GeneHunting:
the search for genes involved in complex human disorders

by

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Background

Professor Lyn Griffiths is a medical researcher who has been studying the genes involved in common human disorders for about fifteen years. Her expertise is in the field of human gene mapping and molecular genetics. She graduated from the University of NSW Biochemistry Department in 1980, and gained a PhD from the Faculty of Medicine, University of Sydney in 1990. Professor Griffiths established and heads the Genomics Research Centre at Griffith University on the Gold Coast, where for the last ten years her research has been focused on identifying the genes involved in complex disorders, including migraine and hypertension. Her research group was the first in the world to show that migraine is a disorder involving multiple genes, implicating genes on chromosomes 19, X and 1, in different migraine families. These studies have also very recently implicated a gene playing a significant role linking susceptibility to stroke in migraine. This gene may help explain the co-morbidity of these disorders, and may have implications to the treatment of migraine. Similar studies have also very recently implicated variants in hormone receptor genes as playing a role in migraine. Professor Griffiths is not only a researcher but also a university lecturer. She lectures in Cell Biology, Molecular Genetics and Molecular Diagnostics to Biomedical and Health Science students, and has given many public lectures to community organisations, support groups, and to both primary and high school students. Professor Griffiths is also a research mentor to many students. To date she has supervised 21 Honours research students, 16 of whom have gained First Class Honours awards and has had 13 PhD students and several MPhil students graduate under her supervision. In addition, Professor Griffiths has been a Director of the Australian Society for Medical Research, is a member of the QIMR Council, and was recently awarded the Centenary Medal for Distinguished Service to Education and Medical Research.

Chromosomes, DNA and Genes

The continuity of life in an individual and a species depends on cycles. These cycles relate to division and replication. There are two processes involved at the cellular level in these cycles, termed mitosis and meiosis. The cycles involve the division of a parent cell to give two daughter cells. If the daughter cells are absolutely identical to the parent cell, the process is called mitosis. Mitosis produces cells for body growth and maintenance. The other process is called meiosis and it results in cells that have half the amount of chromosomes as the parent cells. This process is needed to create gamete cells from each parent that are used for reproductive purposes. These gamete cells are joined to produce offspring with half the chromosomes coming from each parent.

Chromosomes are hence important to the continuity of life and also to inheritance. Chromosomes occur within a centre part of the cell called the nucleus. When the cell is undergoing division and replication, the chromosomes become clearly visible. Under a microscope you can see that the chromosomes separate into the daughter cells. A simple salt extraction procedure reveals that the core of all chromosomes is a single DNA molecule. This was first undertaken in 1868 by Friedrich Miescher, who found that if you treated white blood cells with a high salt concentration, a large amount of a gelatinous substance that he called nuclein, was obtained. We now know that nuclein is in actual fact DNA, and that chromosomes are composed of a long DNA double-helical molecule complexed with proteins.

The structure of DNA was elucidated in detail by Watson and Crick in the 1950s. However much earlier Friedrich Miescher had developed an excellent method to extract DNA in good purity, and he was also able to determine the level of each of its core elements. In terms of the structure of DNA, we now know that DNA is composed of two coiled polymers in a double-helical shape. Each of the monomers within this long polymer is called a nucleotide and each nucleotide is composed of a nitrogenous base, and there are four of these: adenine, guanine, cytosine and thymine, attached to a specific sugar called deoxyribose and to a phosphate group. The sugar and the phosphate make up the backbone of DNA and the bases are stacked internally. The structure is very stable because there is specific hydrogen bonding between the internal bases. This is what makes DNA a very stable structure.

The DNA is packed into chromosomes so that it will fit into a cell. It is packed and coiled and complexed with proteins in a very specific way to form the chromosomes. This specific packing is required because there is a large amount of DNA in each cell in the body. As an example, if you look at a human cell, it contains about 6.4 picograms of DNA - a very small amount in terms of mass. However, if you were to lay the DNA out, not stretching it, simply laying it out, there is actually about 2 metres of DNA in every normal body cell. The average width of a cell is about 1 ten-thousandth of a metre. So it is astonishing that 2 metres of DNA can fit into something that you cannot see with the naked eye and which you need a microscope to view. In fact an average human 70kg body has about 10^{14} cells. So an average human would have about 2×10^{14} metres of DNA in a normal body. That's enough DNA to get you from the Earth to the Sun and back 670 times – a very significant amount of DNA fits within our bodies.

So, how does DNA then relate to genes? Each chromosome contains a molecule of DNA, and that molecule is actually a double stranded helical structure of DNA. Different chromosomes contain different amounts of DNA and also different sequences of the bases that make up the DNA. In the human genome, ie in human cells, there are 46 chromosomes in normal body cells. They come in pairs, with 22 pairs of the autosomes, ie non-sex chromosomes, and a pair of the sex chromosomes - either two X chromosomes if you are a female, or an X and a Y if you are a male. In terms of the numbering of chromosomes, they are numbered from chromosome 1 to chromosome 22, chromosome 1 being the largest and chromosome 22 being the smallest. We have two of each chromosome because one is obtained from each of our parents.

In terms of how this DNA then relates to genes, it is the sequence of bases in the DNA that determines the genes and the traits that make us different to each other. In fact genes lie in a linear fashion along

chromosomes. If you change the DNA sequence of a gene, specifically the bases of the DNA, then you can change the product of the gene, and this can then change the characteristics of an organism. Genes determine many characteristics, including traits like eye colour, blood group, height, blood pressure, what diseases you have or you are prone to. We are all products of the genes that we have inherited.

