Janice Krushner Memorial Lecture 2011

The Importance of IGA Glaucoma Database 2011

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Thank you for inviting me back to give you a progress update on the IGA family database. Searching for genes takes a long time and so has our collaboration with the IGA. We began with samples that Mr Pitts-Crick first helped us collect at King's College Hospital. He had set up the first computerised glaucoma database in the world and we went through, pulled out any family members who had the same name and with that began our work. We needed to find families who had at least two affected members in at least two generations.

We have also benefitted from the supervision of glaucoma experts such as Professor Roger Hitchings, Professor David Garway-Heath and Professor Stephen Vernon. Clinical and Molecular Geneticists have been involved and Professor Mansoor Sarfarazi at the University of Connecticut has been one of our chief collaborators. He managed to obtain funding from the National Institute of Health to do the laboratory work which is always the most expensive. At present we are collaborating with Professor Andrew Lotery, also funded by the IGA, who works in Southampton. We now have 555 families with various types of glaucoma, many of whom are IGA members.

As I said, we were looking for families with two or more affected people in two or more generations, but such families are very rare in the United Kingdom because parents don't tend to be related prior to marriage. We went to Turkey and found three very large families, one had five generations with multiple affected

members and this helped us find three loci for congenital glaucoma and also the first gene for congenital glaucoma. The families also had open angle glaucoma and a few had angle closure glaucoma, but this type of glaucoma is being investigated by another group because they have managed to find many families with angle closure glaucoma. We also had four families with pigment dispersion glaucoma so you will be hearing about those in the future as well as nine families with pseudoexfoliation glaucoma, for which the first gene has now been found.

responsible for their glaucoma as recessive genes which are running in the family have a chance to come from both parents which means that their child may have the condition. This family has been very helpful and, of course, we were able to go back to them with the discovery of the gene and counsel these affected members not to marry first cousins if they can possibly arrange another marriage.

Our work sparked other research around the world so that we now have groups from Pakistan, Turkey, Iran and China reporting their findings

Fig 1.

Fig 2: PCG-100 Turkish Pedigree
This family has strong linkage to the GLC3C region

