We are now moving towards the new era of personalised medicine. In prospect, a DNA-tailored healthcare system will utilise genomic information from patients and populations for the prevention and treatment of diseases. This near-future medicine recognises that there is already evidence of genetic disparity between people of different ethnicity. Our own previous genetic surveys of human genomes have demonstrated abundant genetic variation in New Zealanders. These now include extensive new work on immune system genes. We have produced a comprehensive reference set of genotypes for clinically relevant antigens in transplantation (human leukocyte antigen, major histocompatibility complex class I chain-related gene A and killer cell immunoglobulin-like receptor) and transfusion (blood group and human platelet antigens) medicine in Māori and Polynesians. This report sets these data in context and highlights allelic variations and their implications for ancestry and health. It is hoped that this information may help to resolve some of the present inequalities in Māori and Pacific health status.

Introduction

We have been engaged on a programme of medical genetic research for 30 years. This tests the proposition that the gene pools of Māori and Polynesians are extensively differentiated from those of Europeans. Further, that these differences are markers of ancestry and medically significant. Many of our earlier reports support this position and we now review an extensive body of new evidence drawn from recently published analyses of immune system genes in these populations.

Article

New insights into ancestry and health of Polynesians and New Zealand Māori

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Our research programme has its roots in forensic science via establishment of first-generation genetic profiling via DNA fingerprinting (Hamilton et al. 1996). From 1988 to 1990 we collected a bank of DNA samples from volunteers in the Wellington area with the assistance of the local Blood Transfusion Service. Samples were grouped according to self-declared ethnic affiliation (the forensic standard definition), but supported with an interview questionnaire to better establish genetic ancestry. This latter feature was to prove of paramount significance for effective use of the VUW DNA Bank for medical genetic applications. Our forensic study confirmed the expectation that Polynesians have lower levels of genetic variation and showed that these levels were even further reduced in Māori (Clark et al. 1995). Such initial findings raised concerns about the utility of the then available genetic techniques to build unambiguous missing persons databases. This problem was investigated using mitochondrial DNA (mtDNA) (Whyte et al. 2005) and Y-chromosome (Underhill et al. 2001) markers. There were two remarkable extension findings from these two studies. First, in our Māori subjects approximately 15% of mtDNA genomes and 50% of Y-chromosomes proved to be of European origin, reflecting the gender-biased pattern of recent historic admixture. However, this also implies that minimally 35% of them are of full Māori descent. Second, the maternal and paternal genetic lineages track back to different geographic origins: Taiwan Aboriginal for mtDNA and Papuan for Y-chromosomes. Again this reflects historic gender-biased geneflow, but set much...
Recent insights into ancestry in Māori and Polynesians

The first question that must be answered is: How did the European and Polynesian genepools become so divergent? The primary answer lies in the early separation of genetic lineages as anatomically modern humans first left Africa (Chambers 2008, 2013). Our presently emergent picture of Polynesian origins is captured by the extended version (Chambers & Edinur 2013, 2015) of the ‘Synthetic Total Evidence Model (STEM)’; see Table 1 for this and other competing models.

According to STEM, the distal sources of populations that contribute to the settlement of Remote Oceania can be divided into at least two groups; first an ancient Papuan-speaking Australoid lineage (P-SA; ancestors of Australian Aboriginals, ex-coastal Melanesia, hill tribes of Papua New Guinea, Andaman Islanders and Negritos) moved South from Asia 40,000–70,000 yrs ago and the second group of Austronesian-speaking Mongoloids (A-SM) who first occupied Taiwan around 4000–6000 yrs ago and the second group of Austronesian-speaking Mongoloids (A-SM) who first occupied Taiwan around 4000–6000 yrs ago (see Figure 1). The A-SM people migrated to Island South East Asia (ISEA), coastal Papua New Guinea and Island Melanesia between 5000 to 3500 years ago (Bellwood et al. 2011, Chambers & Edinur 2013, 2015). In coastal settlements around northern mainland Papua New Guinea and the neighbouring islands, matrilineal marriage meant that numerous P-SA men were recruited by the A-SM voyagers and contributed towards a gender-biased genetic admixture process; see Chambers & Edinur (2013) for further details. The newly formed hybrid population (ancestral Polynesians) then moved outwards to Remote Oceania (Polynesia) and eventually occupied New Zealand from around 750–650 years before present (ybp). Genetic admixture continues today as Māori and Polynesian people intermarry with the descendants of Europeans who arrived in New Zealand over the last 150 years. These historical events have left a ‘genetic trail’ that stretches from Taiwan to New Zealand, Hawaii and Easter Island.

