Forty Years From Markers to Genes

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There have been incredible advances made in human genetics over the past 40 years. I have set out in the next few pages to describe just some of these changes and to illustrate how they unfolded through my own experiences.

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There was little specialized training in human genetics for scientists during the 1960s so many of us from that era who are now in the field of human genetics came through other disciplines. Human genetics was relatively retarded compared with developments in some other animal groups; for example, the correct number of human chromosomes was not settled until Tjio & Levan (1956) and G-banding for individual identification of human chromosomes was not available until Sumner et al. (1971). The Genetics Society of Australia held an annual national scientific conference that most of us attended each year because the Human Genetics Society of Australasia was not formed until 1977. It sprang from the impetus generated at the 1976 human genetics conference held in conjunction with the Adelaide Children's Hospital (now Women's and Children's Hospital) Centenary celebrations (Sutherland, 2008).

Human cytogeneticists of that era came into the field from other medical laboratory disciplines or had worked on insect chromosomes. They established human cytogenetics laboratories within health institutions during the 1960s. The feasibility of establishing such laboratories came about from a series of technical breakthroughs in the analysis of mammalian chromosomes, as reviewed by Hammerton (1971) and Hsu (1979).

Fisher (1930), Wright (1931) and Haldane (1932) had laid down much of the population genetic theory during the 1930's. This became experimentally testable with the widespread application of protein gel electrophoresis in the 1960s–1970s (Harris, 1966; Lewontin & Hubby, 1966; Manwell & Baker, 1970). But the use of protein variations as genetic markers remained one significant step removed from the ultimate template, the DNA sequence, because protein electrophoresis detected only those amino acid changes that affected the mobility of the protein through the gel. Watson and Crick (1953) brought DNA onto center stage by unravelling its structure, which fitted nicely with the properties required of a heritable chemical which needed to remain stable when replicated in rapidly dividing cells. As an alternative to laboratory experimentation, the in silico approach using the binary code to simulate genotypes had been established in Australia in 1956 when the first computer SILLIAC was built in an Australian University (Nicholas & Hammond, 2008).

Realization of the extent of previously unsuspected high levels of genetic variation in populations through protein electrophoresis was a defining stage in animal (including human) population genetics. The maintenance of variation according to the mutation-selection balance model soon gave way to debates on selective neutrality and genetic drift on the one hand (Kimura, 1968) versus some form of balancing natural selection with associated genetic loads on the other, in order to adequately account for the levels of genetic variation suddenly unmasked by electrophoresis. Interestingly, in recent times with an inability (as yet) of common genetic variants in populations to adequately account for much of the heritability of the common human disorders with complex genetic architectures, there has been a resurgence in speculation that the mutation-selection balance model might after all play a major role (Kruglyak, 2008). This has been well established for monogenic disorders in animal genetics (including man) for a very long time, and for quantitative traits the only conjecture has been the relative contribution of rare versus common variants (Frankham, 2008).

The Early Days: 1970 to 1978

The Ecological Genetics of Cactophilic *Drosophila*

So what did some of us do during the early days while waiting for recruitment into human genetics? As a major in zoology I started in genetics because that was the first job I could get after discharge from the army. Apart from counting flies in fitness experiments and 'collecting virgins' for *Drosophila* mating experiments,

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I set up my first electrophoretic 'lab' in the maintenance man's workshop by scraping away a pile of tools sufficient to create two free meters of undulating bench space. The outcome was the extensive population genetic survey (Barker & Mulley, 1976) of *Drosophila buzzatii* with its prickly pear host *(Opuntia* species), from 35 locations. From that publication, Nicholas & Hammond (2008) estimated that we covered 160,000 square kilometers, corresponding to more than half of Eastern Australia, and collected more than 5000 flies from which I had manually determined 112,000 electrophoretic phenotypes. I 'industrialized' the screening using multiple home-made electrophoretic tanks made from containers purchased at the supermarket wired with platinum, and I made splitter boxes to run multiple gels off the one power supply, and tried to avoid touching the wicks leading into the buffer tanks when the current was on!

That study marked the first of many significant publications related to the *Drosophila*-cactus-yeast system in the career of Stuart Barker in his quest for answers to questions in evolutionary genetics and fitness, apart from his many practical contributions to the genetics of livestock improvement. During the project we stumbled upon *D. aldrichi,* which had not previously been recorded in Australia. Similar in appearance to *D. buzzatii* it was more narrowly distributed entirely within the *D. buzzatii* range (Mulley & Barker, 1977). Embarrassingly, I initially missed the subtle visual differences when trapping the flies and upon staining an electrophoretogram from the bodies collected where the two species were sympatric, it was evident that my sample was a mixture of two species. For *D.buzzatii* we teased out marker associations with climatic conditions (comprising combinations of weighted environmental components) using a variety of multivariate statistical techniques and we uncovered genotype-environment correlations consistent with selection (Mulley et al., 1979). I left the project knowing that the best was yet to come, experimental perturbation of gene frequencies of an isolated natural population (Barker & East, 1980), again supporting selection rather than the alternative of selective neutrality of markers (or more precisely, their haplotype blocks otherwise known as 'super alleles').

The Evolutionary and Ecological Genetics of Commercial Prawn Species

As an unfulfilled marine biologist, and with the developing urge to apply genetic technology to practical problems, I moved in 1975 to another position. The project was with Barrie Latter funded from his successful ARC grant application (then called ARGC). On my first day he made it clear that his philosophy was to work hard and enjoy the science. So I did. Moreover, such an environment was extremely productive, so from then on I endeavoured to model my own laboratory management on that philosophy. This new position again involved extensive fieldwork, which I loved, especially this time as it was mostly on prawn trawlers as a volunteer deckhand in tropical northern waters. The goal was to assess the use of genetic markers as tags for breeding stock differentiation of commercial prawn species. As an aside, we were able to use the same data to examine evolutionary relationships by cross species comparison of genomes using our crude molecular tools of the day (Mulley & Latter, 1980). But sadly, this approach proved impractical for resolving the breeding stocks (Mulley & Latter, 1981a, 1981b), due we thought to the degree of larval dispersal (gene migration) via the oceanic currents.