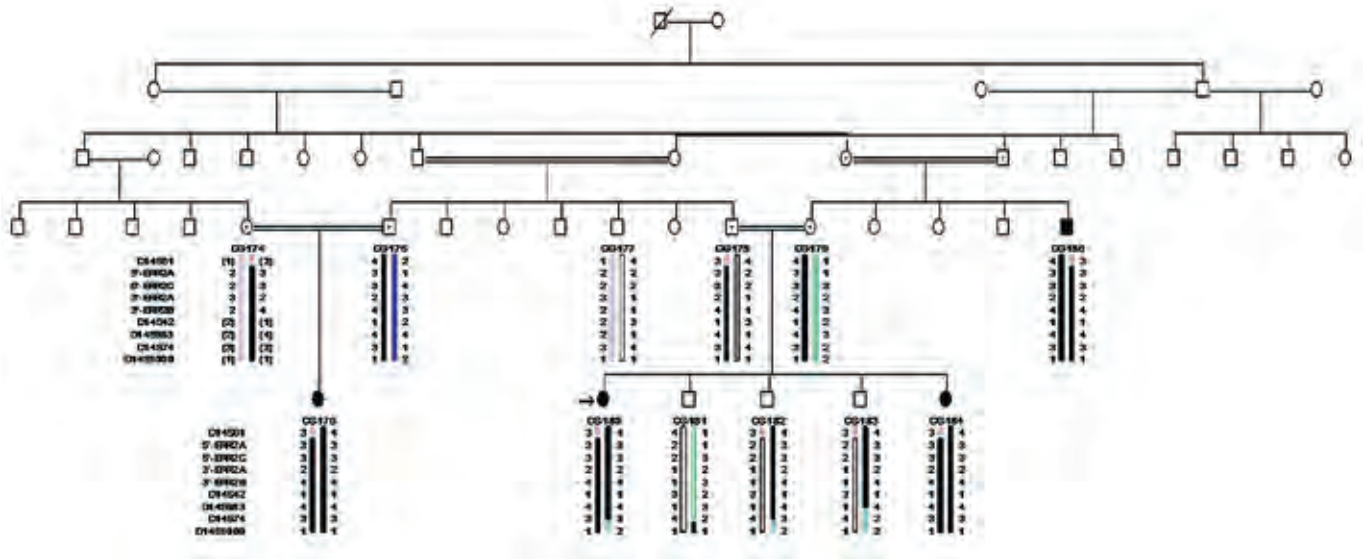


Figure 1 shows the family links of one of our Turkish families in which the first locus for congenital glaucoma was found. The double horizontal lines show where first cousins have married which, although not very good for the families, is good for us as we try to find the genes from their own populations. This is what happens in research, as soon as you publish one thing it's like tossing a pebble into a pond and you find the waves wash up around the world and you start hearing from many other groups.

The 3 loci for congenital glaucoma are GLC3A, GLC3B and GLC3C. The first gene we discovered was 1B1 and there have been papers from around the world confirming that this does indeed play a role in other populations. The third locus, GLC3C, showed promise with genes located on chromosome 14 long arm. Researchers from Pakistan and Iran reported mutations in the gene LTVP2. We checked our congenital glaucoma cases from England but unfortunately none of them had mutations in this gene, so it seems clear that there must be two genes that can create congenital glaucoma very close to each other on chromosome 14. The gene found in the Pakistani/Irani families causes other features as well as glaucoma. We call that a syndrome, which means finding features together that help you identify the cause. We are still looking for the particular gene in children in the UK who just have glaucoma but are otherwise healthy.

All the juvenile and adult onset loci that have been found are in the same basic area and I want to focus on optineurin, which was one of the genes discovered in Professor Sarfarzi's lab. Optineurin was located in the right places to actually cause glaucoma and here in Figure 2 we see a section of the retina with optineurin. The protein is stained in red in the retinal ganglion cells and in the photoreceptor cells and it is

Fig 2: Expression of Optineurin Protein in Retina of an eye Normal Pressure Glaucoma