When we think about DNA, the things that come to mind would be DNA fingerprinting, ie forensic DNA analysis, or perhaps we think about cloning experiments such as those that generated Dolly or those involving extinct organisms, such as the dinosaurs in Jurassic Park or the moa bird. However at the Genomics Research Centre here at Griffith University we investigate DNA, not for cloning or forensic purposes, but to identify the genes that are involved in common human disorders. If we could identify such genes, we could then characterise the structure and the function of the gene, determine whether the gene interacts with other genes or with the environment, and more importantly use gene differences to develop diagnostic tests and better disease treatments.

Common Complex Disorders

Complex disorders are disorders that involve genetic and environmental components. They are very common, that is, they have high prevalence in the population and they often have a late onset, such as high blood pressure, which occurs usually in middle age. Also, as the name implies, they have complex inheritance patterns. This means that it is not clear in each family how they are being inherited. However, they do run in families, indicating that they have a significant genetic component. Quite often the complex inheritance indicates that the disorder involves more than one gene, as well as interacting environmental influences or triggers. The human genome project has provided much useful information outlining the sequence of DNA in our genome. At present though, this information is just a skeleton. Much research is still needed to interpret this information, to define the location and identity of all human genes and to identify which genes are involved in specific disorders.

To identify common disease genes, it is vitally important to have well characterised disease populations. What sorts of populations can you use to try and map genes or hunt for genes involved in disorders? Probably the most useful type of population, would be that comprised of very large families or pedigrees affected with the disorder. The most informative types of families are large, multi-generational ones that involve multiple affected individuals. Sometimes though, it is not always possible to get large multi-generational pedigrees showing the inheritance of a disease gene. An alternative then is to use affected siblings, brothers and sisters, or sometimes relative pairs, such as mothers and daughters, who share the disease genes. Another alternative, instead of using families is the use of case control populations - that is where you use a large number of affected people and compare their genes to a large number of unaffected people. This is called an association study. Finally the last type of population that is useful for gene hunting is a community based population. In particular, isolated founder-effect populations are useful because they tend to have a more homogeneous genetic pool, which makes it easier to track down genes involved in disorders.

Our Genomics Research Centre has been collecting samples for these sorts of studies since 1995. To date we have collected about 6,000 DNA samples, for various sorts of studies on migraine, multiple sclerosis, hypertension and cancer. These include samples for case control studies, as well as large pedigrees, including extended Australian families and isolated founder effect populations, such as that from Norfolk Island.

There are two main approaches used to identify the genes involved in common complex disorders. These are termed association studies and linkage studies. An association study tests a gene that may plausibly be implicated in the disorder, often called a candidate gene, to see if variations in that gene occur more often in those that are affected, compared to in those that are unaffected with the disorder. An association study is

based on the premise that if a gene is primarily involved in causing a disease, then variations of that gene will occur more frequently in individuals who suffer from the disorder, compared to those who do not. It is a case control approach and is usually used to investigate candidate genes.

A linkage study uses families to test for co-inheritance of a tested gene marker with the disease gene. Linkage studies usually use large families to test for co-inheritance or segregation, as larger families provide more information than small pedigrees. This approach can be used to localise genes, but can also be used to determine the distance between genes. These distances are measured in centimorgans (cM), and relate to the amount of co-inheritance between tested gene markers.

Migraine Gene Studies

The Genomics Research Centre has been undertaking gene mapping and gene identification studies on a number of different disorders, including studies on the molecular genetics of migraine, cardiovascular disease gene studies, cancer studies and a relatively new study looking at multiple sclerosis. This presentation will involve a significant discussion on migraine, a briefer outline of our studies on cardiovascular disease and conclude with a very brief description of our recent MS studies.

Migraine is a very common, debilitating and painful neurological disorder. It affects about 12% of the overall population and results in a very significant economic burden. In the Australian population, the Australian Brain Foundation estimates that the economic burden is about $700 million per annum. This is from loss of productivity but also for treatment of the disorder. There are currently no laboratory-based diagnostic tests for migraine and diagnosis is based on symptom descriptions. Even though there are some very good treatments for migraine, these treatments exhibit variable efficacy, indicating that they are not suitable for all sufferers of the disorder. Hence there is a real need to develop better treatments for migraine.

The aim of our migraine research is to identify the genes involved in the disorder, so that new and more appropriate forms of diagnosis and treatment can be developed.

Prevalence

Migraine is a very common disorder. Perhaps the largest epidemiological study undertaken on migraine involved ~36,000 individuals and showed that migraine affects about 18% of women, 6% of men and 4% of children. Even though the exact numbers vary, depending on which particular study and which population around the world was investigated, all migraine studies show a much greater incidence of the disorder in females compared to males. This is particularly obvious if you consider the age of onset. In children, the number of affected individuals is fairly close in males and females. At puberty though there is a dramatic increase in the number of affected females. This could indicate a hormonal influence on the development of the disorder.

Migraine also results in an increased co-morbidity with other disorders, ie people who suffer from migraine have an increased risk of suffering from other disorders. Probably the most common would be psychiatric disorders, depression in particular, but also cardiovascular disorders, and to a lesser degree – epilepsy and diabetes. This may indicate that there are shared genetic factors that play a role in these disorders.

Migraine Classification

The International Headache Society guidelines for the diagnosis of migraine define two main types of migraine - migraine with aura and migraine without aura. Both types of migraine usually involve recurrent attacks of headpain that vary in intensity, frequency and duration. The headpain is usually associated with nausea, vomiting, photophobia and phonophobia, involving aversion to bright lights and loud noise, respectively. The headaches last between 4 and 72 hours in duration and are usually unilateral and pulsating

– meaning they tend to occur on one side of the head and are throbbing. In addition to these symptoms, which occur in both types of migraine, migraine with aura also has additional neurological symptoms. These usually result in visual disturbances, such as flashing lights, wavy lines, blurred vision, tunnel vision or even complete lack of vision. Also, unilateral numbness or weakness and speech defects, may be involved. These neurological symptoms tend to occur just before the headpain or within the first 15 to 20 minutes of a migraine onset.

