that it was one on chromosome 9 and another somewhere else, but when did it become clear that there was a locus on chromosome 16?

JS Very, very late in the day because the problem with most TS families, because it's quite a serious disease, is they tend to be small and so certainly in an era of limited informativity of markers, it was actually difficult to map small families. It would be easier to exclude linkage to a known locus than to find a positive second linkage. And so what actually happened was initially there were two false leads, one emerged from California with suggestion of linkage on chromosome 11 and another from Germany where there was a translocation involving chromosome 12 with some dubious linkage support. But these were not terribly good quality data actually and one of the things that I did when I came to Cardiff was to decide, okay, we've got to sort out whether these things are real or not. And I got a lot of help with that. By then there really was a bit of an international TS community and we put together a meta-analysis of all the data to be generated for the 3 chromosomes: 9, 11 and 12. You know it was something like a thousand families so it was really quite a large amount of data and the Americans and Europeans all put in their primary data; it was fantastic.

And at that point, I expect you remember Lodewijk Sandkuyl had started working with us and so it was great really; that was fantastic. He was undertaking a kind of peripatetic existence but he'd fly over to Cardiff and we assimilated all this data, this international data, and analysed it rigorously. And this must have been 1990 or something like that. And basically this showed that absolutely there was a chromosome 9 locus. And in fact because we had so much data that by using heterogeneity analysis, taking potential types of heterogeneity and multipoint analysis together, we pretty well pinpointed where we thought the chromosome 9 gene would be. And in retrospect we got it absolutely right. But we showed really that there wasn't a gene on chromosome 11 or chromosome 12. And just at the time when we were completing that analysis an American neurologist, Ray Kandt, who had access to a couple of very large families with TS had gone to do a genome-wide VNTR type analysis to try and identify where the locus might be that could account for those families. And that came out at about the same time showing that there was a chromosome 16 locus. But interestingly also at that same time, and just before Ray's paper appeared, we had had a visit from Isabel Cordeiro who told me about a Portuguese family with a chromosome translocation involving chromosome 16.

And I think I'm right that the order was that, at the time she first told us about the family we didn't know that the chromosome 16 linkage had been identified but we heard about that pretty soon after; it was around the same time. So I've bought some bits of paper from that time with me so that I can get the dates and the sequence of events exactly right on this. But all these things, as is often the case, they all kind of happened together. We realised the proposed chromosome 11 and 12 loci were wrong; we heard that there was a chromosome 16 linkage, which subsequently appeared in press, and the chromosome translocation family all

emerged pretty much together. And so that really changed things quite dramatically. I should probably have a quick look and I just wonder which of these bits is the one. Yes so this is 1992, so I think that Ray's paper probably appeared in 1992 and I've got some letters here from July 1992, and I think these are letters that were to my colleagues that we were collaborating with. So basically in Cardiff, our group, which had grown a bit by then, was collaborating with geneticists in Rotterdam, Bart Janssen and Dickey Halley, and we were working together really mainly on the chromosome 9 locus.

And so the letter that I've got here from July '92 is saying that this translocation has come to our notice and that this really might be incredibly useful, and what I was really suggesting was that we ought to prioritise this. We'd been working on chromosome 9 at this stage for 5 years, but I suggested that perhaps now we ought to put our main efforts into exploiting this new resource. So before we go into that I think it would be very useful if I could mention in a bit more detail how this translocation family resource really came to our attention. It had in fact predated Isabel Cordeiro's visit to Cardiff that was primarily in relation to neurofibromatosis research. Heloisa Santos, who headed the Department of Clinical Genetics in Lisbon had some years before, I think perhaps 4 years earlier, seen a patient who she felt had dysmorphism and severe developmental delay. And she had done chromosome analysis on the patient and identified a translocation, an apparently unbalanced translocation involving chromosomes 16 and 22. And Heloisa had investigated him more fully and had found signs of tuberous sclerosis. He had some renal cysts and his brain CT scan, which I can remember seeing (it was pretty early generation, not the sort of scan that you'd have nowadays) had suggested some calcified sub-ependymal nodules.