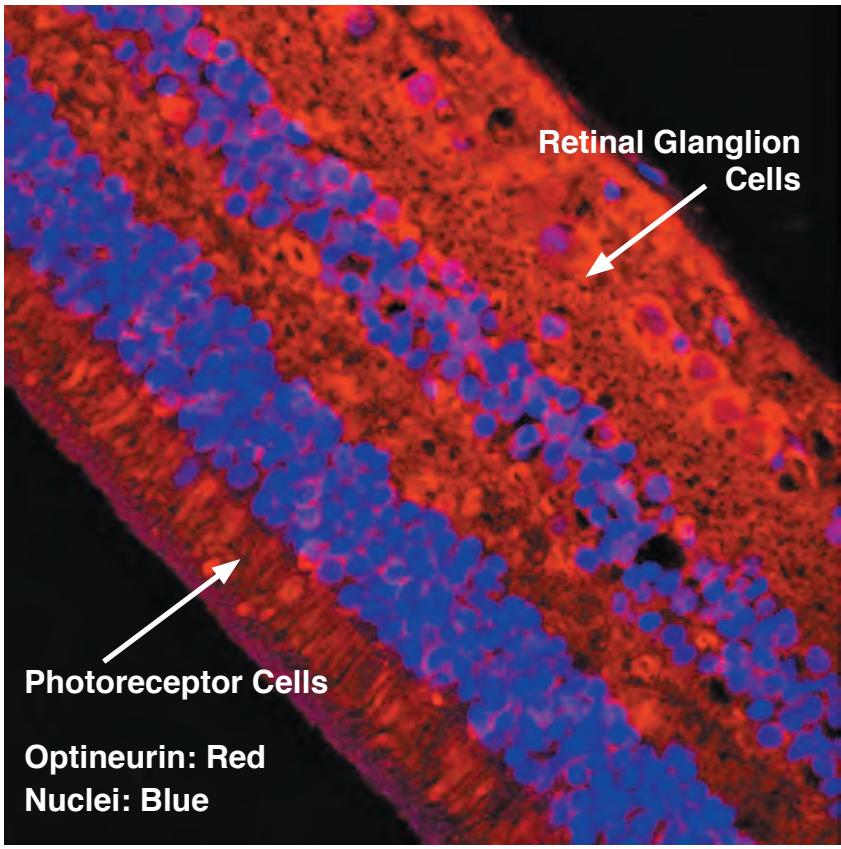
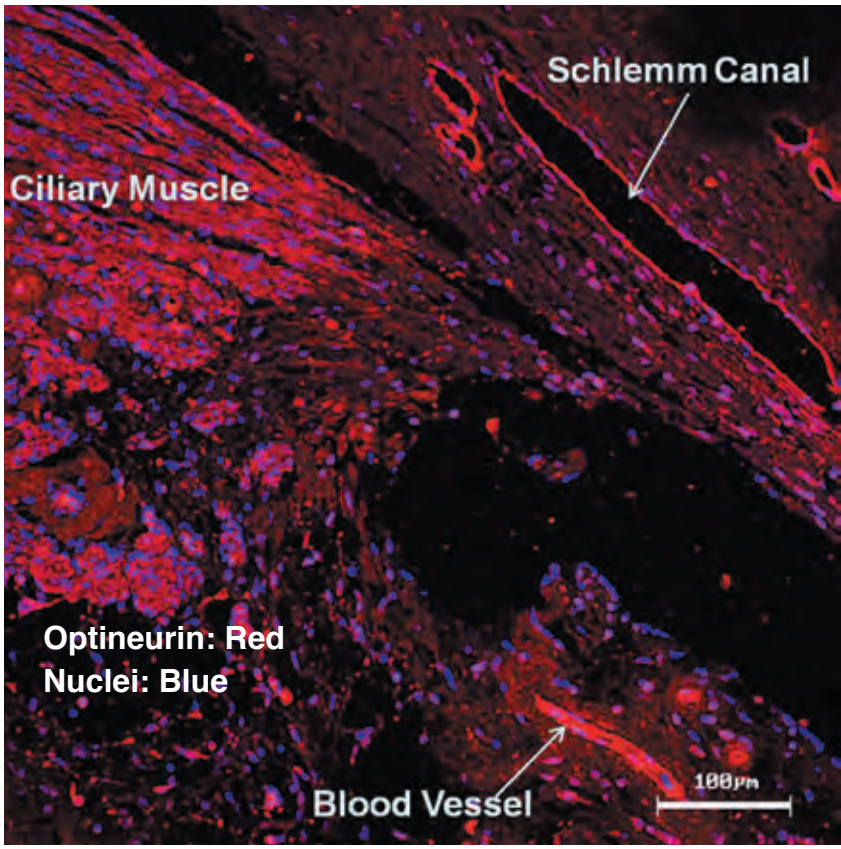