Migraine Genetics

What do we know about the genetics of migraine? We do know that migraine shows strong familial aggregation - meaning that it tends to run through families. About 90% of migraine sufferers have another close relative who suffers from the disorder. About 50% of the time, this is a first degree relative, ie a mother, father, brother or sister. Twin studies have also shown that migraine has a significant genetic component, with a greater concordance of migraine in monozygotic (identical) twins compared to dizygotic (non-identical) twins.

Migraine shows a complex pattern of inheritance. It usually shows dominant inheritance, meaning that it occurs most often in every generation, although there can be reduced penetrance, meaning that occasionally it may skip a generation. It also involves environmental triggers. There are a number of well-known environmental factors that affect migraine. In particular, there are certain types of food that many people can identify as influencing or triggering a migraine headache onset. Examples of these foods include red wine, ripe cheeses, oranges and chocolate, but also strong perfume, cigarette smoke, stress level changes, glare, and changes in barometric pressure can trigger a migraine attack. These environmental factors are believed to trigger the migraine attack in those who are genetically predisposed to the disorder.

The actual number of genes involved in migraine is currently unknown. Also to complicate matters, the two main types of migraine, migraine with aura and migraine without aura, can occur within the same families and also within the same individual at different stages of their life.

Approach

Our approach to investigating migraine has been to collect samples from families and individuals with migraine and also from control individuals who do not suffer from migraine. We extract the DNA from these blood samples and we then investigate specific genes or DNA markers to try and map and identify the genes that are involved in the disorder.

The diagnosis of migraine is undertaken using the International Headaches Society guidelines and has been undertaken by a clinical neurologist within all of our families. We then get gene results, which are analysed in a number of different statistical ways. In terms of the populations that we use for these studies, we use case control or cross-sectional populations, and we have two very large populations for these studies. The first involves 275 affected and 275 age, sex and ethnicity matched unaffected controls, whilst the second population uses 300 affected and 300 matched unaffected controls. Genes that look quite positive in the first population are tested again or replicated in the second independent case-control population. We also have a number of pedigrees or families that are used for these studies. We now have over 100 pedigrees with a significant number of affected people in multiple generations and we use these families to test for linkage to localise or map migraine genes.

To test DNA markers for association or linkage, we undertake genotyping. This involves detecting DNA sequence variations in an individual's DNA. There are various ways to detect DNA variations, but most of these involve some kind of gene analysis equipment that allows us to accurately detect these variations. Once we determine these genotypes, we then undertake pedigree analysis to see whether that variation runs through the family with the disease or undertake association analysis to see whether that variation occurs more often in those who are affected compared to those who are unaffected. Most of this is done by

computer analysis and we use some fairly sophisticated programs to look at the probabilities and the significance of our linkage and association results.

Gene Mapping Results

In terms of our gene mapping studies for migraine, one of the first things that we started investigating was the hypothesis that the X chromosome might be involved in migraine. The reason we started to think about this, was that there is an increased prevalence of migraine in females, with three times more females than males suffering from the disorder. There is also an increased risk of migraine in close relatives of affected male migraine sufferers. Both of these situations could occur if there was an X-linked gene, in fact an X-linked dominant gene, that was involved in migraine. To test this theory, we used three families that showed inheritance of migraine through multiple generations and tested a number of markers that span the X chromosome – actually 28 DNA markers spanning this chromosome. The results from these three families were really quite interesting. Of the three tested families, two of them clearly showed excess allele sharing, that is they provided evidence of significant linkage, to a specific region near the tip of the long arm of the X chromosome. The third family showed no linkage at all to any of the tested X markers. This indicated that there was an X chromosomal gene in 2 of the 3 families we tested. In the third family there was no involvement of the X chromosome at all. These results also strongly suggested that there was more than one gene involved in causing migraine. We have undertaken further fine mapping studies of this X region. We have also investigated a number of other pedigrees and identified another family that has been very useful in refining or narrowing the migraine gene localisation on the X chromosome (1, 2).

We have also been investigating a number of other genomic or chromosomal regions for involvement in migraine. One region in particular on chromosome 19 was originally implicated in studies on a very rare and severe form of migraine called familial hemiplegic migraine. This very rare form of migraine shows a clear autosomal dominant form of inheritance and has been shown to be caused by at least two genes, one of which maps to chromosome 19 and another to chromosome 1. To determine whether these same regions show involvement in the more common and more typical types of migraine, we have been investigating both the chromosome 19 and the chromosome 1 region in our families.

The results, first of all looking at chromosome 19, were very interesting. We tested four families for linkage to DNA markers that spanned the familial hemiplegic migraine region on chromosome 19. Results from these studies indicated that one of these four families showed significant independent linkage to this region on chromosome 19. The other 3 families showed no linkage at all to the chromosome 19 region. This is particularly interesting when you realise that two of those families showed linkage to the X chromosome and did not show linkage to chromosome 19 and the family showing 19-linkage showed no X-chromosomal linkage. Also, we undertook a particular statistical test, which gave us very significant evidence that there were at least two genes involved in migraine. So, these results indicated that there are at least two genes resulting in similar symptoms in migraine, one on the tip of the X chromosome and one around the centromeric (middle) region of chromosome 19. The chromosome 19 gene for familial hemiplegic migraine has been identified as a neuronal calcium channel gene. This gene has mutations that cause familial hemiplegic migraine in some families with this disorder. This was clearly a good candidate for involvement in typical migraine, particularly in the family showing linkage to this region. However, we have investigated this gene seriously, and could not find any mutation resulting in typical migraine. When we undertook further studies of this region, our results indicated that a gene located a little bit further away, called the insulin receptor gene, has gene variations that showed significant association in our Australian populations but also in extended UK populations. These results suggest that this gene, which is expressed in the brain and plays a major signaling role there, may be the chromosome 19 gene involved in typical migraine (3, 4, 5).