The Introduction and Distribution of European carp in Australia

The ability to multitask is one of the keys to a productive life in science. While at a fish biology meeting I met the inland fisheries biologist Karl Shearer, stationed at Narrandera in New South Wales, who had overlapping morphometric and meristic data with shifted means suggestive of strain differentiation between three known populations of European carp in Australia. I provided the definitive genetic confirmation of strain differentiation using electrophoretic markers (Shearer & Mulley, 1978). This resolved an interstate debate over the origin of the escapee carp responsible for the carp population explosion throughout the Murray–Darling river system during the 1960s. It was extremely pleasing to be able to apply genetic tags to answer an important fisheries question.

Value of a Solid Academic Grounding

There were lessons learned from my early years at the University of Sydney, as described above. No experiment or task was too great provided it was realistically matched to resources and operator capabilities, logistically organized down to the last detail and the laboratory tasks competently executed. To succeed, a young scientist had to be technically competent at the bench, because on temporary contracts one lives or dies at that level by the data one is able to generate with one's own hands, analyze and write up to at least draft manuscript stage. Then it becomes important to be able to conceive new projects, take them through to their completion to establish a credible track record, and then to keep a constant eye on what might be on or over the horizon. To put such pontificating into perspective, all of us who are established in our fields of expertise need to remember that for today's new graduates getting started and established, it is now far more competitive than it ever was.

Time for a Change

A change of faculty by Barrie Latter, taking him into wheat breeding, gave me the push start looking elsewhere. As luck would have it, the first newspaper I opened had a position advertized for a scientist to establish and manage a genetic markers laboratory at what was then the Adelaide Children's Hospital, in the field of human genetics. It was Martin Luther King Jr. who once said 'Faith is taking the first step even when

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you don't see the whole staircase'. Having never previously even remotely contemplated a career in human genetics Grant Sutherland interviewed me in The University of New South Wales cafeteria during a lunch break of the Second Annual Scientific Meeting of the HGSA. He emphasized that he published a lot — which I read as a clear message.

Thinking that there would be others in this field with experience in the application of markers to humans, it came as a shock when I found out that I was the successful applicant. I did not realize it at the time, but during the 1980s the field of human genetics began to blossom, and by either luck or design there is nothing like being in the right place at the right time. Later, it could be said that it exploded, especially after completion of the human genome sequence. My initial impression at the end of the 1970s was that the 'genetics' in 'human genetics' was not as strong or rigorous as the 'genetics' in 'animal genetics.' But now, in my field, I believe the tide has turned and animal geneticists can learn from human genetics, particularly since the advent of the Human Genome Project. For me, the start in animal genetics was advantageous, with much of what I learnt about population and quantitative genetics providing insights from another angle later on in human genetics. This is especially relevant now, where the emphasis in humans has shifted to complex disorders.

Marking Time in Human Genetics: 1978–1984

When I took up this new position in 1978 there were too few enzyme and protein polymorphisms and too few linkages between them and mutant human disease genes for immediate application of diagnosis by linkage, the purpose for which I was recruited. Therefore, further disease gene and marker mapping became the priority. Chromosome X had just one known polymorphic marker, the *XG* blood group said to map to Xq28. I was tasked to establish linkage between the fragile site *FRAXA* and *XG*. I never managed that; the reason being that *XG* was subsequently reassigned to the opposite end of the X chromosome! My first lesson in human genetics was this — keep an open mind and don't necessarily believe everything you read!

Compared with what followed later, this period was relatively quiet. I set up the linkage program, LIPED, on the hospital mainframe, the FORTRAN IV based workhorse widely used for linkage analysis in human pedigrees (Ott, 1974). Subsequently, I implemented the LINKAGE package written in PASCAL (Lathrop & Lalouel, 1984; Lathrop et al., 1984). I was immediately struck by its versatility to accommodate specific attributes for any given genetic disorder, making it an indispensable aid for research as it was then being carried out in human genetics, and for diagnostic application to risk determination for any diagnostic laboratory that used DNA markers linked to disease genes. I took turns with the departmental secretary at using the terminal during a lull in word-processing activity to access the hospital mainframe computer to run my linkage programs. Apart from electrophoretically detectable enzyme and protein markers, there were polymorphic blood group markers (Race & Sanger, 1975) that my first research assistant, Catherine Nicholls, typed as additional markers for application to linkage analyses. Toward the end of the 1980s Graham Suthers arrived from Sydney as a PhD student and used his PC (personal computer) wizardry to transfer the LINKAGE programs to an early PC, thereby releasing us from reliance on access to the hospital mainframe computer used primarily as a giant hospital filing cabinet prior to us seeking time on it for scientific purposes.

Gene mapping really took off in the early 1970s, as soon as each of the human chromosomes became individually identifiable through their unique G-banding fingerprints. A succession of biennial International Human Gene Mapping Workshops from 1973 onwards, each with expert committees specializing in updating all available information for each chromosome, greatly facilitated data exchange, which was synonymous with progress. The pace substantially quickened after Southern (1975) and the RFLP paper of Botstein et al. (1980), which opened the way for genome-wide hunts for linkage using DNA sequence markers, although their application was incredibly tedious compared with today's PCR and chip-based SNP technologies. Grant Sutherland walked into the laboratory in 1980 waving the Botstein et al. article, telling me everything I had just established in his cytogenetics unit was suddenly obsolete, and it was! Gene mapping was truly at the crossroads (Lindley, 1979).