So Heloisa had concluded that this patient had a chromosome rearrangement and tuberous sclerosis and she know this could be really important. She had actually tried to interest others in it. She tells me that she had first of all talked to colleagues in Portugal but they didn't feel that they had the resources to investigate this in much more detail. And she then evidently talked to Marcus Pembrey but of course at that time Marcus wasn't working on tuberous sclerosis and at that time it also appeared that there was a gene on chromosome 9 and a gene on chromosome 11 and a gene on chromosome 12. And I think Marcus had said that this is yet another tenuous bit of evidence that there may be something on another chromosome, so he hadn't taken it up. So Heloisa had asked Isabel to talk to me when she visited, because she'd been aware that we were working on tuberous sclerosis. And so that's what Isabel had done. She'd told me about Heloisa's patient. She couldn't remember any great detail but I said, "Okay this is very, very interesting and that really we ought to investigate that family" and eventually arrangements were made and I went off to Lisbon to visit the family and take samples and so on with great help from Heloisa and from Isabel.

And of course the family not only had tuberous sclerosis but also polycystic kidney disease and the two balanced translocation carriers had PKD while the boy with the unbalanced rearrangement had tuberous sclerosis as well as kidney cysts. So it was really clear that this was an absolutely critical family and I was incredibly lucky to happen to be in the right place to talk to Isabel and benefit from Heloisa's astute clinical and cytogenetic observations. So it shows that you should always talk to your visitors, spend time talking with them; I'm very grateful that I sat down with Isabel and we had a chat about this.

PH **It's amazing how, and there are several examples like this, but the importance of a single, critical patient that's well documented both clinically and chromosomally can completely change the picture.**

JS Absolutely. And you know Heloisa in those days did her own chromosome analysis, so she did both the critical clinical and laboratory investigations. So, yes this was what prompted my communication with Bart over in Rotterdam and it raised the question of okay, how do we make the most of this resource. Because we were aware now that Ray Kandt and the American groups had a chromosome 16 locus that they would pursue and they had noticed that this was in the PKD linkage region, and they were aware that a patient with TS could have polycystic kidneys sometimes. They wondered if they were allelic disorders, or something unusual. So we recognised that there was going to be a bit of an issue here over competition/collaboration with the PKD1 research community. You know that was a pretty fiercely competitive community as well with Greg Germino, Steve Reeders and others who had done a huge amount of work on mapping PKD1. Steve Reeders, initially in the UK in Oxford and then in the US had actually drifted into the commercial arena by then and had a company that was pursuing PKD1 because of the perceived potential commercial value.

I think the feeling was that because PKD was such a common disease and such a common cause of renal failure that it was a good target commercial enterprise. I wasn't convinced at all that that would be the case but it was the nature of the competition. So we did have to decide what we were going to do about this finding that you know, was a gift really that Heloisa and Isabel had presented us with. And so I remember

discussing this with quite a few people, yourself for one, and Lodewijk because he had insight into the situation in the Netherlands, and we had years of collaboration on chromosome 9 with the Dutch. And in the end the feeling was, you know, we had a very good PKD group in the UK at the Institute of Molecular Medicine in Oxford with Peter Harris and Doug Higgs who was involved because of the thalassemia genes at 16p. It seemed really sensible to approach them. The Dutch had a group working on PKD in Leiden with Martijn Breuning, and the feeling was that the Rotterdam link ought to be consolidated with Leiden because of the joint interest in the chromosomal area and we ought to approach Oxford. So what we did was we contacted everyone - all three groups. I can remember meeting up with Doug Higgs and Peter Harris; Doug nearly fell off his chair with the prospect. He immediately recognised that the family from Portugal was going to be absolutely critical.