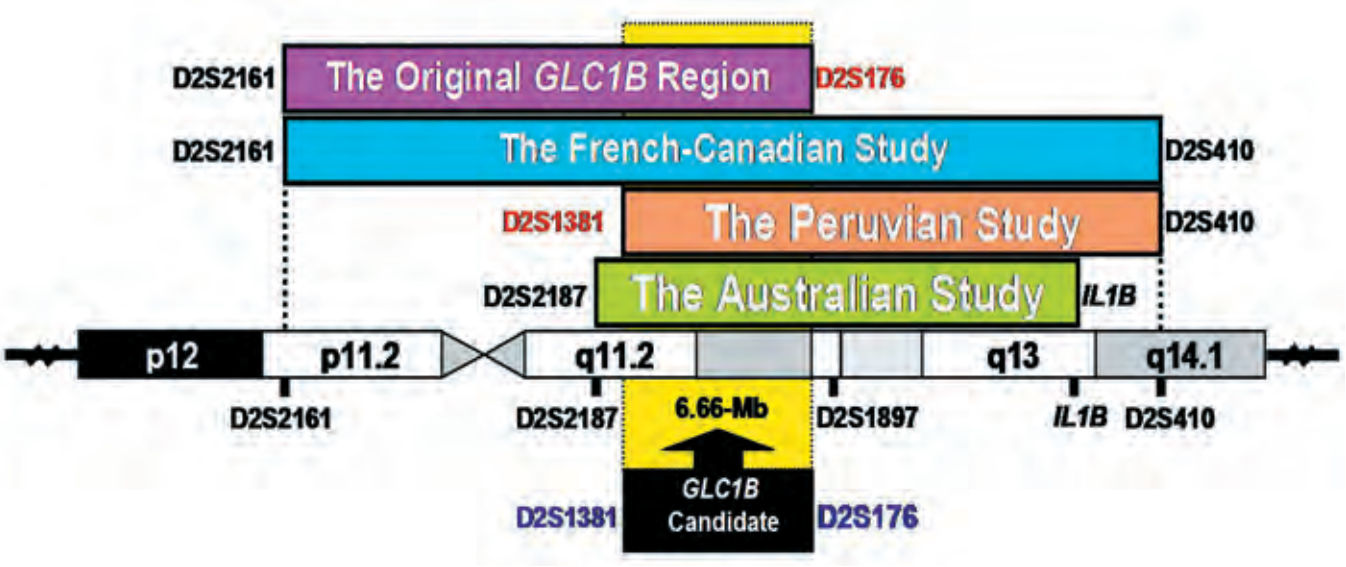
Fig 3: Expression of Optineurin Protein in Anterior Chamber of an eye with Normal Pressure Glaucoma



also present in the anterior structures of the eye (Figure 3) in Schlemm's canal, which carries the fluid out of the eye and back into the blood stream. The international impact of discovering optineurin is reflected in 70 papers from research groups around the world all of which are studying the role of optineurin in various types of glaucoma not just normal tension glaucoma.

Perhaps it would be good to pause here to discuss exactly how we attempt to identify the specific gene that causes the problem. Our PhD student is working on an adult onset glaucoma locus called GLC1B (Figure 4). The original region was found in our families from the IGA database. A French Canadian group reported a bigger location on a chromosome for their group and a study from Peru and another from Australia also reported this region to be important. Consequently, we were pretty sure we had found a gene for glaucoma, or at least its location. If you look at the overlapping regions and draw lines for the minimum area, you end up with the candidate region and we know that the gene must lie in there somewhere (if we are lucky).

Fig 4: A summary combining data from *Stoilova et al., Raymond et al., Fujita et al. and Charlesworth et al.* that shows the *GLC1B* candidate region between markers D2s1381 and D2S176350, 433, 434, 436



The next step is to list all the genes in the region from top to bottom of the chromosome and then we go looking for genes which are actually located in the eye. Here (Figure 5) we have the ciliary body, macular and fovea, retina, lens, cornea, trabecular meshwork and optic nerve head. Within this region there are two genes as a first priority (because they fulfil these criteria). We have worked our way through five genes and haven't found any mutations to date, but that doesn't stop us trying.