The Linkage and Positional Cloning Era: 1980s to 1990s, and Beyond

X-linked Intellectual Disabilities: For Example, Fragile X Syndrome

Initially, gene identification was out of our reach and the perennial question at grant interviews was 'how will you identify the gene once you localize it by linkage?' At that time identification of the pathogenic defect at the DNA sequence level truly was akin to looking for a needle in a haystack. In the interim, I devised the computational procedure using the LINKAGE package of programs for determination of genetic risks in fragile X syndrome using recombination frequencies of flanking genetic markers and incomplete but variable penetrance, with the latter based on the parameters of (Sherman et al., 1985). The procedure was used to provide an ongoing diagnostic service and to pre-diagnose members of all our known fragile X families from the state of South Australia, plus some huge interstate families (Mulley & Sutherland, 1987), pending identification of the pathogenic mutation.

Then, the unstable CCG repeat responsible for Fragile X syndrome was identified within the department (Kremer et al., 1991; Yu et al., 1991). At one

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point we had given up on this, as we had perceived that other groups had too great a lead. The critical step getting us back into the race was accidental. Val Hyland made a genomic library based on a single human chromosome 16 in a mouse somatic cell hybrid made by David Callen. The chromosome 16 was missing its distal 16q sequence, but at that point had human Xq27-q28 translocated onto it. Remarkably, when Val separated out the human clones and mapped them, a disproportionately high number were (and this remains unexplained) from the Xq27-q28 region. Not known to us at the time, there were two more folate sensitive fragile sites close to *FRAXA*, namely *FRAXE* and *FRAXF*. Later we discovered that the human DNA fragment in Val Hyland's clone VK21 which was closely linked to *FRAXA* (Suthers et al., 1989), was in fact part of the *FMR2* gene associated with FRAXE mental retardation (Gecz et al., 1996).

This was a most exciting and historical period. Nobody had anticipated the inheritance of unstable triplet repeat units as responsible for fragile X syndrome, or any other genetic disorder for that matter. Within weeks of this discovery we carried out the first fragile X syndrome prenatal diagnosis, which was of an affected male fragile X fetus with a clear CCG expansion by Southern analysis (Sutherland et al., 1991). Since there was no precedent for diagnosis using triplet repeat expansion, we simultaneously confirmed the diagnosis using linked markers. We had prediagnosed members of all families by linkage from banked DNA in order to have the molecular defect instantly validated as the pathogenic lesion once it was identified (Mulley et al., 1992b; Yu et al., 1992). This new genetic mechanism involving unstable triplet repeat expansion was termed dynamic mutation (Richards & Sutherland, 1992).

It wasn't long before we detected another mechanism for fragile X syndrome, in a boy in whom Michael Partington swore blind had the syndrome on clinical grounds but without any cytogenetic expression of the fragile site. This case had no CCG expansion either, and in fact had no signal whatsoever by Southern analysis using the fragile X probe. It was attributable to deletion of a coding region of the associated gene *FMR1* (Gedeon et al., 1992), and similar cases have subsequently been detected.

X-Linked Mental Disabilities: Nonspecific and Syndromal X-Linked Mental Retardations (XLMRs)

One a Saturday morning in about 1987 Grant Sutherland and I met Gillian Turner, at her instigation, as she was passing through Adelaide. Discussions led to the initiation of collaboration between Sydney and Adelaide, which had major repercussions for the careers of several of us. We took the early international lead in the X-linked mental retardations (XLMRs) beyond Fragile X Syndrome, and we established the MRX field with the first gene localization, for MRX1 (Suthers et al., 1988). This Program on intellectual disability and Programs of the other Section Heads, David Callen, Rob Richards and Elizabeth Baker, transformed Sutherland's Unit within the Department of Histopathology into a fully fledged Department of Cytogenetics and Molecular Genetics in its own right, up until 2004 when it was merged with two other departments to form a super department, The Department of Genetic Medicine, under Eric Haan.

During an international workshop in Strasbourg in 1992 on fragile X syndrome and X-linked mental retardation, I was part of a subcommittee invited to establish international nomenclature guidelines for the XLMRs, other than fragile X syndrome. We formally established the incremental MRX numbering system for the nonsyndromal XLMRs (cognitive impairment with no other consistent clinical feature), defined their inclusion criteria, and we assigned syndrome names to the mapped but unnamed syndromal XLMRs (Mulley et al., 1992a).

The syndromes we named included three from Australia: Partington syndrome (Partington et al., 1988a), which was later to be incorporated within the *ARX* phenotypic spectrum (Strømme et al., 2002), Wilson-Turner syndrome (Wilson et al., 1991) with the gene still unidentified but the syndrome confirmed from other centers by clinical description and mapping, and Sutherland-Haan syndrome (Sutherland et al., 1988) subsequently found to be caused by mutations in *PQBP1* (Kalscheuer et al., 2003). Author names incorporated into the syndrome name were restricted to the subset responsible for characterizing the clinical aspects of the syndrome. Thus, the format in place pregene mapping for some of the classic syndromes of XLMR was retained, as in Coffin Lowry syndrome named earlier and mapped later (Partington et al., 1988b), and Borjeson-Forssman-Lehmann syndrome which we mapped (Turner et al., 1989) and went on to identify the gene (Lower et al., 2002).

Arguably the most common gene for pure nonspecific XLMR does not have an MRX symbol because it was definitively defined cytogenetically by a fragile site (*FRAXE*). The gene for fragile XE nonspecific mental retardation was the first of the nonspecific XLMRs to be identified (Gecz et al., 1996). A relationship between mild mental impairment and *FRAXE* has often been questioned because male *FRAXE* carriers were frequently within the normal range of intelligence. We regarded the distribution of intellectual functioning as simply having been shifted downwards, below where it would have been without the fragile site. Not all individuals with the fragile site are shifted down far enough to enter the intellectual disability range. If this conceptualization is correct, then there are likely to be other mild mutations in the community with similar effects, but these would remain unrecognized without an independent marker to delineate them, as we had with the fragile XE site. We named the *FRAXE* associated gene *FMR2* (Gecz et al., 1996). Relationship of *FRAXE* with mental disability is reinforced with study

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of affected families (Mulley et al., 1995), one of which was unusual in segregating both *FRAXA* and *FRAXE*.