Having looked at the chromosome 19 familial hemiplegic migraine gene, we also decided to investigate the chromosome 1 gene and similarly we found a family that showed quite significant linkage to this region. This region has now been refined to an 8.2cM region on chromosome 1. More interestingly we have shown

an interaction effect in this family. It seems that this family, which is quite large, shows independent significant linkage to two genomic regions - on the X chromosome and on chromosome 1. Thus it appears that two genes in these regions may be interacting to result in the migraine symptoms in this family (6).

To summarise these gene mapping results, our studies indicate that there are at least three migraine genes. They are located on chromosome 1, chromosome 19 and the X chromosome. We have implicated a gene in one of these regions, the insulin receptor gene, a gene that is expressed in the brain and plays a role in controlling sugars levels. It is interesting to note that in some migraineurs fasting can trigger a migraine attack and sugar cravings are also associated with migraine. Our results have also shown that of the other two genes involved in the disorder, the chromosome 1 and the X chromosomal genes appear to be interacting to cause migraine symptoms in at least one large pedigree.

So, how many genes are involved in migraine? These results indicate that there are at least 3 genes, but there may be more. Looking at that particular question, we have recently undertaken a full chromosomal genome scan to try to identify how many genes are involved in migraine. These results have implicated some of the known regions that we have already discussed, but have also identified a number of novel or new chromosomal regions. We are currently investigating these regions, fine mapping them to try to confirm those new regions, but we are also investigating candidate genes in these regions.

Candidate Gene Studies

So what do we mean by candidate genes? A candidate gene is basically a gene that may plausibly be implicated in the disease. Examples of these in migraine would be: neurotransmitter genes, ie genes that control the chemicals released in the brain, such as serotonin related genes; or genes that could be involved in the nausea and vomiting symptoms that you find in migraine, such as dopamine related genes; or genes that are involved in controlling pain processing, such as the nitric oxide synthase genes. We have investigated many of these candidate genes, over 50, and most have given negative results, indicating that they do not play a role in migraine (7-12). However, some genes do appear to play a role. One early example that we identified involves the dopamine beta hydroxylase gene. This gene has variations that showed association in our migraine samples and could be important in affecting neurotransmitter release and nausea associated symptoms (13).

Vascular Related Genes

Another interesting and fairly recent result relates to the similarities that you find in migraine and stroke. Both migraine and stroke involve vascular or blood flow disturbances. There are also some symptoms that you can find in both disorders– loss of vision, numbness, speech problems and also headpain. There is another disorder called CADASIL, which is a type of cardiovascular-stroke disorder that is caused by mutations in a gene called Notch 3 (14). This disorder results in both severe migraine and also in stroke. There is also a well known co-morbidity of migraine and stroke, with individuals who suffer from migraine being more likely to suffer from stroke. The methylenetetrahydrofolate reductase (MTHFR) gene contains a mutation that results in a high level of a particular biochemical called homocysteine and high levels of this have been shown to increase the risk of cardiovascular disease and stroke. We investigated the MTHFR gene to determine whether the same MTHFR mutation also occurred in people who suffer from migraine. A couple of small studies in Japanese and Turkish populations indicated that this may be the case. We tested this in our migraine association population in ~270 cases and controls and found that, particularly in migraine with aura, there was an increased level of this mutation - 19% of migraine with aura sufferers compared to 9% of controls had the mutation. We also tested this in a number of families and again found there was an increased mutation level in migraine with aura members compared to controls. From these results we realised that genes that play a role in stroke susceptibility may also play a role in migraine (15).

Another gene that is considered to play a role in cardiovascular disease and stroke is the ACE or angiotensin converting enzyme gene, which has an insertion-deletion variation that tends to occur more often in those who are at risk of cardiovascular disease. We tested this particular marker and again found a

significant difference in migraine sufferers compared to controls. Also our results indicated that both of these vascular related genes – ACE and MTHFR - appeared to interact or act synergistically to increase the risk of migraine. In migraine with aura sufferers in particular, there was a big difference in the number of those who had the MTHFR mutation and the deletion mutation of the ACE gene, 16% compared to 6% in controls. We believe that this indicates that there is a link between migraine and vascular function and that the MTHFR and ACE genes could be the common genetic factors that link migraine and stroke.

In terms of treatment these results could have some very significant benefits. Increased folate levels in the diet have been shown to reduce the levels of homocysteine in the blood which occurs as a result of the MTHFR mutation, and also ACE inhibitors which can affect the ACE gene product may also play a useful role in treating some migraine sufferers. Both of these should be considered in clinical trials to test whether they would be useful in treating migraine severity and onset.

Hormone Related Genes

The last group of candidate genes that we have been looking at, are hormone related genes. Hormones have long been considered to play a role in migraine. We know that there is a significant migraine onset that occurs during puberty, but there is also a group of migraine sufferers who first develop symptoms during another hormonal variation phase, that is through menopause. There is also a particular type of migraine called menstrual migraine, in which hormonal levels tend to trigger the migraine onset. Also migraine severity has been seen to vary during pregnancy, when taking the pill and during hormone replacement therapy. Thus it is quite possible that hormone related genes may play a role in migraine. To investigate this possibility, we tested a variation in a particularly important hormone gene – the estrogen receptor gene.

