

LENScience Senior Biology Seminar Series
Insight into Blindness: Whanau - Science Partnership for Discovery
 Jacquie Bay, Marion Maw, Patricia Lndon-Treweek, Peter Dearden, Waiora Port



Good eyesight is something all parents hope for their children. For one branch of a large New Zealand whanau who are descendents from Spanish whaler, José Manuel, the chance of a newborn child being vision impaired is very high. Five generations of the family have been plagued by blindness.

José Manuel deserted from a whaling ship in the 1830's and went to live with a Māori tribal community in the East Cape of Aotearoa-New Zealand. He took multiple wives (as was normal for this community) and now over a 180 years later has more than 10,000 descendents. In one branch of this whanau, more than 40 of the family who are alive today are either blind or have extremely poor vision. Their vision may be so bad that they can hardly distinguish between light and dark. While having a serious vision impairment makes life very challenging, for some members of the whanau there is an additional inherited challenge. As well as their vision problems, a number of the boys in more recent generations are affected by intellectual disabilities and autism.

Dr Marion Maw, a geneticist from the Department of Biochemistry at the University of Otago studying molecular genetics of inherited disorders of the retina was a part of the team of scientists who worked with this whanau to help discover why so many of them suffer from blindness and vision impairment.

Marion's work within the team focussed on understanding at a genetic level what is causing the problem. The research team consisted of whanau members, clinicians, geneticists and biochemists. Key people in this team were:

- **Patricia Lndon-Treweek**, a whanau member and co-author of the research who coordinated the partnership between clinicians, scientists and family members
- **Drs Carolyn Hope and Dianne Sharpe**, ophthalmologists from Auckland Medical School and Auckland Hospital who conducted the eye examinations and interpreted the clinical findings
- Post-graduate student **Ariana Hemara-Wahanui** who identified the mutation in the gene as part of her MSc thesis studies
- **Dr Peter Dearden**, a geneticist from the University of Otago
- **Dr Steffen Hering** from Vienna who collaborated with the Otago team to investigate the effect of the gene mutation on calcium channels.

AUTISM is a lifelong developmental disability that affects the way a person communicates and relates to people around them. There is a wide range of variation in the extent to which autism affects people. Some have intellectual disabilities. The desire for routine and development of repetitive behaviour patterns is common in autism. Autism Spectrum Disorder is thought to be the result of abnormal development in the brain, likely to be genetic in origin.



The **RETINA** is the light sensitive layer at the back of the eye. It contains the light sensitive cells called **rods** and **cones** as well as nerves. The rods and cones are connected to nerve fibres which leave the top layer of the retina to join up and form the optic nerve. Cones are sensitive to colour and work best in brighter lights, rods are not sensitive to colour and work best in dim light.



Na matou te rourou,
Na koutou te rourou,
Ka ora ai te iwi



With our contribution,
With your contribution,
The people will be well.

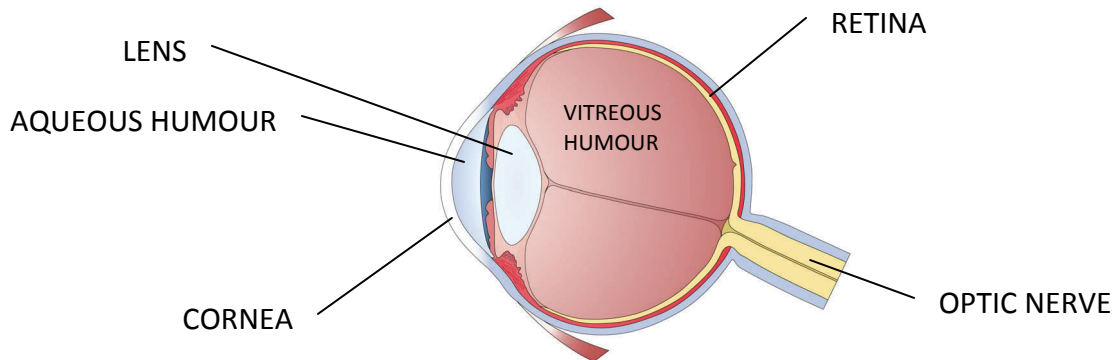




How do we see?

Light enters the eye through the **cornea**, passes through the **pupil** and the **aqueous humour**, **lens** and **vitreous humour** before it reaches the **retina**. As the light hits the cornea and then the lens, it **refracts** or bends to form an upside down image on the retina (which is a bit like a projector screen). While the cornea is fixed, the lens is adjustable and just like a camera, the eye can adjust the lens (using muscles) to **focus** the image.

The light that hits the retina stimulate nerves which carry an electrical signal to the brain. The brain translates the information it has received into an image.



Disease	Cause	Effect on Vision
Cataract	The lens of the eye becomes cloudy.	Blurring - leading to blindness
Glaucoma	Normal fluid pressure inside the eye slowly increases, damaging the optic nerve.	Reduced field of vision - leading to blindness
Age related macular degeneration	The macula (a point in the centre of the retina) deteriorates, creating loss of sharp vision.	Straight ahead vision is blurred. Fine detail is difficult to see.
Diabetic retinopathy	Damage to blood vessels in the retina caused by complications of diabetes	Vision loss potentially leading to blindness.