Using the flanking marker data arising from the MRX mapping studies, we translated the gene localizations for each family to diagnosis, by linkage. Every family represented a unique 'private syndrome', involving mutation in a different unidentified gene. By using informative flanking markers one could follow the haplotype of linked markers surrounding the disease gene through a family (sometimes called gene tracking) and make a predictive diagnosis with very high accuracy, with error rate less than the chance of double crossover between the closest flanking markers. Much is mentioned these days of personalized medicine, but looking back, what we were doing then was an early form of personalized genetic medicine. This was further précised by applying direct diagnosis for the mutation once we, or others, identified the gene in each family.

We mapped a number of MRX genes on chromosome X (Gedeon et al., 1996), facilitated by our early in-house development of a PCR-based linkage map of chromosome X (Donnelly et al., 1994b). Our first estimate for the number of MRX genes based on nonoverlapping localization intervals was a minimum number of genes for nonspecific XLMR not less than 8 (Gedeon et al., 1996). This estimate soon rose empirically to 11, and we argued that this number could reasonably be doubled to at least 22 (Gecz & Mulley, 2000). The European XLMR Consortium took over the international lead through sheer weight of family material and investigators in a concerted and well-coordinated multicenter approach to MRX. This, together with the application of large scale genome sequencing approaches to gene discovery (Chiurazzi et al., 2008; de Brouwer et al., 2007; Ropers, 2006), has led to 25 specified nonspecific XLMR genes in about half of all known MRX families (de Brouwer et al., 2007). Given the extent of genetic heterogeneity, with only about half of the families solved, then as many as 50 MRX genes on chromosome X could reasonably be predicted. This provides some insight into the complexity of the human brain just to ensure its normality in one of its functions, cognition. For the other brain disorders like schizophrenia, bipolar disorder and epilepsy as examples, and all with complex phenotypes and complex genetics, complexity at least as great as for cognition can be predicted.

Jozef Gecz refers to the approach where every gene on chromosome X is sequenced in every putative X-linked disability family (Raymond & Tarpey, 2006) as 'Tour de Force'. The field promises to take yet another leap forward through array based exon capture technology targeted at index cases with X-linked disabilities (Albert et al., 2007; Hodges et al., 2007). Eluted DNA will then be subjected to high throughput sequencing to cover the same coding regions as the initial Tour de Force approach, but far more economically. Theoretically then, for any mutation within any exon for any monogenic disorder, especially for but no longer limited to chromosome X, there appears to be no escape for the remaining disease genes!

For the 12 MRX family localizations we made in the initial mapping era, the manual positional candidate approach resolved the gene identity for just two of them: *PAK3* for MRX30 (Allen et al., 1998), and *RSK2* for MRX19 (Merienne et al., 1999). In contrast, the Tour de Force approach has recently found the genes for another six. These are *HUWE1* for MRX17 and MRX31 (Froyen et al., 2008), and IQ gene for MRX1 and MRX18, *IL1RAPL1* for MRX11 and *JARIDIC* for MRX13 (J. Gecz, personal communication).

This Tour de Force approach led to detection of mutation in *PCDH19*, both an epilepsy and intellectual disability gene (Dibbens et al., 2008). This convergence between epilepsy and intellectual disability might represent a lead into a new gene family with members responsible for either or both; epilepsy is not an uncommon component of the phenotype of many intellectual disability genes.

The distinction between nonspecific and syndromal XLMR was a useful operational separation for some years, to categorize the complexity we saw before us. But like many artificial systems of classification there are exceptions. *RSK2* mutation in MRX19 was the first exception (Merienne et al., 1999), when it was already the established gene for Coffin-Lowry syndrome. I initiated this collaboration for no good reason other than we had mapped MRX19 to an interval which crossed the *RSK2* location (Donnelly et al., 1994a), Andre Hanauer in Strasbourg was testing patients with Coffin Lowry syndrome and there was no reason why a milder mutation might not confer a milder phenotype, like intellectual disability alone (as in the MRX series of disorders). Subsequently other XLMR genes responsible for syndromes have been found to have a similar phenotypic spectrum including MRX (de Brouwer et al., 2007). Of the 2% to 3% of the population with ID, at this time only a few of the biochemical ones like PKU deficiency are treatable. A broader overview of XLMR is given elsewhere (Chiurazzi et al., 2008; Sutherland et al., 2007).

Other Disorders: X-Linked and Autosomal

The experience gained working with a disorder with unstable repeating units inherited through families (fragile X syndrome) led to solution of another long standing mystery associated with an unrelated disorder. From our diagnostic referrals of families with myotonic dystrophy (another unstable DNA repeat disorder) I observed that the transmission behavior of the larger heritable expansions differed depending upon whether they were passed on maternally or paternally. As a general rule, maternal transmissions of large expansions expanded further accounting for infants with severe congenital myotonic dystrophy; whereas paternal transmissions of similarly large expansions contracted closer to the normal range. Thus, the diagnostic laboratory resolved the mecha-

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nism for the long seen clinical conundrum of exclusive maternal origin for severe congenital myotonic muscular dystrophy (Mulley et al., 1993).

Throughout the 1980s to 1990s a command of linkage-based analyses were as strategically important to a genetics laboratory as data mining is to the modern day molecular genetics laboratory. Working in close proximity to a medical genetics unit and the laboratory staff enthusiastic about collaboration, either with the next room or across the world, there were now many opportunities.

Apart from genes for X-linked disability, genes for a variety of other X-linked recessive and autosomal disorders were assigned to chromosomes, and localized to specific regions within them. Genes have been identified for most of them by now, sometimes by us, sometimes by the rest of the scientific community. Either through linkage or direct mutation detection we then provided where appropriate diagnostic feedback to the families. These other X-linked disorders included a blood group suppressor gene (Mulley et al., 1988), reticulate pigmentary disorder (Gedeon et al., 1994), X-linked fatal infantile cardiomyopathy (Gedeon et al., 1995b) and X-linked spondyloepiphyseal dysplasia tarda (Gedeon et al., 1999). The autosomal disorders included central core disease (Haan et al., 1990), nemaline myopathy (Laing et al., 1992), FGFR3 craniosynostosis and FGFR3 deafness (Hollway et al., 1995, 1998) familial periodic fever (Mulley et al., 1998), hyperphosphatasia (Cundy et al., 2002) and various defined epilepsy syndromes as shown below (Table 1).