Results from this particular gene variant showed a positive association in the first population we tested, and also positive results were replicated in the second large test population (16). Thus there was support for our hypothesis that genes involved in hormonal pathways may play a role in migraine. There are a number of other hormone receptor genes and we have very recently, over the last few months, started investigating these. To date, we have investigated variations in the estrogen, progesterone and androgen receptor genes. Results from both the estrogen and progesterone studies have proved to be positive, whilst those from the androgen receptor gene are quite negative. Again it appears that both the estrogen and progesterone gene variants are interacting and individuals who have at least one copy of both risk alleles are 3.2 times more likely to suffer from migraine. We are currently looking at other variations in these hormone receptor genes and also investigating the significance of these variations in terms of hormone function.

Certainly our migraine candidate and gene mapping results over the last few years have been very exciting. We have shown that the disorder involves multiple modifier and interacting susceptibility genes. We have mapped a number of migraine genes, implicating a number of chromosomal regions. We have also implicated specific candidate genes, the insulin receptor, dopamine hydroxylase, MTHFR and ACE and hormone receptor genes, as playing a role in migraine. Our studies have shown that different genes play specific roles in different individuals and families to result in migraine symptoms. Our aim is to define all of the genes that result in migraine so that gene profiling can be used for definitive migraine diagnosis and so that in future, migraine treatments can be tailored to the actual gene problems in each individual. This is part of the emerging field of pharmacogenomics (17) and it should aid in providing better diagnosis and more suitable treatment for this common and debilitating disorder

The Norfolk Island Cardiovascular Study

The Genomics Research Centre has also undertaking other complex disease studies. One of these relates to a long-standing interest - going back to the 1980s (18 - 31) - in the genes that cause high blood pressure or hypertension. Hypertension is a very common, life threatening disorder and similar to migraine, it is a complex disorder that involves multiple genes, as well as environmental influences. Our approach to

investigating this particular disorder has involved case control association studies, but we have also been interested in using families to look at genes involved in hypertension.

Unfortunately though, hypertension is a late onset disorder and so it is very difficult to get multiple affected generations. We have thus used affected siblings, brothers and sisters, in a linkage or family-based approach. Recently we undertook a full genome scan to map hypertension genes, using these siblings and testing DNA markers regularly spaced at 10cM intervals across all the chromosomes. These studies identified two novel hypertension gene regions (32, 33). To investigate these regions further and to refine the localization of these genes, it would be very useful to identify a large hypertension affected family. However these are very difficult to find as the disorder has such a late, middle-age onset. Isolated founder effect populations though, can be considered large pedigrees and hence can be useful for these sorts of studies. These populations tend to have multiple generations all within one geographical area and this more homogeneous gene pool can enrich for specific genes. Such populations are considered very powerful resources for conducting studies of genetically inherited conditions. The Norfolk Island population is an isolated founder effect population that could be very useful for such studies.

The Norfolk Island population contains about 1500 residents, ~1200 of which are permanent residents and about 900 of which are aged greater than twenty years. The Islanders have very strong family groupings and well-documented family histories. They are also geographically remote and have known founder effect going back to the mutiny on the Bounty, as well as the Tahitian/Polynesian maternal influence. The Island also has extensive isolation and strict quarantine customs which could reduce environmental differences and make it easier to identify the genetic factors involved in disorders like hypertension and cardiovascular disease.

Norfolk Islanders are very proud of their history and have kept a very good genealogical history or record, that goes back 12 generations to the 1780s and involves about 6300 individuals. There are 5 major family groupings and about 70% of the current population of Norfolk can trace their ancestry directly back to the original Pitcarn Islanders who later relocated across to Norfolk Island. The original founders were Tahitian, and Caucasian Bounty mutineers, involving 8 maternal and 12 paternal individuals. The Norfolk Island Cardiovascular Project was undertaken to obtain genotypic and phenotypic information that could aid in the identification of the genetic factors that play a role in the development of cardiovascular disease. Wide community consultation was undertaken prior to commencing this project. The island had very limited healthcare and no information available for public health planning. So, it was agreed that the project could incorporate the collection of relevant public health information that would be useful for the future public health planning of the island.

In the year 2000, whilst I was on sabbatical or research study leave, we set up a clinic on the island and we undertook studies to phenotype, that is to get measurements, from the island population. This involved taking blood samples for cholesterol, testing for diabetes, determining body mass index and body fat levels, measuring blood pressure, doing full dietary assessments and getting questionnaire information on medical and family histories and lifestyle factors, such as occupation, smoking, alcohol use and exercise levels. About 600, that is about two-thirds of the adult population were involved in this study. All individuals had a full medical check with results relayed back to individuals and an anonymous health database set up to provide information for future healthcare planning.

Also, there was significant biochemistry analysis undertaken on all blood samples and DNA was extracted from white cells to enable gene studies. Of the many measurements that were taken, we were obviously very interested in looking at blood pressure. About 18% of the population had high blood pressure levels. Since high blood pressure tends to cluster with other cardiovascular risk disorders such as high cholesterol, obesity and diabetes in a metabolic syndrome sometimes termed syndrome X, we investigated these related phenotypes as well. There were significant numbers in the population that had high cholesterol and high body fat levels, and we detected a number of previously unknown diabetics. All of these factors are

important risk factors for developing cardiovascular disease. In terms of body mass index, there were significantly more males than females considered to be overweight, although there were quite significant numbers of both sexes that were overweight on the island and in fact these levels were higher than in the Australian population. We also looked at lifestyle risk factors for cardiovascular disease, such as smoking levels. There were quite a significant number of smokers on the island, which may have some relation to the lack of island taxes and the greater affordability of cigarettes. About 55% of the population were current or ex-smokers, with 22% being current smokers. In terms of exercise levels, this varied, but there was about 16% of the population that indicated they never exercised.