Condition	Cause	Effect on Vision
Albinism	Recessive allele that results in little or no pigment in the eyes, skin and hair. Sight problems are caused because the retina does not develop properly.	Uncontrolled eye movement; squinting; bright light is uncomfortable; blurred vision; varying degrees of partial sightedness
Leber Congenital Amaurosis	LCA is a family of inherited retinal degenerative diseases that cause the rod and cone cells to deteriorate and die in early childhood.	Severe visual impairment (blindness). Sight is limited to detecting bright lights or hand motions.
X-Linked Congenital Stationary Night Blindness (CSNB)	X-linked CSNB is caused by a mutation on the X chromosome. There are 2 genes known to be mutated in the condition. This affects the ability of the retina to function properly. Congenital refers to the fact it is present from birth. Stationary means it does not get worse over a life time.	Difficulty seeing in low light, reduced acuity (sharp vision), low vision— usually severe short-sightedness, involuntary eye movements, eyes that do not look in the same direction.



Fig. 1 Effect of disease on vision Images courtesy National Eye Institute, National Institute of Health



Living with Blindness


Blindness has been a big part of the whanau for so many generations that it is almost normal. Kaumātua had for many generations believed the whanau was cursed. Patricia’s Mum would take every young baby into a darkened room and light a match. If the baby followed the light with their eyes, she knew they were not affected. While being part of a large whanau with a common medical condition means that the whanau know how to cope with it and there are plenty of cousins, aunts and tūpuna (grandparents) who can offer awhi (wisdom and support) and aroha (all encompassing love) “*but basically you were expected to get on with it because it was just there.*”



Insight into Blindness: Using Observation as a Scientific Tool

Making observations and asking questions are key skills for any scientist. A locum paediatrician who met Patricia’s sons at Homai noticed that their eyesight problems were the same as hers and started asking questions. Dr Carolyn Hope, a consultant ophthalmologist held clinics at the Blind and Low Vision Education Network (formally Homai National School for the Blind and Vision Impaired) in Manurewa. Carolyn saw many children from the whanau who had very poor vision from birth in her work at the clinic. She observed that their conditions didn’t quite fit the usual categories of vision impairment. This observation led Carolyn and her colleague Dr Dianne Sharpe to ask why that was. **They were puzzled that for affected people in this whanau the eyes looked normal, examination of the eyes did not show any structural damage, yet they were vision impaired or blind.**

Working together: Whanau, Clinicians, Scientists

 *Kaua e rangiruatia te hā o te hoe; e kore tō tātou waka e ū ki uta.*
Do not lift the paddle out of unison or our canoe will never reach the shore.

Māori use whakatauki to express concepts of their tikanga (belief systems). This whakatauki reminds us that in order to succeed we need to work together. If the mystery of the blindness was to be unravelled, the collective wisdom and knowledge of the whanau, clinicians and scientists would be required. However before that could happen, the whanau needed to decide together whether they wanted to find out.

Finding out the cause of a family condition such as this is a major step because it affects everyone in the family. In Māori culture, decision making is collective. It is usual that whanau share in the process and come to a decision together because ownership of the genes is shared. In making a decision a family needs to think about many things. ***Does everyone want to know? What will happen as a result of knowing? Could anyone be hurt or upset by knowing? Could knowing help whanau members? What will the whanau have to go through to find out? How will the partners of the extended family feel?***

To Māori people, DNA and genes are taonga. Members of a family who are biologically related share the same genes. Genes are precious as they represent the biological makeup of a family. By allowing genes to be studied, a whanau is sharing with non-family something that is unique and precious. Advances in science that allow families to find out about their genes mean that families all around the world are opening up knowledge that in the past humans would never have had access to. When a decision is made to find out about genetics, that decision affects the whole whanau or family. Each person can then decide what they want to do with whanau support.

Patricia Lundon-Treweek is a whanau member affected by vision impairment herself. She has twin sons who in addition to vision impairment, have autism. Patricia had many unanswered questions and was motivated to find answers. A social scientist by training, Patricia coordinated the idea of finding an answer within the whanau. She talked with whanau members and once a decision was made to go ahead, she traced the whakapapa of the family detailing the relevant medical history of the whanau over the past five generations and working with clinicians and geneticists to unravel the mystery of the condition and find out what was causing it.

Supporting people as they make decisions

Dr Waiora Port, (Te Aupouri [Ngāti Pinaki], Te Rarawa [Ngāti Maroki]), is a respected Kuia with long-standing community knowledge of Māori health issues. Waiora was awarded her PhD in Molecular Medicine/Māori and Pacific Health in 2007 after investigating the cultural and spiritual issues around DNA testing for Māori with a genetic predisposition to cancer. Waiora is Kai Arataki (Cultural Advisor) for the Northern Regional Genetic Service at Auckland Hospital and was a member of Toi te Taiao: the Bioethics Council of New Zealand for 6 years. In her work Waiora provides support by liaising between whanau and clinicians for those who are going through the process of making decisions about genetic testing.