And shortly afterwards everyone came to Cardiff. We had a brief meeting in Cardiff. Andrew Wilkie was working in Cardiff at that time as a trainee but had worked with Doug on the thalassaemia mental retardation syndrome in Oxford and I remember he chaired the meeting as someone with a foot in both UK camps. So the stage was kind of set that those 4 groups would collaborate on this. And actually, I don't' know whether it was a good thing or not, but I did it anyway, I really formalised that collaboration. Again I've brought along, there's some bits of paper but I wrote out what was virtually a contract and got the leaders of the 4 groups to sign up to a commitment to see this project through and that we wouldn't involve other groups unless everyone agreed. I was a little bit paranoid but I didn't really know the Oxford group and I didn't really know the Leiden group at all. I was worried that in sharing the biological resource and the information with everyone it would be very important that we all felt we were part of a single team with a unified purpose. And you know we really didn't want someone going off unilaterally to some other group.

PH **It was very important to get this agreed before results started to come out rather than afterwards.**

JS Yes, so it was right at the beginning. I've never done anything like it ever again since but the situation was unusual in that it was focused around one very specific resource and one very specific aim. Well, in a way there were 2 aims; there were 2 groups who were primarily interested in TS and another 2 groups primarily interested in PKD. But there was still the formal possibility that they might be allelic disorders. We didn't really think so because the boy with the unbalanced translocation was the only one with TS so the feeling was that probably he'd lost the TS gene and the hypothesis was that the balanced translocation was through the PKD1 gene or very close to it and it was disrupting its activity in some way. So actually in that collaboration, we worked very solidly for about a year and a half. I can remember I think the only day that I took off and probably the other people took off was Christmas day. But every other day, I think 7 days a week for about a year and a half we went at it. And they were quite long days as well.

But the collaboration did hang together and towards the end of the period I think other groups in the US became aware that we were onto something as it's impossible to keep something completely secret. And I remember one of the things that happened was David Cox flew in, in his commercial guise for a company whose name I can't remember but I think I've got a letter here somewhere. He arrived with his corporate lawyer from the US to have a meeting with us all. And I do remember this quite distinctly, because we felt at this point we'd worked probably for about a year or so already and we'd really cloned out the region, mapped it very accurately, had genes that we were then examining as candidates. And I think David's proposal was that basically his company would join the collaboration in return for some significant financial remuneration which would keep our TS and PKD research going for evermore, so he said. We sent him away again. Now whether that was a sensible thing or not, who knows? But we said no and obviously we didn't need the help right then as we did succeed with the existing collaborators, but of course maybe his help would have been very useful after the gene had been cloned. But I think we were so blinded by the whole thing we couldn't really see past the point at which the genes would be identified. We weren't thinking much past that. At least I wasn't.

And it worked out and of course the way it worked out was complex because the TS gene was identified first, even though the translocation was through the PKD1 gene. This was because the PKD1 gene turned out to be largely present in multiple copies further down the same chromosome and this complicated the analysis a lot. So we got TS first and that caused some difficulties because we couldn't hang around. So we had to report it but we had to report it in a way that didn't give away the location of the PKD1 gene. So it was an interesting paper to write [laughs] but that went in separately and then you know obviously we didn't give too much away because then the PKD1 gene emerged some months later. And so I think it was a very successful if rather exhausting period. And it came to, I think to a pretty good conclusion and we've all

remained good friends and colleagues since that time. Although after that piece of work really the PKD work was pursued by the PKD groups and the TS groups pursued the TS work; we didn't develop an on-going mutual interest really except perhaps in relation to the contiguous gene syndrome, a deletion syndrome, which we described, when both genes were involved.

PH What had happened during this time to the TSC1 locus?

JS Well obviously it was in abeyance to a very considerable extent in Rotterdam and Cardiff, but not completely; we were still refining the location by linkage analysis. I mentioned when I moved to Cardiff I went back up to Scotland to create a resource of pulse field gel electrophoresis DNA blocks from TS patients and they proved to be absolutely critical really to finding the TSC2 gene within the translocation region that was highlighted by the Portuguese family. This was because we were able to find, I think it was initially 5 deletions on pulse field and by looking at the overlapping regions of deletion, we identified which gene from the region must be the TS gene. So of course we also looked in the pulse filed blocks at the region of the TSC1 locus but actually didn't find anything. And we know now that there are deletions at the TSC1 locus but they're much, much more rare. So TSC1 didn't progress very much. However, we'd found what turned out to be a very critical recombinant in Cardiff in one of the large chromosome 9 linked families. We then spent a lot of time on pulse field mapping of what was really quite a big candidate region without being able to find any large deletions that stood up to rigorous testing.