In terms of cardiovascular disease, the risk to the individual increases with the number of risk factors. When we put this risk factor information together in a demographic overview of the population, it was clear that Norfolk men had a greater number of risk factors, but there were quite a significant number of risk factors across the entire population. Well over 50% of the population had two or more risk factors for cardiovascular disease. This indicates that the Norfolk population that we have tested has quite high levels of cardiovascular risk. The population has been carefully and well phenotyped to define these risk profiles and we believe that it should be very helpful in defining the genes that play a role in developing hypertension and other cardiovascular conditions. We are just at the early stages of the gene studies in this research. We have the phenotypic and pedigree information and have recently prepared DNA samples to commence the gene studies. It will be interesting to see if the large Norfolk pedigree can help identify the genes involved in common cardiovascular disorders.

Multiple Sclerosis

The last study that I will very briefly outline relates to Multiple Sclerosis. Multiple Sclerosis is a serious neurological disorder that affects about one in a thousand Australians. We are using a completely different approach to investigate this disorder. This approach involves the investigation of genes expressed in MS brain tissue to identify those that show expression differences. Rather than investigate DNA directly, we are looking at the products of the DNA - this is gene expression. The brain tissue of MS sufferers contains regions that are quite abnormal in shape and appearance - these regions are called plaques. They can be detected by MRI and are characteristic of the disorder. We are using plaques from deceased MS sufferers to investigate the expression of genes in these regions, compared to expression in normal human brain tissue. We use a microarray screening approach to investigate this expression. This approach allows us to investigate thousands of genes simultaneously and to determine which genes show over- or under-expression in the MS tissue compared to normal matched tissue.

Early results from these studies using six different MS brain samples, has allowed us to detect some consistent differences between MS and normal brain samples. From these studies we have been able to define a number of genes that show expression differences in these plaque samples (34) and identified about 20 genes that show consistent differences in the samples tested so far. We are currently validating these results and investigating these genes and others in a greater number of MS brain tissue and genomic DNA samples (35, 36). This will allow us to accurately measure or quantitate the gene expression levels and may aid in determining whether our results implicate genes that play primary or a secondary roles in the development of the disorder.

MS is a disease that affects the central nervous system, mostly in young adults. It has a high prevalence and there are no simple diagnostic tests or effective treatments for the disorder. There really is a need to develop more effective treatments for MS and we hope that our studies will aid in this endeavour.

Conclusions and Acknowledgements

I have provided you with an overview of some of the studies that I have been involved in over the last ten years. Of course none of this research would have been possible without many collaborators including clinicians, such as Peter Brimage and John MacMillan, who have been involved in the diagnosis of migraine pedigrees and Wally Tortelotte and Tony Tannenburg, who have undertaken MS tissue morphology and assessment. Also this research has involved the help and the work of some wonderful students and researchers. I have been very lucky to have many excellent postgraduate and honours students in my laboratory and none of this work would have been possible without them. So, I would like to pay tribute to the many students who have worked with me and also to the research coordinators, particularly Sharon Quinlan, who have been involved in collecting samples through our Genomics Centre Clinic. None of these studies would have been possible without their help and without the help of the Gold Coast public, who have been astonishingly good at coming forward and helping us get hold of DNA samples to enable these studies. Finally I would also like to thank Griffith University, notably Professors Roy Webb, Michael Irving, Roger Holmes, Dennis Lincoln and Max Standage, who have given me the space, the opportunity and the support to undertake and to grow this research. My very last thanks must go to my family- Mum, Dug and Nan, Kerry, Molly and Phil - for their support and inspiration and my husband Kit and children, Carl and Lauren, who put up with the many late nights.

Selected Relevant Publications

1 Nyholt, D.R., Dawkins, J.L., Brimage, P.J., Goadsby, P.J., Nicholson, G.A. and Griffiths L.R. (1998) Evidence for an X-linked genetic component in familial typical migraine *Human Molecular Genetics* 7: 459-463.

2 Nyholt, D.R., Curtain, R.P. and Griffiths, L.R. (2000) Familial typical migraine: Significant linkage and localisation of a gene to Xq24-28. *Human Genetics* 107: 18 - 23.

3 Nyholt, D.R., Lea, R.A., Goadsby, P.J., Brimage, P.J. and Griffiths, L.R. (1998) Familial typical migraine: linkage to chromosome 19p13 and evidence for genetic heterogeneity. *Neurology* 50(5): 1428-1432.

4 McCarthy, L.C., Hosford, D.A., Riley, J.H., Bird, M.I., White, N.J., Hewtt, D.R., Peroutka, S., Griffiths, L.R., Boyd, P.R., Lea, R.A., Bhatti, S.M., Hosking, L.K., Hood, C.M., Jones, K.W., Handley, A.R., Rallan, R., Lewis, K.F., Yeo, A.J.M., Williams, P.M., Priest, R.C., Khan, P., Donnelly, C., Lumsden, S.M., O'Sullivan, J., See, C.G., Smart, D.H., Shaw-Hawkins, S., Patel, J., Lnagrish, T.C., Feniuk, W., Knowles, R.G., Thomas, M., Libir, V., Montgomery, D.S., Manasco, P.K., Xu, C., Dykes, C., Humphrey, P.P.A., Roses, A.D., and Purvis, I.J.(2001) Single nucleotide polymorphism (SNP) alleles in the INSR gene are associated with typical migraine. *Genomics* 78(3): 135-149.