Whakapapa of a Gene—The Story in a Family Tree

The tree shown in Fig. 2 was developed in consultation with whanau members using information from family history, and the results of testing and consultation during the research process. The fact that this is a large family has helped to determine the pattern of inheritance. The first known case of the problem in the family was a granddaughter of Jose Manuel. To make it easier for you to follow her story, we will call her Aroha (not her real name). Aroha* had 3 partners and 10 children in her lifetime. Note—only the family members are shown. Their partners are not shown. None of their partners are affected by the condition.

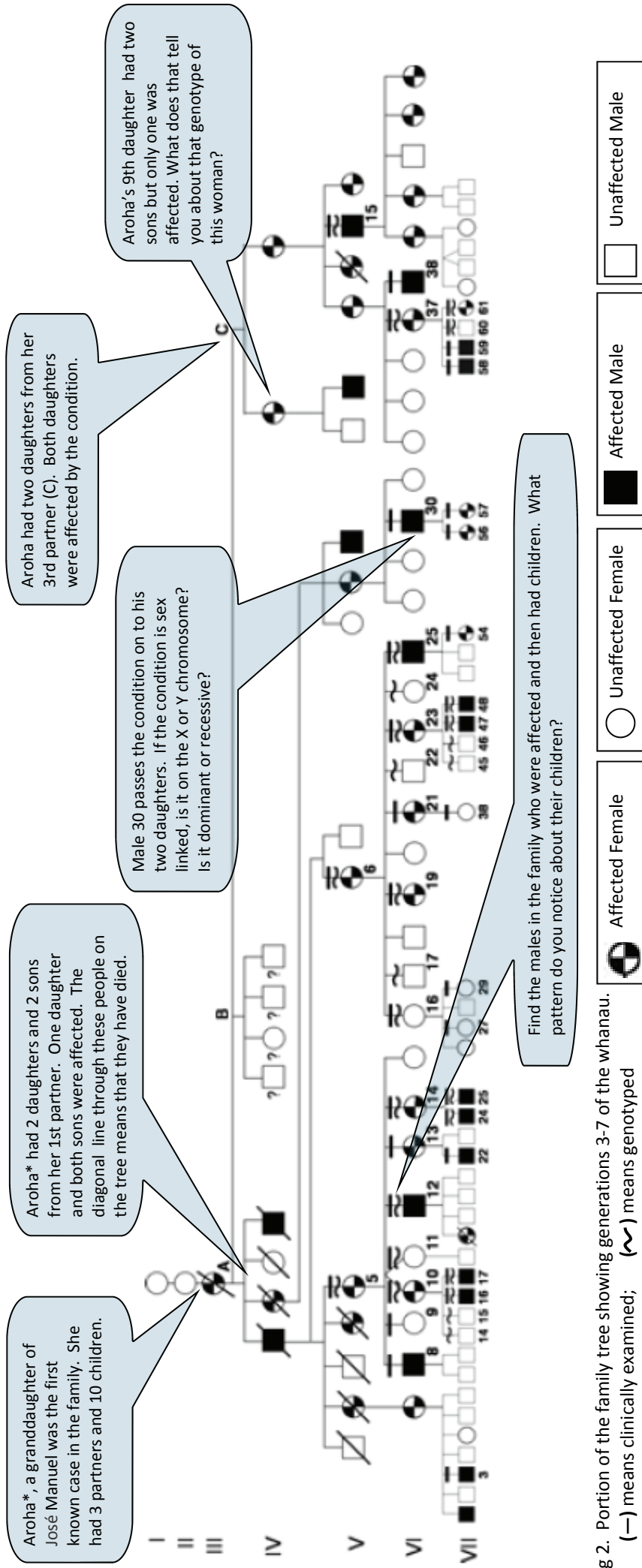


Fig 2. Portion of the family tree showing generations 3-7 of the whanau. (—) means clinically examined; (◐) means genotyped

Creating the genetic tree showing the members of the whanau who were affected confirmed for everyone that this was clearly an inherited condition. The fact that the males in the family who were affected only pass the condition on to their daughters tells us that it is found on the x-chromosome. The team could see that they needed a geneticist to unravel the mystery. It was once the tree was built that Otago geneticist Marion Maw was invited to come into the project and offer her expertise.

Research Questions / Aims

The aim of this study was to find out what the genetic condition was being caused by and therefore hopefully in time find a possible cure or method of treatment for the condition for this whanau. Along side the whanau needs, being able to study a variation in vision like this helps science to understand more about how healthy vision works. A big research question like this needs to be split up into small, achievable steps.