And so TSC1 was actually eventually cloned out through another international collaboration led by David Kwiatkowski from Harvard who made the critical finding. And the way that that was done was though a consortium of groups, again involving Rotterdam and Cardiff and people from Boston and people from Cambridge and London as well. We'd cloned out essentially the whole of the TSC1 candidate region and mapped it in quite some detail and looked at a lot of genes from within the region without being able to find any mutations. What David Kwiatkowski managed to negotiate was that this contig across the region would be sequenced by the Whitehead Institute, really in the primary phase of the human genome project. And so that was done and the gene was found by sequencing of the genes in the candidate region in a panel of patients. And mutations were identified in one of many, many exons that were looked at. This was quite early on really. I think it was in the 64th exon that was being looked at from this very large region. If I remember right, if we'd gone at it from the other end it would have taken a lot longer, so it was lucky it was done the way around that it was.

And so those initial mutations in that exon were found by David Kwiatkowski and he immediately notified us saying that's the exon and this must be the gene. And then we all worked together to characterise the rest of the TSC1 gene. So it was a completely different way of going about things really. You know that was a 10 year effort whereas the TSC2 effort was really 18 months of intensive effort. So that was the difference that having a chromosome rearrangement and pulse field deletions made; it's a huge difference because both areas were quite comprehensively mapped and similar in other ways.

PH Tell me just a bit about how the research has gone since the genes came out and how the focus has changed and developed.

JS It really has changed very much for tuberous sclerosis. So I guess because there were 2 genes and they were identified 3 and a bit years apart - it was several years apart - there were quite a lot of obvious things to do initially genetically. It became immediately apparent to us when we identified TSC1 that the phenotype was milder in TSC1 patients. Initially it was quite a lot of work to characterise how often is TSC2 involved? How often is TSC1 involved? And what are the phenotypic risks associated with a mutation at either locus and so on? So quite a lot of genotype/phenotype work was done. And obviously the big interest was well, what do these genes do? TSC2 had a region of homology to GTPase activating proteins so we thought this might well be a critical function. That was apparent from the predicted protein sequence at gene cloning. TSC1 really didn't look like anything that was known and it was very unclear what that it would do. So initially the focus was on the issue of TSC2 perhaps being a GTPase activating protein. A lot of effort went into trying to establish whether it was because it was not typical; it lacked certain predicted features that you might have expected of a GTPase activating protein, but there was significant homology. And what was the target? What was it acting on? There were quite a few false leads that emerged from that time.

In Cardiff we didn't really have any prior expertise in this area. We asked the questions but we took molecular genetic approaches to trying to address them. So for instance we looked at the distribution of mutations across the genes; could it tell us anything about potential functional domains and so on ? But

really it wasn't until the Drosophila geneticists identified TSC1 and 2 orthologues that functional work took off properly. They were really not pursuing them as any sort of TS determining genes but through a phenotype driven approach they ended up finding that TSC1 and TSC2 regulated cell and organ size. And that was quite a lot of years later, but it was a critical observation and it put the genes into the same regulatory pathway as PTEN - the insulin signalling pathway. But it wasn't immediately clear how they might mediate that regulation of this growth pathway, and that was nailed by a number of people simultaneously including Andy Tee who now works here in Cardiff, who identified the target for the GTPase activity as RHEB, a molecule that regulated mTOR. And I'm trying to think when that was. That must have been I think about 2002/2003.

And the great thing about that was that it immediately flagged up the possibility of using mTOR inhibitors to treat tuberous sclerosis because it was clear from the cell biology that TS mutations would lead to mTOR over activity, and this might well be the basis of many of the phenotypic effects in tuberous sclerosis.