Genomics of Human Chromosome 16

To assign disease genes to chromosomes and refine their chromosomal localizations, genetic maps of densely distributed markers are required. We chose as our genomic contribution human chromosome 16. It had three different types of fragile site and a selectable marker APRT in order to construct single whole or partial chromosome 16's in somatic cell hybrids, for ease of physical mapping of new markers (Callen et al., 1988, 1989). Next we did a correlation of the genetic and physical maps of chromosome 16 (Kozman & Mulley, 1996; Kozman et al., 1993), then a PCR based genetic map (Shen et al., 1994) and finally we coordinated the international multicenter consortium map of chromosome 16 to create the first definitive genetic map for human chromosome 16 (Kozman et al., 1995) incorporating all recognized genetic markers known at that time which had been genotyped on the same standard set of family DNA. These whole chromosome maps were placed in the public domain as fundamental

Table 1

Genes and Susceptibility Loci for 'Monogenic' and Complex Epileptic Channelopathies

Gene	Syndrome	Year of discovery (References)
Monogenic channelopathies:		
CHRNA4	ADNFLE	1995 (Steinlein et al., 1995)
KCNQ2	BFNS	1998
KCNQ3	BFNS	1998
SCN1B	GEFS$^+$	1998 (Wallace et al., 1998)
SCN1A	GEFS$^+$/(SMEI)	2000/(2001)
CHRNB2	ADNFLE	2000 (Phillips et al., 2001)
GABRG2	CAE/FS/GEFS$^+$	2001 (Wallace et al., 2001)
SCN2A	GEFS$^+$?/BFNIS	2001/2002 (Heron et al., 2002)
GABRA1	ADJME, CAE	2002/2006
CLCN2	IGE	2003
CHRNA2	ADNFLE	2006
Monogenic, closely aligned with Channelopathies:		
LGI1	ADPEAF	2002
Monogenic or polygenic (?), and the relationship to ion transport is unclear:		
EFHC1	JME	2004
Complex epilepsy: Components of polygenic Channelopathies:		
CACNA1H	CAE, IGE	2003/2004 (Heron et al., 2007)
GABRD	IGE, GEFS$^+$	2004 (Dibbens et al., 2004)
KCND2	TLE	2006
GABRB3	CAE	2006

Note: Abbreviations in order of appearance:

Genes: *CHRNA4*: acetylcholine receptor subunit, α4; *KCNQ2/KCNQ3*: potassium channel subunits; *SCN1B*: sodium channel β1 subunit; *SCN1A*: sodium channel α1 subunit; *CHRNB2*: acetylcholine receptor subunit, β2; *GABRG2*: $GABA_A$ receptor subunit γ2; *SCN2A*: sodium channel α2 subunit; *GABRA1*: $GABA_A$ receptor subunit α1; *CLCN2*: chloride channel gene 2; *CHRNA2*: acetylcholine receptor subunit α2; *LGI1*: leucine-rich, glioma inactivated 1 gene; *EFHC1*: protein with an EF-hand motif; *CACNA1H*: $Ca_v3.2$ T-type calcium channel; *GABRD*: $GABA_A$ receptor subunit δ; *KCND2*: $K_v4.2$ α subunit voltage gated potassium channel of the *sha*l subfamily; *GABRB3*: $GABA_A$ receptor subunit β3

Syndromes: ADNFLE: autosomal dominant nocturnal frontal lobe epilepsy; BFNS: benign familial neonatal seizures; GEFS$^+$: generalized epilepsy with febrile seizures plus; SMEI: severe myoclonic epilepsy of infancy; CAE: childhood absence epilepsy; FS: febrile seizures; BFNIS: benign familial neonatal–infantile seizures; ADJME: autosomal dominant juvenile myoclonic epilepsy; IGE: idiopathic generalised epilepsy; ADPEAF: autosomal dominant partial epilepsy with auditory features; JME: juvenile myoclonic epilepsy; TLE: temporal lobe epilepsy.

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resources for the international disease gene-mapping community for locating the genes for the many Mendelian genetic disorders to specific chromosomes that were without a home at that time.

The Positional Candidate Era and Epilepsy: 1990s to Present

As fate would have it, in about 1994 I received another lucky break. Samuel Berkovic heard of the genomic work carried out by Grant Sutherland's department and arrived with his briefcase bulging with epilepsy pedigrees for a meeting with us. Thus we met an academic neurologist adept at taking family histories, realized the way forward in his field was through molecular genetics, and he wanted to collaborate. This collaboration between Adelaide and Melbourne remains very much alive and well today.

The Epilepsy Program, like that of the Intellectual Disability Program, now has links with a multidisciplinary network of national and international clinical and scientific collaborators. Like intellectual disability, the frequency of epilepsy in all communities is about 2% to 3% of the population. But for epilepsy this applies as a whole of life figure, because at any one time less than 1% of the population are affected due to late onset in some cases, and effective drug treatments in others.

Some of the areas where we have made most impact are as follows.

Gene Identification

Table 1 shows the big picture outcome for the epilepsy collaboration. After our identification of the first gene for idiopathic epilepsy (Phillips et al., 1995; Steinlein et al., 1995) we subsequently played a major role in establishing the molecular basis for other 'Mendelian' epilepsies. We have identified mutations of large effect segregating through autosomal dominant pedigrees in acetylcholine receptor subunits, sodium channel subunits and $GABA_A$ receptors subunits (Table 1). Details of these studies plus those which have identified potassium channel subunits and other functionally validated epilepsy genes can be found in comprehensive reviews (Helbig et al., 2008; Heron et al., 2007c; Mulley et al., 2003, 2005a, 2005b).