Fig 5: GENOMIC CONVERGENCE:

List of 23 GLC1B Candidate Genes PRIORITIZED from a Total of 220

	GLC1B Candidate Genes	Microarray Data [1]	Microarray Data [2]	Microarray Data [3]	Representative Ocular Gene Expression Obtained From Public Electronic Databases (including the NEI Eye Bank) or Published cDNA Microarray Papers
					Expression Codes
1	CAPG	-	-	-	Glaucomatous ONH Astrocytes
2	VAMP8	-	-	-	cDNA from Human TM Cell Library
3	ST3GAL5	1st. Priority	-	1st. Priority	LEN; ONH; RET; MRF; RPE
4	POLR1A	-	-	-	cDNA from Human TM Cell Library
5	REEP1	-	2nd. Priority	-	FET; LEN; ONH; RET; MRF; RPE;
6	VPS24	2nd. Priority	-	-	FET; LEN; ONH; MFR; RPE; NPRE; TM;
7	FLJ10916	-	3rd. Priority	-	LEN; ONH; MFR; NPRE; TM; FET; Iris
8	LOC440886	3rd. Priority	-	-	Iris; TM; LEN; ONH; RET; MRF; RPE
9	FLJ14082	-	2nd. Priority	-	Whole Eye
10	STARD7	3rd. Priority	2nd. Priority	2nd. Priority	RPE; NPRE; FET; LEN; ONH; RET; MRF;
11	TMEM127	3rd. Priority	-	3rd. Priority	LEN; FET; ONH; RET; MFR; RPE;
12	ASCC3L1	-	2nd. Priority	2nd. Priority	LEN; NRPE; ONH; MFR; RPE; Iris; COR
13	INPP4A	-	-	2nd. Priority	Adult Retinal Ganglion Cells Microarray
14	UNC50	1st. Priority	-	2nd. Priority	MRF; FET
15	C2orf15	-	2nd. Priority	-	Lacrimal Gland
16	RPL31	-	-	2nd. Priority	cDNA from Human TM Cell Library
17	FHL2	1st. Priority	-	1st. Priority	NPRE; MFR; RPE; LEN; FET; ONH; RET
18	PLGLA1	-	3rd. Priority	-	RET
19	LIMS1	2nd. Priority	-	-	Ocular Pericytes; RPE; Whole Eye
20	RANBP2	-	1st. Priority	-	RET; RPE; LEN: ONH; TM; MRF; COR
21	POLR1B	-	3rd. Priority	-	NPRE; RET; FET; ONH; RET; MFR; RPE
22	IL1F6	-	-	-	Mechanical Strain of Lamina Cribrosa
23	ACTR3	1st. Priority	-	2nd. Priority	TM; RPE; FET; LEN; Ocular Pericytes

1. Microarray data based on Age effect (old vs. Young), Disease effect (POAG vs. Normal) and Differential gene expression levels after Dexamethasone induction
2. Microarray obtained from TM, Ciliary Body, Retina as well as SAGE data from Retina
3. Microarray data obtained from Retinal Ganglion Cells in Rat and Mouse Models of Glaucoma

C.B. = Ciliary Body
HEE = Highest Expression is in the Eye
MFR = Macular and Foveal Retina
LEN = Lens Tissue
COR = Cornea (UniGene Data)
SAG = Retina, Macula & RPE (GDS =117)
T.M. = Trabecular Meshwork
ONH = Optic Nerve Head
FET = foetal Eye Tissue
RET = Retina (UniGene Data)
RPE = Retinal Pigment Epithelium/Choroid