So once Andy and others had made that critical observation the emphasis really went more onto therapeutic research and that has been the focus for the last 10 years. And that's been very interesting because you know [laughs] it's what we all hoped for but it was nothing that we knew anything about. So we then had to enter into the world of clinical trials and interventional experimental medicine which for us was an entirely new thing. We had established mouse models, as had lots of people, and we used agents including mTOR inhibtors to treat them, and then started clinical trials. And you know that's been very interesting. It's very slow, it's also been particularly hard I think trying to operate competitively from the UK (although I think to some extent we have been successful), because the regulatory issues are so massive and because tuberous sclerosis is a rare disease. We also had a lot of trouble selling our idea to major funders, which I think is a great pity because I think it illustrates a very important principle about how targetable single gene disorders might be. And I think it's an area that medical genetics has really got to get to grips with, but it has not been easy. So the initial clinical trial that we undertook, led from Cardiff, was to use mTOR inhibitors to treat patients with tuberous sclerosis who had the renal tumours associated with this condition because these at least are something that's easily measured.

It was literally a pragmatic decision; the renal tumours are not necessarily the most important manifestation of TS, although urology and nephrology friends certainly think they are, but I don't think patients think they're the most important thing. But they were certainly something that could be approached in a fairly standard oncological trial setting. So the first trial that we did we proposed at the same sort of time as the Americans, I mean everyone was thinking the same thing at the same point. But it took a lot of time to get the funding and then a lot of time to get ethical approval. I remember we went back 3 times to get ethical approval for that study because of concerns, really I think ill-founded ones, in the ethical committees. This wasn't in Wales; this was in an ethics committee somewhere else. Because this was a genetic disorder there seemed to be concerns that there was somehow an inherent extra risk. The ethics committee seemed very risk averse in relation to dealing with genetic disorders even though these drugs had been used in many other contexts. There were concerns that perhaps people with TS would react in some terrible way to them because their mTOR signalling was impaired. But that was the very reason we might expect them to work.

But we did complete a Phase 2 trial which was extremely successful. We had put a research fellow, Mark Davies to work on it and it was brought to a successful conclusion. However we were treating our patients for 2 years and we hadn't finished our study when the Americans finished theirs so we had to publish initially an unplanned analysis part way through the study and publish that and then publish the full thing some time later. One of the things we did in our study was we also looked at neurocognitive measures in the patients with tuberous sclerosis which was suggested by a colleague, Petrus De Vries who was then working in Cambridge, now a Professor in Child and Adolescent Psychiatry in South Africa. He was keen to use this trial looking at kidney outcomes to also get some initial exploratory idea about whether we could approach neurocognitive problems in tuberous sclerosis at the same time, which we did and the results were interesting but because this was an open label Phase 2 study the neurocognitive data were really pretty impossible to interpret. It clearly didn't make everyone worse but what we found was the people who had deficits, generally those improved during the trial whereas in people who were functioning quite well to start with didn't.

On the basis of those findings, we immediately started applying for funding to undertake a bigger study looking specifically at neurocognitive aspects of tuberous sclerosis. But I think we were just too early. I don't think the wider community was ready. I think the TS community was ready but I don't think the wider

research community was really quite ready for it. And I think the idea that you could perhaps inhibit a signalling pathway and improve things like autism was just too much. So we actually made 3 funding applications before we were successful, again over several years. By that time, what really changed was that preclinical data emerged from a group in the US showing that mTOR inhibitors could reverse learning deficits in mice, transgenic mice. And that really captivated everyone's imagination and they thought okay, well this is something that clearly is worth looking at. So finally that trial is underway here in Cardiff and patients are being recruited from across the UK. There's a parallel trial in younger patients in the US and a third trial on-going in the Netherlands looking at even younger patients. So yes, things are going on. I'm a little bit sad because I think we could have been quicker had the wider research community seen the potential that we saw. I think we could have got going on this years earlier than we did.