As was observed for the XLMRs, each validated epilepsy gene is a rare cause of familial epilepsy. Complicating gene localization for epilepsy however, apart from the rarity of large families, is the often extreme range of phenotypic expression within families associated with the same primary mutation. Moreover, a number of epilepsy syndromes are genetically heterogeneous caused by mutations in more than one gene (Table 1).

Mutations in most of the known epilepsy genes discovered by us and others (Helbig et al., 2008; Heron et al., 2007c; Mulley et al., 2003; Table 1) lead to dysfunctional ion channels interfering with normal transmission of electrical signals through the brain. Apart from translation of this research to the application of new molecular diagnostic tests creating the basis for a molecular system of classification and diagnosis for these clinically complex and genetically heterogeneous disorders, by the time we identified *SCN1B* (Wallace et al., 1998) it had become apparent that we were teasing out members of a family of channelopathies. All of this was based on rare large families we believe have provided valuable insights into what to look for to unravel the genetic architecture of the epilepsies with complex genetics.

SCN1A and Dravet Syndrome

The majority of cases with *SCN1A* mutations have a severe degenerative brain disease associated with their seizures but symptoms can be wide ranging, with merely a mild form of generalized epilepsy at the other end of the spectrum (Mulley et al., 2005b). Since about 20% of cases at the severe end of the spectrum do not have detectable defects in the gene primarily responsible for this clinically defined group of disorders, the question is raised whether these syndromes are best classified in the laboratory taking advantage of the new molecular genetic tools rather than continuing to rely solely on clinical assessments. The answer is that a mixture of both approaches is now required. Clinical patterns are used to suggest the laboratory test required and the laboratory test clinches the diagnosis where there might be clinical ambiguity, or makes the differential diagnosis (Berkovic et al., 2004).

We found that infants and adults have detectable molecular defects in the sodium channel gene *SCN1A* in a greater variety of epilepsies than previously suspected (Harkin et al., 2007; Wallace et al., 2003). Translation of this knowledge to the provision of diagnostic services has allowed earlier diagnostic confirmation for a wider range of epilepsies, thus eliminating a raft of further costly investigations that would otherwise be necessary to arrive at a diagnosis. The faster the diagnosis can be made the quicker the family gains closure in their quest for an explanation for problems in their family member and the quicker the most effective medication can be prescribed to alleviate the effects of the seizures.

Solution to Alleged Cases of 'Vaccine Encephalopathy'

Whooping cough vaccination has long been causally implicated in progressive brain disease associated with intellectual disability and seizures that do not respond to conventional treatments. This has serious medico–legal implications for companies that manufacture and supply vaccines. We found that such cases had detectable genetic defects in the known epilepsy gene *SCN1A* that predisposes individuals to these adverse effects, providing evidence that the vaccine itself is not the causative factor (Berkovic et al., 2006). This finding has major societal implications that relate to the acceptance of vaccination for the infant population, and should eliminate inappropriate litigation directed at vaccine manufacturers.

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Extending the Range of Genetic Mechanisms for Childhood Epilepsies

We found that the two genes most commonly involved in heritable childhood epilepsies (*SCN1A* and *KCNQ2*) now account for significantly more cases of certain epilepsy syndromes than previously thought (Heron et al., 2007a; Mulley et al., 2006). This has led to the development of a single-step second-tier testing strategy (after sequencing) using MLPA (multiplex ligation-dependent probe amplification) in order to diagnose with greater specificity a substantial number of additional cases caused by deletions and duplications. For those deletions that extend beyond either end of the target gene, array CGH (comparative genome hybridisation) is useful for further characterization for size and gene content (unpublished data).

Susceptibility Genes for Epilepsies With Complex Genetics

Most of the epilepsies in the community are the common types with complex genetics. Terminology is crucial here, to distinguish epilepsies with complex genetics from complex epilepsies, which are complex in the clinical context, and not necessarily in the genetic sense. As for many of the complex brain disorders, association studies in epilepsy are yet to be informative (Tan et al., 2004, 2006). In contrast, we have made early inroads with a functional approach, prompted by our extension of the channelopathy paradigm to the common epilepsies (Dibbens et al., 2004; Heron et al., 2007b). Functional characterization of suspected susceptibility variants is slow, expensive and labour intensive, involving cell lines and animal models with no guarantee of detecting what would only be relatively small effects and no a priori knowledge of which experiment will be the definitive experiment. None of the proposed susceptibility variants could have been detected by association studies because of their rarity among cases of the syndrome examined.

Thus, we proposed the genetic heterogeneity model for epilepsy with complex genetics (Dibbens et al., 2007; Mulley et al., 2005a). The genetic basis is likely to be a large number of susceptibility genes each of small but additive or interactive effect, with a heritable subset of these polygenes being responsible for epilepsy in any given patient, often modulated by presence, absence or intensity of environmental triggers. Such epilepsies are not amenable to conventional gene mapping and gene identification technologies. Therefore, there has been very slow, and very little, progress in working toward knowledge of their underlying genetic architecture, with success of association studies unlikely if the polygenic heterogeneity model is correct.

Many do not share my view on association studies as applied to epilepsy. Although I have serious doubts about the approach, I have hedged my bets by encouraging and participating in international multicenter studies looking for associations in order to fully explore that possibility with greater statistical power (Cavalleri et al., 2007a, 2007b; Hempelmann et al., 2006). The model under test is the common variant common epilepsy model (Mulley et al., 2005a). So far the utility and robustness of our polygenic heterogeneity model has been reinforced by failure to find solid evidence in support of the alternative model. A stream of contemporary genome-wide association studies present positive associations for a range of disorders, but these account for only a small proportion of the heritable component of the phenotype (Kruglyak, 2008).