1. What is the extent of the disorder in the family and is there a **pattern of inheritance**?
Researching a family tree will help answer this question
2. What is the **phenotype and how is it different in males and females**?
Clinical examinations of affected family member could answer this question.
3. What is the **genotype of the disorder—what gene is involved**?
To answer this the team needed to analyse the DNA of family members
4. How is the change in the gene affecting the function of the protein it codes for and ultimately **causing the phenotype**?
Once the genotype was established—the next big question is to find out HOW this different genotype is causing the problems that are seen in the phenotype.
5. The final question is how can we **predict / prevent / treat this condition**?

Methods and Findings

Question 1. Pattern of Inheritance:

Patricia Lundon-Treweek facilitated the process of building the family tree, ensuring that cultural and social concerns relating to the study were respected. The result of this work is seen in Fig 2 on page 4.

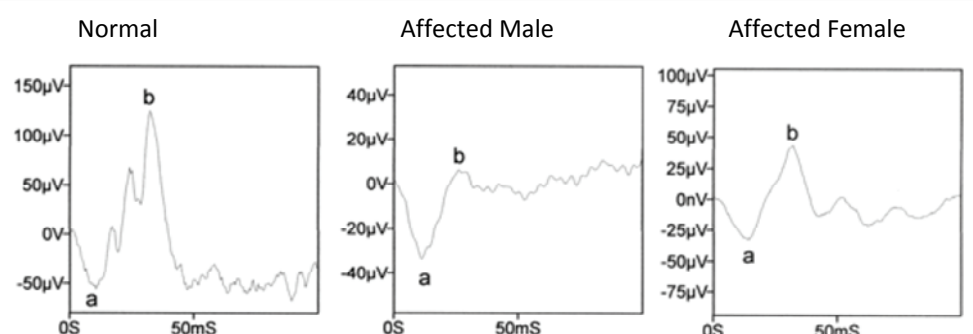
Question 2. Recording the phenotype in detail:

Thirty-six members of the whanau were recruited to the study for clinical evaluation, 17 male and 19 female. Information about their medical history and symptoms were obtained. Detailed eye examinations were used to find information about how well their retina was functioning (Fig 3). Table 3 compares the phenotype of the whanau members with the phenotype of typical X-linked Congenital Stationary Night Blindness and shows that this whanau had a similar condition to this but there were many differences.

Table 3: Comparison of clinical finding for NZ whanau compared to X-linked Congenital Stationary Night Blindness (CSNB)

Clinical Finding	CSNB Males	NZ Whanau Males	CSNB Females	NZ Whanau Females
Night Blindness	Sometimes	No complaint; Glare a problem	No symptoms	Most have poor vision, all have involuntary eye movement, many are very short-sighted.
Short / long sighted	Sometimes	Often longsighted		
Visual acuity (clarity and sharpness of vision)	Varies	Poor—All profoundly affected. Some children can perceive light		
Colour Vision	Unaffected	Abnormal		
Involuntary eye movement	Sometimes	Always		
Cataract	Not associated	Cataracts present		
Neurological Conditions	with the condition	Some with autism, intellectual disability & epilepsy		

Fig 3: Electroretinopathy tests showed that the rod and cone cells sensed the light a-wave but it was not sending a signal to the optic nerve b-wave. Notice that the scale on the Y-axis is different for each graph. The b-wave is flat in the male (no signal). In the female there is a smaller signal.



Key findings

- Both males and females were affected
- Neither all male nor all female offspring were affected
- The eyesight of affected males was more severely impaired than the eyesight of affected females
- The condition had a phenotype similar to but not the same as a well known condition called X-linked congenital stationary night blindness (Table 3).
- Electroretinopathy tests showed that the retina was detecting the light but the signal was not being transmitted to the optic nerve (Fig. 3)
- The gene causing the effect is on the X-chromosome—deduced from observation of the pedigree that the males never passed on to sons and always passed on to daughters.
- Affected females are heterozygous.
- Affected males are hemizygous (Hemizygous means having only one copy of an allele in a diploid organism. Males only have one copy of the X chromosome, therefore only have one copy of any gene found on the X-chromosome).
- Some males with affected eyesight were affected by intellectual disabilities

Question 3: Finding the Genotype

DNA from family members was extracted from blood samples. A DNA profile was created by comparing regions of the DNA called microsatellites in affected and unaffected family members. The team had worked out from the inheritance pattern in the family tree that the gene was on the X-chromosome, so they knew where to start.

Microsatellites or Variable Number Tandem Repeats (VNTR's) are small non-coding repeat sequences found throughout the genome that have high levels of variation between individuals. In a family, this is a good place to look for variation. Copies of these sequences (eg CAG, CAG, CAG, CAG.....) are repeated in tandem (one after another) in the genome. The number of repeats will vary from one person to another. The scientists wanted to find out whether they could see a pattern that was only in affected individuals. If they could, that would give a clue as to where on the X-chromosome the gene was sitting. The scientists used the fact that genes that are physically close together on the chromosome (linked) tend to stay together during meiosis to look for microsatellites that were found together.