5 Lea, R.A., Hilton, D.A., MacMillan, J.C. and Griffiths, L.R. (2003). An analysis of clinical characteristics in migraine affected pedigrees. *Cephalalgia* 23:808-813

6 Lea, R.A., Shepherd, A.G., Curtain, R.P., Nyholt, D.R., Quinlan, S., Brimage, P.J. and Griffiths, L.R. (2002). A typical migraine susceptibility region localises to chromosome 1q31. *Neurogenet* 4:17-22.

7 Nyholt, D.R., Curtain, R.P., Gaffney, P.T., Brimage, P, Goadsby P.J. and Griffiths L.R. (1996) Migraine association and linkage analyses of the human 5-hydroxytryptamine ($5HT_{2A}$) receptor gene. *Cephalagia* (1996) 16: 463-467.

8 Griffiths, L.R., Nyholt, D.R., Curtain, R. P., Goadsby, P.J. and Brimage, P.J. (1997) Migraine association and linkage studies of an endothelial nitric oxide synthase (NOS_3) gene polymorphism. *Neurology* 49: 614-617.

9 Lea, R.A., Curtain, R.P., Shepherd, A.G., Brimage, P.J. and Griffiths, L.R. (2001). No evidence for involvement of the human inducible nitric oxide synthase (*i*NOS) gene in susceptibility for typical migraine. *Am. J. Med. Genet.* 105: 110 - 113.

10 Lea, R.A., Curtain, R.P., Hutchins, C., Brimage, P.J. and Griffiths, L.R. (2001). An investigation of the *CACNA1A* gene as a candidate for typical migraine susceptibility. *Am. J. Med. Genet.* 105(8): 707-712.

11 Shepherd, A.G., Lea, R.A., Hutchins, C., Jordan, K., Brimage, P.J. and Griffiths, L.R. (2002). Dopamine receptor genes and migraine with or without aura: an association study. *Headache* 42(5):346-351.

12 Johnson, M.P., Lea, R.A., Curtain, R.P., MacMillan, J.C., and Griffiths, L.R. (2003) An investigation of the *5-HT$_{2c}$* receptor gene as a migraine candidate gene. *Am J of Medical Genetics* 117:86-9.

13 Lea, R.A., Dohy, A., Jordan, K., Quinlan, S., Brimage, P.J. and Griffiths, L.R. (2000) Evidence for allelic association of the Dopamine B-Hydroxylase Gene (DBH) with susceptibility to typical migraine. *Neurogenetics* 3:35 - 40.

14 Grigg, R.F., Lea, R.A., Sullivan, A.A., Curtain, R.P., MacMillan, J. and Griffiths, L.R.. (2000) Identification of a Novel C144F Mutation in the *Notch3* Gene in an Australian CADASIL pedigree. *Human Mutation* 16(5): 449 - 50.

15 Lea, R.A., Ovcaric, M., Sundholm, J., MacMillan, J. and Griffiths, L.R**.** (2004). The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura. *BMC Medicine* 2:3, 1-8

16 Colson, N., Lea R.A., Quinlan S., Brimage P., MacMillan J., and Griffiths, L.R. (2004). The estrogen receptor (Erα) G594A polymorphism is assciated with migraine susceptibility in two independent Caucasian populations. *Neurogenetics* 5:129-133

17 Rogers, K.L., Lea, R.A., and Griffiths, L.R., (2003). Molecular mechanisms of migraine: prospects for pharmacogenomics. *American Journal of Pharmacogenomics* 3:329-43

18 Morris, B J and Griffiths, L. R. (1988) Frequency in hypertensives of alleles for a RFLP associated with the rennin gene. *Biochem. Biophys. Res. Comm.* 150: 219-224.

19 Zee, R.Y.L., Lou, Y.K., Griffiths, L.R. and Morris, B.J. (1992). Association of an insertion/ deletion polymorphism of the angiotensin I-converting gene with essential hypertension. *Biochem. Biophys. Res. Comm.* 184: 9-15.

20 Zee, R.Y.L., Griffiths, L.R. and Morris, B.J. (1992). Marked association of an RFLP for the low-density lipoprotein receptor gene with obesity in essential hypertension. *Biophys. Res. Comm.* 189: 965-971.

21 Ying, L-H. , Zee, R.Y.L., Griffiths, L.R. and Morris, B.J. (1993). Non-linkage of insulin receptor locus with essential hypertension in an affected pedigree. *Hypertens. Res.* 16:25-28.

22 Morris, B.J., Zee, R.Y.L., Ying, L-H.and Griffiths, L.R. (1993). Independent, marked associations of alleles of the insulin receptor and dipeptidyl carboxylase 1 genes with essential hypertension. *Clin. Sci.* 85: 189-195.

23 Zee, R.Y.L., Lou, Y.K., Griffiths, L.R. and Morris, B.J. (1993). Molecular genetic analyses of RFLPs for PCR-amplified growth hormone gene, renal kallikrein gene and atrial natriuretic factor gene in essential hypertension. *Hypertens. Res.* 16:113-120.

24 Zee, R.Y.L., Schrader, A.P., Robinson, B.G., Griffiths, L.R. and Morris, B.J. (1995). Association of *Hin*cII RFLP of low density lipoprotein receptor gene with obesity in essential hypertensives. *Clin.Genet.* 47:118-121.