You have to proceed with caution in any of these clinical settings. But now the results of these trials are very eagerly awaited, including by the commercial sector. In the end most of the funding, not all of the funding, but most of the funding for this trial has come from a drug company. You know, we tried to get this funded through public means on two occasions. The irony is how in TS the majority of this research at a clinical level is now being driven by commercial money and that means that the mTOR inhibitors that are being used are expensive patented drugs. And there are generics like rapamycin which are infinitely cheaper, and which, if the approach works, would be much less expensive for the health service and for the country to support. But the evidence is all being generated with the more expensive drugs and I think that's a great pity. In fact these expensive drugs are now licensed. The trials have worked sufficiently well that the drug everolimus, which is a rapamycin derivative, is now licensed in Europe and the US for the treatment of renal tumours and brain tumours for patients with TS. And trials, commercial trials, are going to start looking at the effects on epilepsy this year.

The first application made to use one of these approved drugs in a non-research setting to treat a patient with TS in Wales was for a girl with TS associated brain tumours and it was turned down. I got international experts to look at her case and her scans and their agreement was she needed drug treatment with an mTOR inhibitor, but the local panel did not agree to use of the approved drug. I was just amazed when the application to use the newly licensed drug was turned down by the individual patient funding request panel here in Wales. We appealed it and it was turned down again. And that's very disheartening. The patient is now being treated with the unapproved generic. But you know somehow it seems like all this research has gone on using the expensive drugs because only pharma will fund the research (for their own drugs). And then although supposedly the decision for drug use in service here in Wales hasn't been made on financial grounds one does wonder, because she's ended up being treated with a cheaper but much less investigated drug. It just seems to be a great pity that the public sector hasn't funded much research using generic drugs; we've had to go to the commercial sector for that. But then the public sector turns down the first application for using the approved drug in a service setting. But this hasn't happened in England where patients have been receiving the approved treatment. [laugh] I guess that's life.

PH Julian, could you spare a few minutes to say something about the colorectal cancer work, particularly your discovery of the recessively inherited form?

JS Yes certainly. So this was I guess another gene discovery story, though somewhat different. I've got some documentation here. I'm just trying to remember what date this was, but certainly genetics had moved on by the time this story kicked off. You encouraged me to take an interest in polyposis syndromes when I first arrived in Cardiff and that was of course in 1989. The gene for FAP, familial adenomatous polyposis, hadn't been identified then. But a register had been started in Wales and you encouraged me to take an interest in this and I did, but very much from the service development side for a long time. But the research interest in colorectal cancer really developed from that. I was just going to look in these notes to see when this story really got going. 1998 it looks like. So the year before that we had identified TSC1 and so I suppose it was quite likely from the TS story that I would be thinking about multiple genes for similar phenotypes. I was seeing all the patients with polyposis in clinics and a family, well a patient, was referred into the Aberystwyth clinic with multiple bowel polyps and a family history of bowel cancer that affected this patient's brother at an early age. But the phenotype wasn't as florid as typical FAP.

There is an attenuated form of FAP so the question the surgeon was asking was, "Is this polyposis or isn't it?" And I was very interested when I saw this family because I thought, well, they seem to be phenotypically somewhere between Lynch Syndrome and FAP. And of course there are families like that that with attenuated FAP but there wasn't a family history in preceding generations of colorectal cancer so it was

rather odd. And I thought probably it was something genetic and wrote back to the surgeon saying, "It is very odd but either it's going to be an unusual APC mutation or it might be a mutation at a different locus altogether and we ought to explore this". And this was done with Jerry Cheadle, now Professor Cheadle, my colleague here in the Institute of Medical Genetics. And really because by this time genome resources were much more widely available, (it was 1998 this started). We didn't yet have the direct genome sequence but things had certainly moved on. So we approached this in a very exploratory way, just in a very small group within the institute here in Cardiff, you know, as a PhD project with Nada al Tassan who was a PhD student tasked with characterising the somatic mutations in the colorectal polyps from this family.