Microsatellites at different sites (loci) on a chromosome can be compared between individuals. The number of repeats of each microsatellite will vary from individual to individual. E.g. Site 1 Person X: CA CA CA vs Person Y: CA CA CA CA CA CA CA CA

T A S K :

Look at Fig 5 on page 7.

- The roman numerals on the left represent the generations
 - The symbols are the same as in the tree on page 4
 - The numbers beside the symbols represent a person
 - The numbers in columns under the symbols represent different alleles of each of the microsatellite markers that were tested.
1. Find the pattern **2 1 4 1 1 3 2 2** on female 5 and 6 in generation V.
 2. Highlight every place in the family tree where this pattern is seen
 3. Crossing over has occurred in person 17 and person 48. Draw yourself a diagram to show this cross over.

Crossing Over

Crossing over is the exchange of genetic material between two homologous chromosomes during meiosis. It happens when the homologous pairs line up side by side at the equator. The inner chromatids in the pairs can exchange genetic material and produce recombinant alleles. The closer genes are together on the chromosome, the less likely they are to be separated during crossing over.

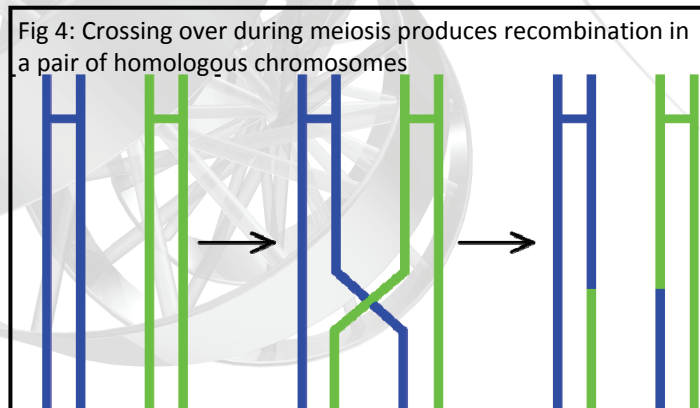
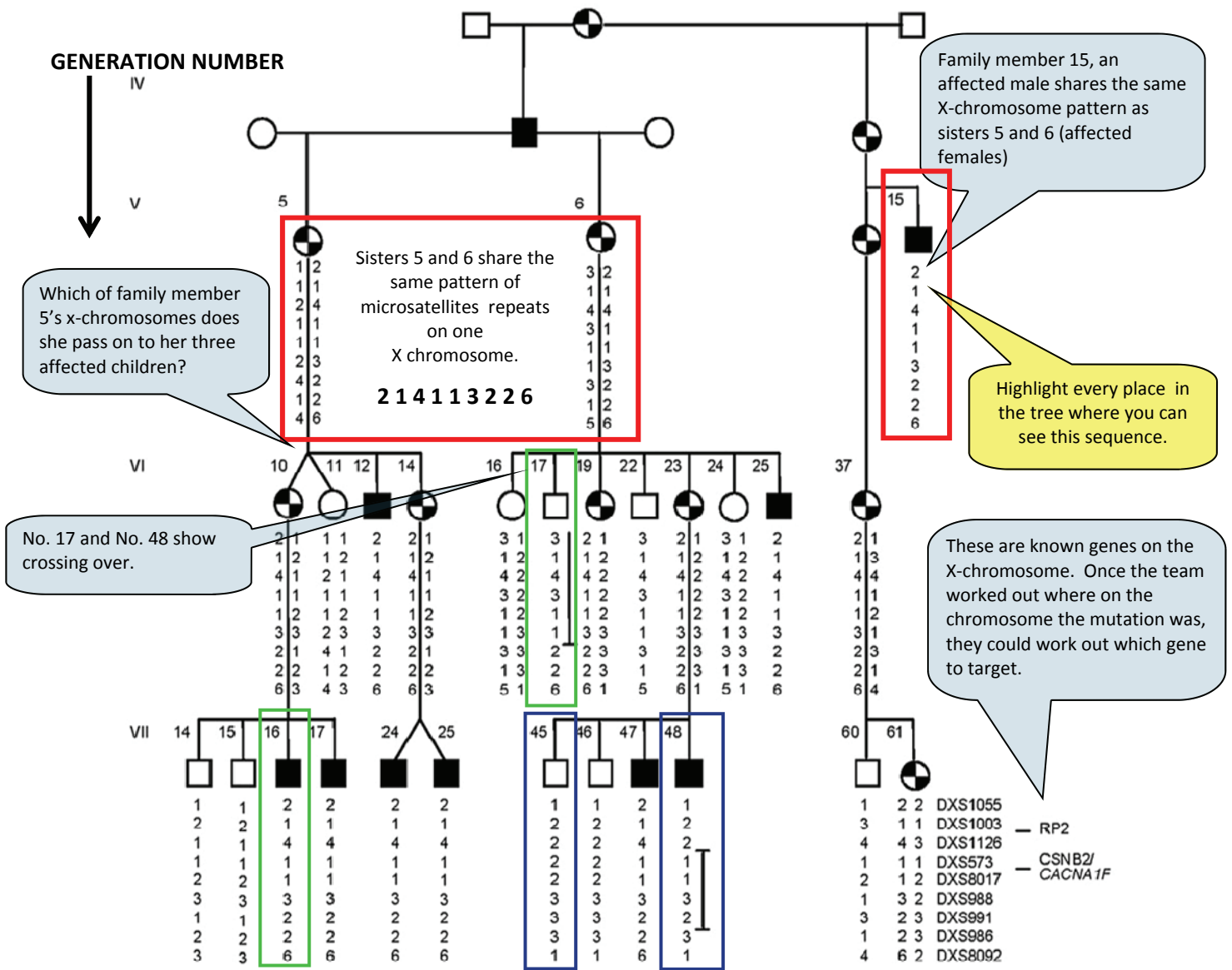