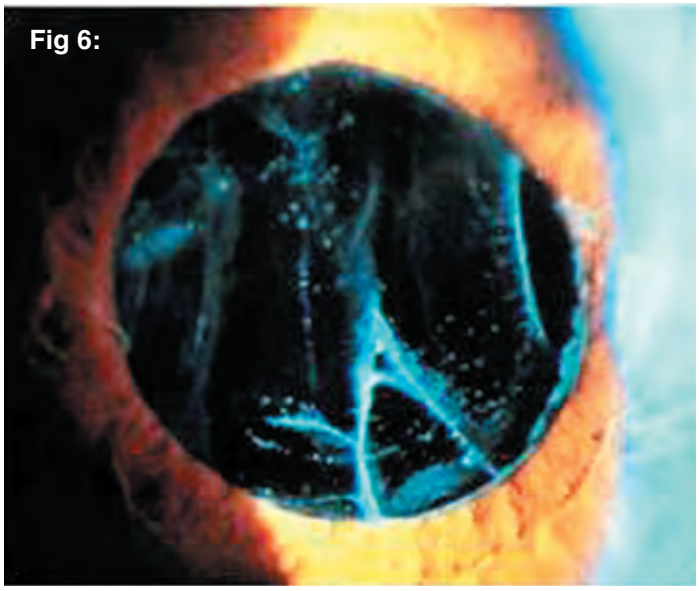


Fig 6:

There are secondary types of glaucoma that are also of interest and one of them is called pseudo-exfoliation glaucoma (Figure 6). You can see the whitish deposit on the eye which identifies it. What we did was to take a gene that had been described by another group and look in our cases: LOXL 1 (Lysyl Oxidase), first described in 1917, is now known to play a role in this whitish deposit on the anterior segment of the eye and is associated with a damaged trabecular meshwork. The glaucoma is often advanced at diagnosis, but not all patients develop glaucoma so this suggested that perhaps there was a gene predisposed to glaucoma, but which didn't actually cause it. However, increased risk in families with the condition is known, so we felt there was a genetic component to this disease. Without going into detail I will just say that the LOXL 1 gene contains polymorphisms in it: these are little variations that don't actually cause disease, but can be associated with disease. When we lined up the different types of glaucomas we showed that one of these variations, namely GG, was important. If a patient had both copies of GG that were identical on both chromosomes, this was of increased significance in patients who had this type of glaucoma. That was at one end of our region where we felt the gene lay and there was another marker at the other end of the region which also had increased prevalence in this particular group of patients. So we feel that we have found (internationally) a gene that predisposes to pseudoexfoliative glaucoma, but doesn't actually cause it, therefore there is probably another unidentified gene nearby or perhaps another chromosome with which this gene interacts to produce the glaucoma. We can't really tell patients what they are predisposed to until we find that second gene, but we are well on the way.

Last year, a very generous grant from the IGA answered a real worry of ours. We had four freezers full of samples from affected and unaffected glaucoma people and families, organised through the IGA and we gathered these between 1995 and 2005. My Professor informed me that we had to downsize and move our department which brought up the question of why we needed four freezers. It was clear that we had to do something about this quickly. We had been concerned about our

samples for another reason as well – which was that DNA degenerates over time if it is kept as a blood sample, but once you have extracted it from the blood sample it lasts pretty much forever (if you can keep it in the freezer). So we came back to the IGA and said 'here's our problem, it's not terribly exciting as a research topic but it is absolutely essential.

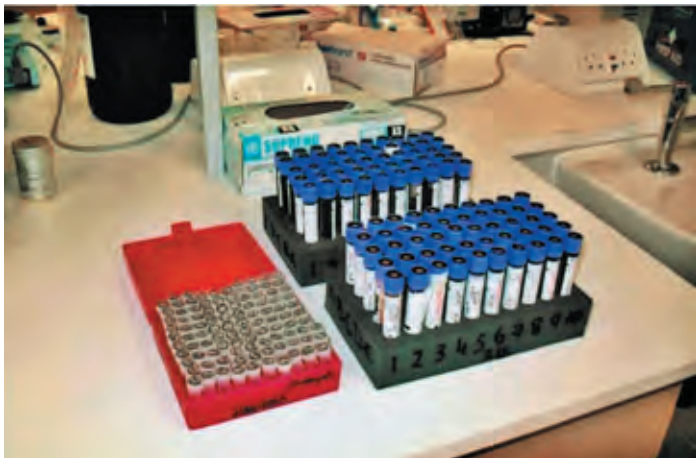
Every possession requires maintenance and this database needs to be maintained so that we can keep the samples for future collaboration. The IGA Grant paid for the extraction of the DNA from the blood samples and in Figure 7 you can see Pippa busy in the lab doing so. Figure 8 shows a blood sample and the amount of DNA that is extracted from it which is, as you can see, a much smaller volume. Figure 9 shows a rack of blood samples and a rack of DNA samples side by side: we now have everything stored in one freezer and the samples are now safe from deterioration so that they can be used way into the future.