In my view, the technology is not yet here to determine the genetic architecture for any but those epilepsies at the 'simplest' end of the spectrum of disorders with 'complex genetics'. Of the brain disorders, of which epilepsy is but one, it is difficult to foresee how clinical endophenotyping can sufficiently homogenize underlying polygenic architectures such that common variants will emerge and be detectable by association studies. The time of feasible and economical whole genome sequencing of large numbers of cases is here and is happening. In the genome community attempts are being made to develop analytical algorithms predictive of function for dealing with the large numbers of rare variants identified by such mass sequencing (Ng, 2006). However, knowledge of three-dimensional protein structures and characterization of their functional domains is only in the early stages so this computational approach remains some distance over the horizon.

Intuitively, it is my prediction that much of the genetic susceptibility for epilepsies with complex genetics is simply an extension of the channelopathy concept for 'monogenic epilepsies' to a polygenic suite of variants each of somewhat lesser effect than the mutations detected in the monogenic epilepsies. The table demonstrates that with what little is so far known about the identity of susceptibility genes, results so far are consistent with the prediction. Gargus (2003) highlights the fact that there are an abundance of neuronal ion channels throughout the genome as potential polygenic susceptibility gene candidates. If that is the case, we are still unable to hazard a guess at this early stage as to how much of the susceptibility to common idiopathic epilepsies is modulated by genes other than ion channels.

Kryukov et al. (2007) propose association studies from a different angle. Their premise is that most variation present in the population at frequencies of less that 1% is at least mildly deleterious. Thus, for a complex disorder like epilepsy where there are likely to be numerous individually rare mildly deleterious variants, the expectation would be enrichment of rare variants within a patient group as compared with a control group. There are at least two large-scale sequencing initiatives underway for ion channels, so the outcome of these will be awaited with much interest.

The rich supply of copy number variations (CNVs) now recognized as 'normal' in all individuals (Iafrate et al., 2004; Sebat et al., 2004) may indeed modulate the expression of any epilepsy susceptibility gene, supplementing the conventional coding variation detectable by sequencing as another potential source

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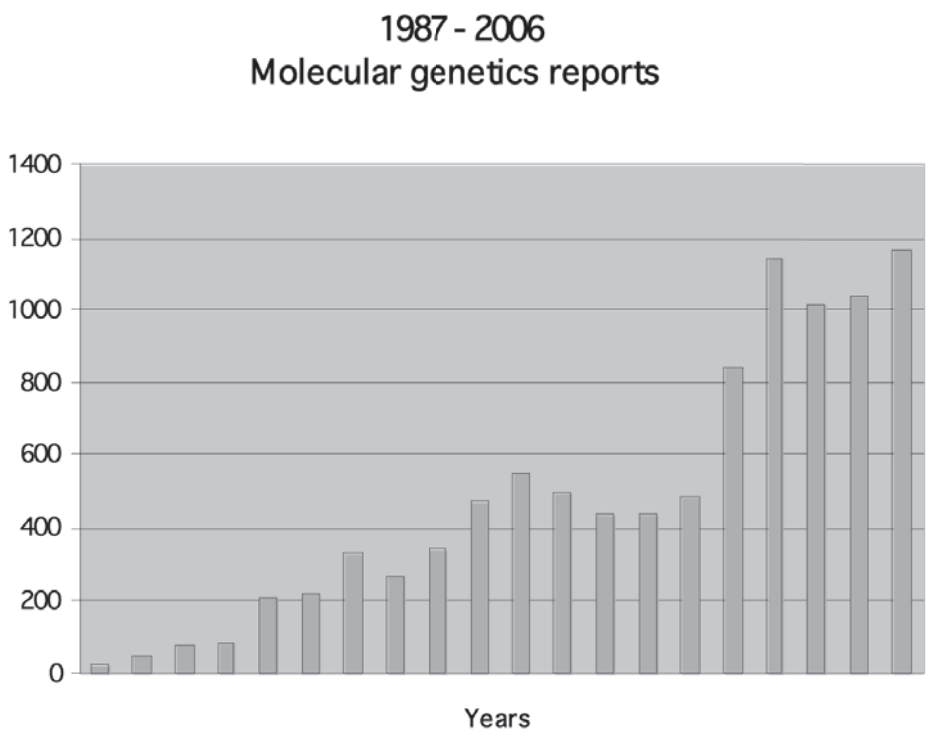

Figure 1

Continuous rise in molecular diagnostic workload based on annual number of diagnostic reports for the period 1987–2006.

of susceptibility for disorders with complex genetics (Sebat, 2007).

We sometimes use terms like 'personalized medicine' in our grant applications. If risk of susceptibility is really the summation of many small contributions from over a large number of susceptibility genes, very few of which are yet known or suspected, then what will genotyping be able to tell us about risk modification? What loci in fact are we supposed to genotype? Will that help determine treatment any more precisely or any faster than just prescribing a succession of therapeutic drugs until one is found by trial and error to control seizures? There seems no point at this time in offering genome-wide genotyping to anyone, despite its present and improving technical feasibility as evidenced through recent sequencing of 'celebrity genotypes' (Check, 2007).

Flirtation With Commercial Biotechnology: 2000 to 2005

This period was the most challenging and stimulating years I have had in science, even though I know that I personally do not fit the mould for a career in mainline commercial biotechnology. This meant that in a normal day for a period of 6 years I wore three distinct hats across two laboratory sites located in different suburbs, North Adelaide and Thebarton. One hat was with the hospital diagnostic molecular genetics service, another hat involved the most appropriate use of NH&MRC-derived research funding in pursuit of knowledge for the long-term benefit to humankind, and the third hat was as a hospital-approved consultant to Bionomics Ltd, as Head of Epilepsy Genetics. There, my research-funded scientists were colocated with company employees, with some of them funded by the company.

The bottom line for the company, by necessity for its survival, was: what can you give us to sell? Although I always remained a full-time hospital employee, I was integrated into the management group meetings, scientific reviews and biannual Scientific Advisory Board Meetings of Bionomics Ltd where I made regular presentations. The company provided us with state-of-the-art equipment, unsurpassed spatial facilities, and all without the burgeoning bureaucratic impediments now choking us in the public sector. This worked particularly well for both sides, with the Epilepsy Program remaining academically competitive with greatly increased funding and better working conditions. In return, we provided Intellectual Property to facilitate the early development and sustainability of the company. This academic–health–industry collaboration represented a unique relationship in Australia at the time. It ended in mid-2005 with the company moving away from genomics and forward into drug discovery.