Fig 5: DNA Profile of the Family: The pattern of microsatellite repeats on the X-chromosome for each family member whose DNA was profiled is shown. Females have two copies of the X-chromosome, males one copy.



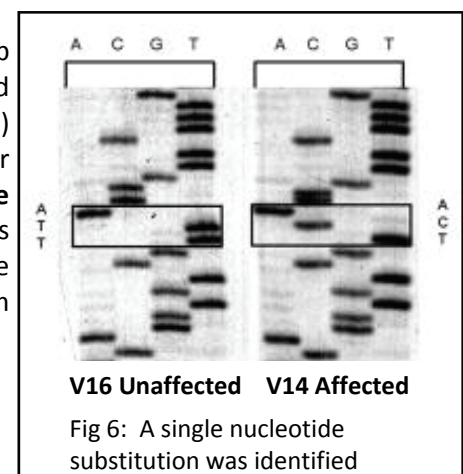
TASK cont'd.....:

- Compare person 16 in generation VII (affected) with person 17 in generation VI (unaffected). What does the difference between the microsatellite pattern for these two individuals tells us about where on the chromosome the mutation is? Look at the sequence "2 2 6".
- Compare person 45 in generation VII (unaffected) with person 48 in generation VII (affected). The difference between these two individuals confirmed for the scientists that the mutation must be in the middle section of the chromosome where the satellite pattern "1 1 3 2" was found. What evidence did they use to come to this conclusion?

Knowing which region in the X-chromosome the mutation was in was a big step forward. The team knew that the gene that causes one form of X-linked Congenital Stationary Night Blindness (the condition similar to that of the whanau) was also found in the same region. They compared the DNA sequence of exons for that gene in affected and unaffected individuals looking for differences. **One substitution in the DNA sequence of the gene was found.** This substitution of as shown in Fig 6 on the right, causes a change in an amino acid that changed the function of the protein. The substitution causes a change in amino acid from isoleucine to threonine.

Key Findings:

- The mutation is caused by one substitution in exon 17 of the gene CACNA1F
- ATT is changed to ACT, changing one amino acid in the protein.