And it got more interesting and more interesting as time went on because initially we had the index patient whose brother had died and we only had a very limited amount of archived material from the brother. But it was a biggish sibship and when I got the other siblings investigated clinically his sister had multiple polyps as well, about 50 bowel polyps, adenomas, as did he in the end. So we had quite a wealth of sample material to investigate and that was Nada's project. And there was a missense variant in the APC gene in some members of the family that we initially thought perhaps had caused the phenotype. We wondered whether this was having an unusual effect on the APC protein. Normally APC has to be affected by a specific type of truncating mutation to produce polyposis. So we thought we'd look at the somatic mutations in APC in the tumours and see if there was an atypical pattern there and there was but it wasn't what we were expecting at all. We discussed this with Ian Tomlinson on one of his visits to Cardiff, but although he noted that the mutations were virtually all G to T transversion mutations he didn't seem to think it was very startling. But we looked into this and felt that statistically it was just so unlikely, really impossible that this could be a chance event and we reasoned that it all pointed to a defect, a very specific defect, in DNA repair. This was pursued by looking at genes involved in repairing 8 oxo-G associated DNA damage, which was the mechanism we thought must underlie this somatic mutation pattern.

And of course we found mutations in the family in MUTYH which fitted with our hypothesis, so at least we had now a very good hypothesis as to what was going on in the family: that there was a recessive colorectal cancer predisposition due a specific DNA repair defect because MUTYH was compromised. And by this stage, it was by now 2001 or so, you couldn't really publish genetic findings without functional work, so we had to try and work on this DNA repair defect in a functional setting. So actually we spent quite a bit of time trying to do that unsuccessfully and then turned then to someone called Sheila David in the Department of Chemistry in Utah. We just found her on the internet really and okay, she worked in this area but in bacteria. And her group were then able to, with the mutations that we'd identified, show that in bacteria these were defective at a functional level. So that was kind of the icing on the cake and off it went as a new colorectal cancer gene.

PH Can I ask Julian, was that gene already known in other organisms at all, or was it completely new?

JS No, it was completely known. Indeed, the DNA repair pathway was quite well established in for instance E. coli. So it was very different to the TS setting where we were pursuing something on a positional basis and then landed in mTOR regulation entirely unexpectedly. Actually, in retrospect one might have been able to predict the TS pathway too, but certainly at the time we didn't expect that. Whereas here things were really very different, we had a likely mechanism before we had the gene and then the mechanism told us which genes to look at through knowledge from model organisms. So it was completely the opposite way round, and of course much quicker and much more straightforward in many ways. So it was a very small and entirely internal project really right until the end when we needed the functional icing on the cake. So yes, a completely different sort of approach.

PH How frequent has this form of polyposis turned out to be? Because being recessive it could quite easily get overlooked or not recognised as being Mendelian among the large dominant families.

JS That's right and it absolutely had been overlooked. I think all people who have polyposis registers essentially had some families where they perhaps had a couple of siblings with polyposis and normal parents but it was always put down to germline mosaicism for APC. It's interesting, the question of how common it is. Because of the disease allele frequency in the population, it should be about as common as FAP but we don't see it as much within polyposis registers. So for instance in Wales I think we have got something like you know 150 families with polyposis on our books and only 20 with MAP as opposed to FAP. And almost certainly a lot of these individuals who have MUTYH-associated colorectal cancer are not recognised, probably because their polyp burden is low or in some cases they developed cancer without polyposis or before polyposis has developed. So it's under recognised at the moment and you know obviously that's a pity because their

siblings are at 1 in 4 risk and would benefit from early identification on screening. So I think now many centres are taking quite a proactive approach to testing for MAP because of the potential to then offer better surveillance for siblings. So yes it's under recognised, definitely still under recognised.

PH **We must draw things to a close soon but to me it's very striking that here are two disorders where, I mean you've spent most of your career working on them, and it seems to me that the whole success of the work has depended on a combined clinical and molecular approach and that without the clinical insights really these advances either wouldn't have occurred or would have occurred an awful lot later. I mean to me it tells me that this is really important and, I mean, do you feel that that's why your work has been so important, having the combination of the clinical and the molecular skills?**

JS Well I'm sure it's been a help. Molecular skills, I'm not necessarily sure about, because obviously as you take on other roles your practical skills very rapidly get out-dated and dwindle. But in terms of having I guess grown up with an insight as to how one might apply molecular genetic approaches to a clinical problem and an insight into how important it is to select the right clinical material to put your efforts into, then I think that has been very helpful in determining success. But you know also the success is very dependent on other people's clinical insights. After all the critical TS family was brought to us and the first MAP family was simply referred into my clinic as one where the diagnosis was unclear. So I think both disorders have involved working with teams of people with complementary skills. But for me it was just very interesting to see the difference between the very arduous positional cloning of the tuberous sclerosis genes, and the much more slick moving from the apparent mechanism to confirming this at a molecular level in the case of MYH.