Molecular diagnostics

When my involvement with molecular diagnostics ended in 2006, the spectrum of diagnostic tests offered was extensive. Figure 1 shows the relentless expansion

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of workload over time expressed simply in terms of number of reports issued per annum up until 2006. In 2008 this bar graph continues to rise and now is fully coordinated by Kathie Friend as it is now a fulltime job with no scope for research.

I recall two incredible outcomes derived from the diagnostic laboratory. The first arose when we were testing for fragile X syndrome in a developmentally delayed male. The fragile X sequence was normal, but the signal on the southern blot from the control probe was absent. This led us by chance to what had previously been the elusive *FMR2* gene (Gecz et al., 1996; Gedeon et al., 1995a). The second time serendipity struck was when we were carrier testing using linked markers in two families with X-linked spondyloepiphyseal dysplasia tarda. Remarkably, we observed that the closest distal Xp recombinant in one family and the closest Xp proximal recombinant in the other family were virtually on top of each another. This was timely, as the region had been sequenced and deposited on the public sequence database only a week earlier. The region contained only four genes, and the first one to be sequenced disclosed the pathogenic mutations, and therefore the responsible gene (Gedeon et al., 1999, 2001). This was yet another gene test that we introduced which now has worldwide significance. Its mode of discovery and much, much more, fully vindicates the resources poured into completion of the Human Genome Project.

The test menu by 2006 included the following: fragile XA syndrome, FRAXE mental retardation, myotonic muscular dystrophy, Duchenne and Becker muscular dystrophies, Craniofacial disorders (various), achondroplasia, spinocerebellar ataxias (SCA1, SCA2, SCA3, SCA6, SCA7 and SCA8), dentatorubral pallidoluysian atrophy, Kennedy spinobulbar muscular atrophy, Friedreich ataxia, familial Mediterranean fever, connexin 26, spinal muscular atrophy, Angelman/Prader Willi syndrome, incontinenti pigmenti, other rare X-linked mental retardations, other X-linked disorders and other rare autosomal disorders after prior consultations.

Based on nearly 40 years of experience as scientist in charge of laboratories, there is much more I would like to say about the management of diagnostic molecular genetics, especially over the past decade, but since it would not be complimentary, this is not the place. I found out repeatedly that as a scientist in the present climate nothing more can be done about this from our level. The onus therefore lies fairly and squarely on those now empowered to manage, to do so.

Life's Branching Points

Recall the film *Sliding Doors* in 1998, directed by Peter Howitt, where Helen (played by Gwyneth Paltrow) misses a tube train by a few seconds? Throughout the film we see her life unfold in two separate but parallel universes, one when she just missed the train and the other when she just caught the train. The branch points we encounter in life and in our careers can only ever be explored by choosing and following just one of the roads before us. This is an area of speculation that fascinates me. Human genetics, largely through molecular applications which we embraced from the mid 1980s onwards, just kept on evolving and transforming to such an extent that one did not need to change jobs or professions to seek stimulation and remain totally committed.

Life's Synergies (Acknowledgments)

Of those mentors who led me, Stuart Barker ran a tough school with a heavy work ethic, as well as being a great teacher providing a solid academic foundation to those setting out on a career path of population genetics, or quantitative genetics applied to animal production. Barrie Latter was a scientist in every aspect of the word, and a pleasure to work with. He had the ability to communicate complex concepts in population genetics in understandable language. Rodney Carter was department head and a pathologist with vision, responsible for promoting much of the scientific excellence for which the hospital through the laboratories became noted for, but in years now past. Grant Sutherland was mentored by Carter to rise up as a scientist department head with incredible capacity to get things done and with audacious objectives dragging the performance of the rest of us along to greater heights than might have been the case if left to our own devices. We section heads adapted well to productively using the grant funding he attracted; especially me, when he founded Bionomics Ltd, which allowed major expansion of the Epilepsy Program. He recruited us to do a job then let us get on with it. Eric Haan shines like a beacon among clinicians and as the present head of department in his tangible encouragement of scientific research and patient but determined restoration of realistic staffing levels and decent work conditions in diagnostic molecular genetics. There remain a few outposts of research, mainly in his department, clinging to a few cracks in the cliff face, despite past best and very direct efforts of some of our masters to dislodge us.

'Genetic nosology' in clinical genetics is the delineation of genetic diseases (McKusick, 1978). Gillian Turner and Michael Partington were crucial for clinically well-characterized material involving XLMR, and now they continue to supply this to my colleague Jozef Gecz. Similarly, Samuel Berkovic and Ingrid Scheffer were and remain crucial for the provision of well-characterized material involving epilepsy. Grant Sutherland either attracted or set up many of the clinical collaborations. Numerous others provided valuable clinical material and I apologize in advance to all of those who I am indebted to but have not named here.

Of those who succeeded from my laboratory in gaining professional fulfilment and international recognition for their work, I name but a few in chronological order. Agi Gedeon (scientist to PhD student to postdoc-

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toral scientist over 18 years), Helen Heddle (nee Kozman, scientist to PhD student to postdoctoral scientist for ~7 years), Jozef Gecz (postdoctoral scientist to co-chief investigator for 12 years and ongoing), Robyn Wallace (PhD student to postdoctoral scientist for ~7 years), Hilary Phillips (scientist colleague for ~15 years), Louise Harkin (scientist colleague for 6 years), Georgina Hollway (scientist to PhD student to postdoctoral scientist for ~5 years), Sarah Heron (scientist and currently PhD student, spanning 8 continuous years so far), Leanne Dibbens (postdoctoral scientist for 8 years and ongoing) and Kathie Friend (from scientist to postdoctoral scientist, now head of molecular diagnostics). Five of the above gained their PhDs in-house. My sincere apologies go to the large number of additional staff past and present who I have not mentioned by name.

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