Question 4—How does the mutation affect the function of the protein

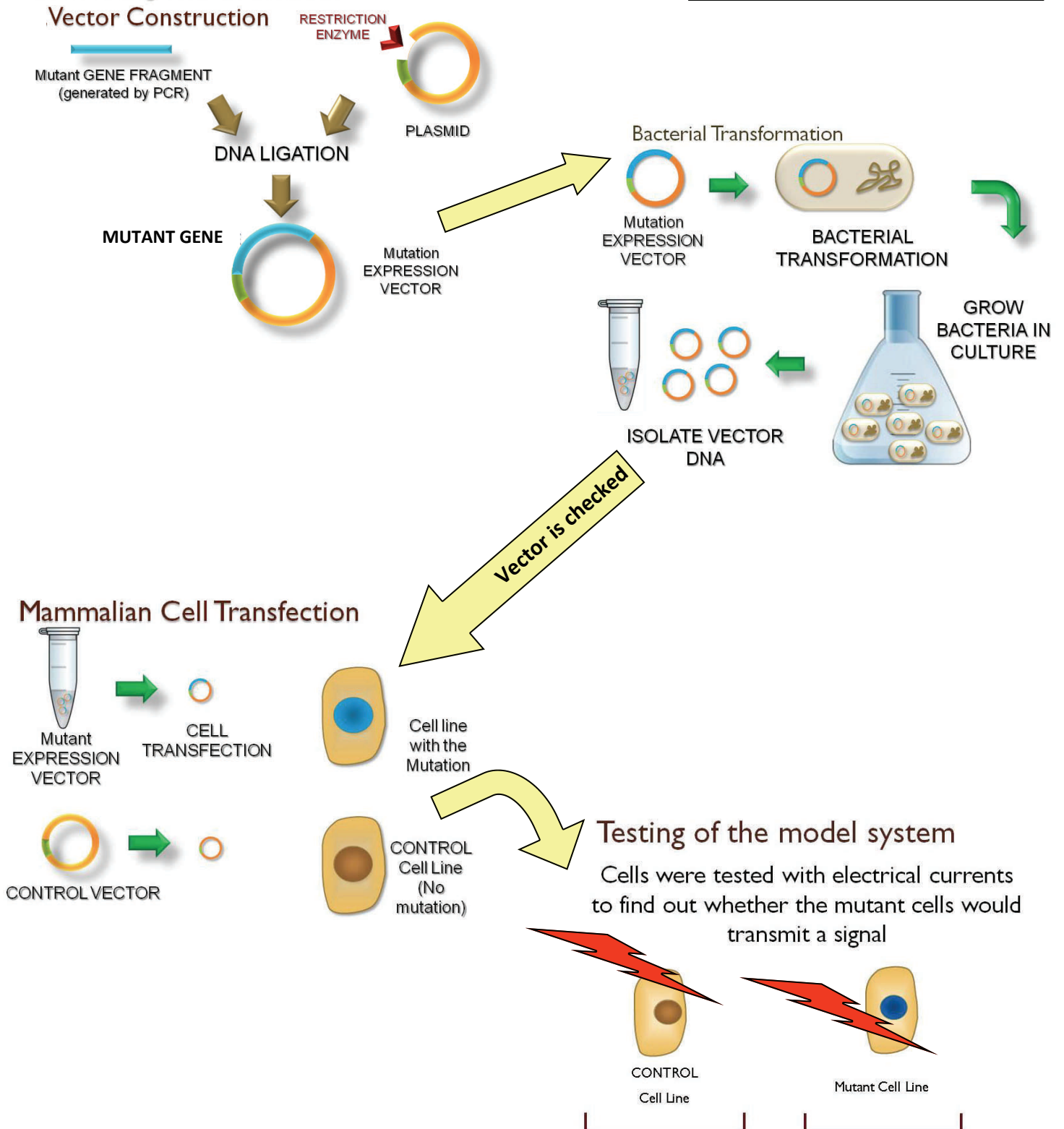
Cell culture studies were used to find out what effect the mutation was having on the functioning of the cells.

A human cell line was used to create a cell tissue culture model that contained the mutant gene. These cells were tested to find out the effect of the mutation. Remember, right back in the beginning, the clinicians had observed that the retinal cells were sensing the light, but they were not transmitting a signal to the optic nerve and eventually the brain. (Fig 3). By comparing mutant cells with control cells, the scientists can try to find out what is happening.



Fig 7: Human Tissue Culture Studies

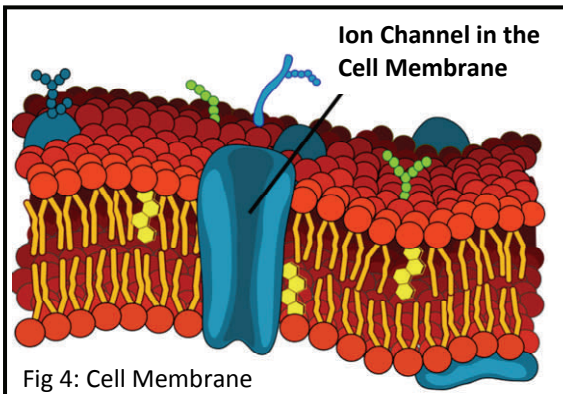
Generating The Cell Model



Understanding Signal Transmission

The clinical team had found that the problem was poor transmission of the electrical signal between the retinal cells and the optic nerve. The retina was sensing the light, but the signal was not being transmitted through the nerve cells to the optic nerve and on to the brain. Why was that happening?

Ion Channels in the Cell Membrane:



Like most cells, nerve cells have a cell membrane which contains specialist proteins which help to transport substances across the membrane. Some of these proteins form ion channels which are like gates, opening to allow ions such as calcium, sodium or magnesium to flow across the cell membrane.

The gate opens as a result of an electrical signal. The ions flow into the cell. The calcium ions bind to proteins, causing the release of special chemicals called neurotransmitters which help send the nerve impulse between cells (Fig 6).