25 Friend, L.R., Morris, B.J., Gaffney, P.T., and Griffiths, L.R. (1996) Examination of the role of nitric oxide synthase and renal kallikrein as candidate genes for essential hypertension. *Clin Exp. Pharmacol and Physiol* 23: 564-566

26 Morris, B.J., Jeyasingam, C.L., Zhang, W., Curtain, R.P., and Griffiths, L.R. (1997) Influence of family history on frequency of glucagon receptor Gly40Ser mutation in hypertensive subjects. *Hypertension* 30:1640-1641.

27 Morris, B.J. and Griffiths, L.R. (1998) Scanning the genome for essential hypertension loci. (Invited review; Fetschrift for John P. Loghlan) *Clin Exp Pharmacol and Physiol* 24 S72-S78.

28 Rutherford, S., Boatwright, SD., Samwell, G., Morris, B.J. and Griffiths L.R.(1998) A linkage and cross-sectional study of hypertension and obesity using a poly (A) Alu repeat polymorphism at the glucagon receptor gene locus (17q25). *Clinical and Exp. Pharmacol and Physiol.* 25, 627-629.

29 Benjafield, A.V., Jeyasingam, C.L., Nyholt, D.R., Griffiths, L.R. and Morris, B.J. (1998)G-Protein B3 Subunit Gene (GNB3) Variant in Causation of Essential Hypertension. *Hypertension.* 32, 1094-1097.

30 Wright, K., Tajouri, L., Lea, R.A., Ovcaric, M., Heux, S., Headrick, J., and Griffiths, L.R. (2004) The role of adenosine-related genes variants in susceptibility to essential hypertension. *Journal of Hypertension* 22:1-4

31 Heux, S., Morin, F., Lea, R.A., Ovcaric, M., Tajouri, L., and Griffiths, L.R. (2004). The methylentetrahydrofolate reductase (MTHFR) gene variant (C677T) as a risk factor for essential hypertension. *Hypertension Research* 27 (accepted 01/06/04)

32 Rutherford, S., Johnson, M.P., Curtain, R.P. and Griffiths, L.R. (2001). Chromosome 17 and the inducible nitric oxide synthase gene in human essential hypertension. *Human Genetics.* 109: 408-415.

33 Rutherford, S., Johnson, M.P. and Griffiths, L.R. (2004). Sibpair studies implicate Chromosome 18 in essential hypertension. *American Journal of Medical Genetics* 126: 241 - 247.

34 Tajouri, L., Mellick, A.S., Ashton, K., Tannenberg, A.E., Tourtellotte, W., and Griffiths, L. R. (2003) Quantitative and qualitative changes in gene expression patterns characterize the activity of plaques in multiple sclerosis. *Molecular Brain Research* 119:170-183

35 Tajouri, L. Ferreira, L., Ovcaric, M., Curtain, R., Lea, R.A., Csurhes, P., Pender, M., and Griffiths, L.R. (2004). Investigation of a neuronal nitric oxide synthase gene (NOS1) polymorphism in a multiple sclerosis population. *Journal of the Neurological Sciences* 218; 25-28

36 Tajouri, L., Martin, V., Ovcaric, M., Curtain, R., Lea, R.A., Csurhes, P., Pender, M., and Griffiths, L.R. (2004) Investigation of an inducible nitric oxide synthase gene (NOS2A) polymorphism in a multiple sclerosis population. *Brain Research Bulletin* 64:9-11

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PROFILE

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Career History

1969-1974	Fort Street Girls High, Sydney, School Captain, Dux, English, History.
1975-1978	*BSc (Hons).* Double Major in Biochemistry and Microbiology, UNSW.
1980-1983	*Research Assist*: Med. Dept, Sydney University & Duke University, NC, USA
1984-1988	*NH&MRC and NSW Dept of Health Biomedical Postgraduate Scholar* Department of Medicine, University of Sydney. PhD Thesis: Chromosome 1 Gene Mapping with reference to CMT Disease.
1989-1991	*Chief Investigator, NH&MRC Project Grant*: "Molecular Genetic Abnormalities in Human Hypertension".Department of Physiology, The University of Sydney.
1990-1991	*Lecturer-Genetics/AssocDirector Biology 1*: School of Biological Sciences, Uni of Sydney.
1992-1994	*Lecturer-Molecular Genetics*, *Cell Biology,Biochemistry:* Applied Science, Griffith Gold Coast.
1995-1998	*Senior Lecturer in Molecular Genetics and Cell Biology*: Health Science, Griffith Gold Coast
1996 -1999	*Head*, School of Health Science, Griffith University Gold Coast.
1999-2001	*Director*, Australian Society Medical Research
1998-2001	*Associate Professor* in Molecular Genetics
2002-present	*Professor* in Molecular Genetics
1997- present	*Director*, Genomics Research Centre, Griffith University Gold Coast.

Research Overview

- Invited speaker at various international and national conferences plus regular reviewer for many international journals
- Chair, Scientific Program Committee for next International Congress of Human Genetics. Convenor: Australasian Gene Mapping Conference (2001); Convenor Australian Society Medical Research Conference (2001)
- Significant R&D contracts with overseas biotechnology (Gemini Genomics) and pharmaceutical (GlaxoSmithKline) companies, as well as significant NCG funding.
- Centenary Medal Award for Distinguished Service to Education and Medical Research
- Extensive media coverage particularly on migraine research including, A Current Affair, The Today Show and nightly news features plus a National Geographic 13 part series entitled "The GeneHunters" featured the GRC Norfolk Island genetic studies as Part 1 of the series.

Publications

BSc Honours Thesis: Leigh's Disease: Biochemical Studies.

PhD Thesis: Chromosome 1 Gene Mapping with reference to Charcot-Marie-Tooth Disease.

93 papers in international journals, after regular submission and review.

201 presentation abstracts: Australian scientific meetings (161), International scientific meetings (40)