Another nice thing about our database is that we have the pedigrees of the families and even if people weren't old enough to have reached the age of onset of glaucoma in the family yet, we still have them on our map. We haven't taken blood samples yet, but we can always go back and look at their eyes and ask for it later. So if any of these studies

Fig 7:

Fig 8:

Fig 9:



discovered the gene mutation in that particular family, they can benefit directly because we can go back and screen the youngsters and find out if they're actually carrying this gene and whether they have a predisposition to glaucoma, in which case we can monitor them and treat it optimally.

We have so many different points of contact with the families that should they move, we can just 'phone the next one and then perhaps pick up on the family pedigree from there.
The young people who worked on these projects are also very grateful and I would like to mention them.
We have now four PhDs and a Masters over this program and all these students have obtained their Degree successfully and gone on to have permanent posts in research work. Our latest PhD candidate, Roshanak (Figure 10), is going to stay on at the University of Connecticut and take charge of the glaucoma research program, so she has all these marvellous samples to work with, with no delay at all. She is going to keep working on the third locus for congenital glaucoma and prioritise adult onset foci identified from our families. Professor Sarfarazi has supervised the work of all our candidates, including Roshanak's husband to be. The future project we have planned is named "Roshnak's Work": She is going to work on GLC1H and GLC1B, which have both been reported and identified from our families and then collaborate with us here in England on this replication study.

Fig 10:

Roshnak (centre) at her engagement with her Fiance (right) and Professor Sarfarazi (left)

Just lastly I would like to mention Xeon sequencing. There has been a bit of a delay in finding new genes for glaucoma mainly because we were waiting for a new technique to speed things up. If you aren't finding anything, the good thing about DNA is that you can just put it back in the freezer and wait, something's bound to turn up, and Xeon sequencing has turned up. The way this works is that if you have a family with glaucoma you take at least two members who are affected, you sequence the entire genome and you will get about 60,000 variations in the gene and because they are family members they will share about 600 of those. If you can add a third family member you can get it down to five or six in the region where you have already identified a gene must be sitting, and then you just prioritise those genes as Roshanak did and start working and looking for a mutation. This is a very powerful new technique, which is not terribly expensive and can be incorporated into our usual classical approach of linkage in families, to see which genes are travelling with the disease in that particular family. Of course the benefit is immediate

for the family involved and benefiting them should be our main aim.

Well, today I have stressed the importance and impact of this database and to conclude I would like to mention many papers we have published. From 1996 to the present we've had 27 major papers, at least 27 abstracts, 3 book chapters and, as I mentioned, 4 successful PhDs and one MSC. These count as publications because they're lodged with the University and we're very very grateful to the IGA for funding this at a time when very few people were listening. We're also grateful to all the patients, their families and their ophthalmologists, and I would like to stress that this database will be important for many years to come, not only benefiting the families but also hopefully leading to specific preventive therapy, once we understand the mechanisms that have gone wrong. Lastly, I would just like to acknowledge the associated funding which we've been able to attract. The National Institute of Health have more than matched the English contribution, which is lovely because they took on the most expensive part and the University of Connecticut Health Centre has supported Professor Sarfarazi. In addition, in England we found a family that had glaucoma who came forward and offered to help us as well, that's the Bluff Charitable Fund. So we are extremely grateful for all your hard work in raising the funds and in making sure that this very valuable database is maintained for years to come.

Thank you.