And the MAP story coincided with a period for a few years where the TS research was really in the domain of the cell biologists; it wasn't stuff that we were going to do here. And so that kind of tided us over until the mechanism of disease in TS was clear then the research re-emerged very quickly into a clinical domain with clinical trials. And so I think being able to be involved with molecular genetics and clinical genetics in clinical research has been extremely handy for getting through what otherwise might be lean times. But I'm definitely not a skilled molecular geneticist; there's absolutely no way. I think having done a little bit and perhaps being able to see where you can work effectively has been useful. But the issue for me now personally is just that the technology has moved on so much that the prejudices that I would have about what is doable and what isn't doable are just being shot down now because if you can get the genomic technologies working you can apply them to things that would have been absolutely insolvable just a few years ago.

I remember with the MYH story, I was invited over to a Dutch genetics meeting you know shortly after that paper came out. I went over and told them about it and I remember a couple of the older geneticists there could hardly believe it, they said to me, "What, you got this gene with one family with two affected people and one dead person? How has that happened?" And yet now we've got to the point where new disease genes can be found by sequencing perhaps one patient or one patient and their parents. So things that were completely impossible have become possible.

PH **And then one final thing you've said a little bit about before. When you started off this work, after you'd come from Glasgow to Cardiff, you were an NHS trainee and then for a number of years after that you were an NHS consultant?**

JS Ah yes, but working in a very nice environment. [laughter]

PH **How did it happen? I do remember that we did our best to kind of shelter you but even so it's been quite a juggling act.**

JS No, and this is very lucky. The first thing was, when I came to Cardiff to complete training in clinical genetics it was before this became very formalised. And the Wales Medical Genetics Service was a relatively big, well-resourced machine you know, compared with many other regions nationally and internationally. So I think my time could be used pretty effectively. I was able to set up the TS research group while doing clinical training. Perhaps in a smaller set up I wouldn't have had a research structure on the university side that my students could operate in, within a culture and a context that was doing the same things and providing a lot of mutual support. And then from the point of view of consultants and trainees, there were Alan Fryer and Oliver Quarrell, who was actually off in Vancouver for quite a bit of the time, and yourself, Helen and Angus were all in consultant posts - so there were a lot of people to provide advice and training.

And also I think we had a good group of genetic counsellors, so things on the clinical side were quite effective. And I was then appointed into a consultant NHS post. I'm just trying to remember when that was, yes in 1991, so there it was then that the TS research moved into a very intensive phase. But during that intensive phase the reality is that I had very good colleagues around to help with all the NHS work; Andrew Wilkie was here as an incredibly able clinical trainee and he helped greatly with the clinical work. So you know we had very, very good people around to just share the load with. So yes I think it wouldn't have been possible in many other settings, first of all because I don't think the early days as a senior registrar would have worked without the university environment and I don't think the possibility of working as an NHS consultant while undertaking the TS work would have worked had we not had such a robust clinical team. So you know it's just extremely lucky. And similar things are very difficult to do now of course, though I think that legacy of mutuality across the NHS and University side still is definitely here in the Institute of Medical Genetics to a big extent, but at a higher, bigger level within the organisations there are tensions. The flexibility is definitely not there in the way that it was. I think now, for people who want to pursue this sort of approach, as we do now have in genetics, they really they have to be in a more formal academic training position.

PH **Julian, there are later things we could talk about but I think we'll finish it there for the time being.**

JS Thank you, Peter.

PH **Well thank you very much.**

JS It's been quite nice to relive some of those periods of time!