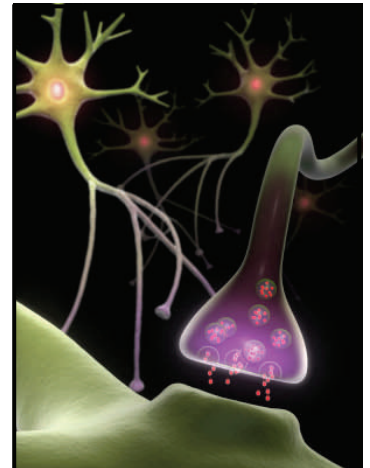
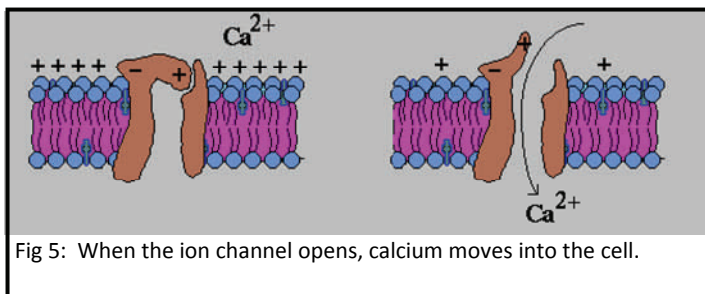
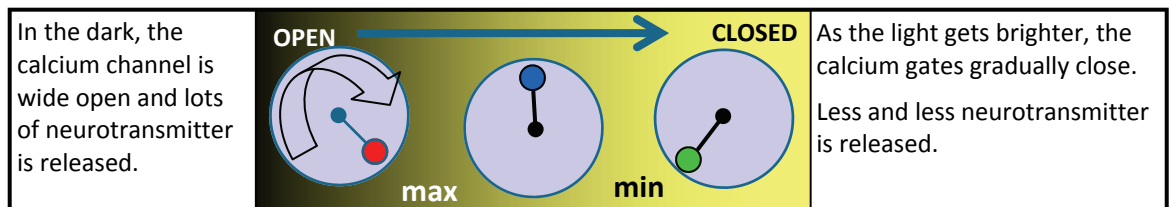


Fig 6: When a nerve impulse reaches the end of a nerve cell, a chemical called a neurotransmitter crosses the gap between cells and the signal continues.



Most nerves only have on or off. Photoreceptors are different. They have a volume dial!



The research team discovered that the mutation affects the calcium channel. In the mutant cell line, far more calcium was getting into the cell because the channel was open more often.

The findings in the cell line led the scientists to suspect that in the rod and cone cells of the retina the channel fails to close in the light and neurotransmitter continues to be released. This is likely to be causing the vision problem.

Key Findings:

- The cells with the mutant gene use less energy to open the calcium channel.
- The channel opens more easily and shuts more slowly.
- This means sometimes it is open when it should be closed.
- The amount of calcium that can move into the light sensing cells of the retina through the calcium channel increases, reducing vision.
- These findings suggest that the change in phenotype seen in the family are likely to be caused by this mutation.



Gene testing offers choices

For the whanau, this knowledge has given them information about the cause of this condition and information that they can use to make decisions. The whanau have always known that baby boys have a very high chance of serious vision impairment and may also have intellectual disabilities and autism. Testing during early pregnancy to identify the sex of the embryo has been a choice open to the whanau for some years. When the gene sequence was determined, Genetic Services at Auckland Hospital offered to create a Pre-implantation Genetic Diagnostic test (PGD). This means that if they wanted to, pregnant whanau members could use IVF treatment with PGD to select embryos that were unaffected by the gene.

Where to from here?

There are still many questions to answer.

- The whanau and research team would love to find out the cause of the intellectual disability that is seen in males. This is a long way off yet.
- Could a calcium channel blocker be created to reverse the effect of the mutation?
The Otago team have developed a mouse model that contains the mutant gene. This means that if a possible solution such as a calcium blocker is developed in the future, it can be tested in a small animal model to find out whether it would work in a whole organism (rather than just cell cultures).
Having established what is happening in the cell line, the mouse model is being studied to find out about the development, structure and function of the retina.
- Through the experience of finding out, the whanau has grown stronger.



Acknowledgements:

The authors would like to thank the whanau for sharing their story with rangatahi throughout NZ schools.

LENScience would like to thank all those involved in the project for their willingness to share their science with teachers and students in NZ schools.

<http://whakaahua.maori.org.nz> (for use of clipart images)

References and further reading:

Hemara-Wahanui A et al (2005) CACNA1F mutation identified in an x-linked retinal disorder shifts the voltage dependence of Ca_v1.4 channel activation *PNAS* vol 102 no. 21 7553–7558 <http://www.pnas.org/content/102/21/7553.full.pdf+html>

Hope, C. I., Sharp, D. M., Hemara-Wahanui, A., Sisingh, J. I., Ludson, P., Mitchell, E. A., Maw, M. A. & Clover, G. M. (2005) Clinical Manifestations of a unique X-linked retinal disorder in a large NZ family with a novel mutation in CACNA1F the gene responsible for CSBN2 *Clin. Exp. Ophthalmol.* **33**, 129–136 (**Note this is not available via open access but please contact the authors if a school and would like to access a copy**).

McConnell R, McConnell V. *Olive Branches Revised: A Record of the Descendants of Manuel Josef, Whaler and Trader of Port Awanui, East Coast, NZ.* Te Araroa: National Library of New Zealand, 1991.

Port Rv et al (2007) Cultural Enhancement of clinical service to meet the needs of indigenous people; genetic service development in response to issues for New Zealand Māori. *Social and Behavioural Research in Clinical Genetics* **73**: 132–138

For further information please contact:

Jacque Bay j.bay@auckland.ac.nz or Peter Dearden peter.dearden@stonebow.otago.ac.nz

LENScience

Bringing Schools and Scientists Together

Copyright © Liggins Institute 2009

<http://LENS.auckland.ac.nz>