The general account above, and other recent New Zealand commentators (Gosling et al. 2015, Matisoo-Smith 2015), has received independent confirmation from large-scale phylogenetic studies of Pacific and East Asian languages (Gray et al. 2009, 2011) and autosomal genes (Friedlaender et al. 2008, Kimura et al. 2008, Lipson et al. 2014, Wollstein et al. 2010). The story that developed from analyses of these autosomal markers showed a hybrid A-SM:P-SA origin of Polynesians. They also clarify the enigma arising from works on maternal (mtDNA) and paternal (Y-chromosome) markers. As noted previously, many mtDNA analyses link Polynesians to Taiwan, but Y-chromosome data showed a predominantly ‘Melanesian’ influence in the Polynesian paternal genepool (Benton et al. 2006).

Table 1. Models of population expansion to Oceania.

<table>
<thead>
<tr>
<th>Model</th>
<th>Descriptions</th>
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<tbody>
<tr>
<td>The Express Train to Polynesia model by Diamond (1988)</td>
<td>Based on the ‘Out of Taiwan’ archaeological hypothesis proposed by Bellwood (1985). According to this hypothesis, common Austronesian ancestors are hill tribe aboriginal people of Taiwan who migrated southwards via the Batanes Islands, Philippines to Remote Oceania without measurable pause in Near Oceania.</td>
</tr>
<tr>
<td>The Slow Boat Model by Oppenheimer &amp; Richards (2001)</td>
<td>This model suggested Island Southeast Asia as the origin of Polynesians, which is in marked conflict with archaeological chronology.</td>
</tr>
<tr>
<td>The Synthetic Total Evidence Model (STEM) by Chambers (2006)</td>
<td>This model embraces all pattern and process elements including Out of Taiwan, gender-biased and reticulated admixture process in Near Oceania and genepool refinement via bottlenecks and founder effects.</td>
</tr>
</tbody>
</table>
Figure 1. Migration patterns of A-SM in Island South East Asia, Near and Remote Oceania based on archaeological dating. The directions of movements are inferred from sequential chronology and linguistic evidence. Abbreviations: A-SM (Austronesian-speaking Mongoloid), P-SA (Papuan-speaking Australoid) and E (Europeans). This figure is reproduced after Bellwood et al. (2011).

New insights into ancestry in Māori and Polynesians

With this background in mind we can now turn our attention to the ways our recent reports on immune system markers further illustrate this story. Our genetic surveys on human leukocyte antigen (HLA), major histocompatibility complex class I chain-related gene A (MICA), killer cell immunoglobulin-like receptor (KIR), blood group and human platelet antigen (HPA) provide novel ancestry-informative data for Māori and Polynesian people and can be used to test reconstructions of Pacific colonisation history (Askar et al. 2013, Edmur et al. 2012, 2013a, 2013b, 2013c, Nemat-Gorgani et al. 2014, Riccio et al. 2013). All these new datasets were compared with those reported for Asia Pacific populations, including their putative ancestors, A-SM and P-SA. Phylogenetic and principal component analyses showed that Polynesian sub-populations are more closely related to one another than either one is to those from other Asia Pacific regions. Estimations made based on HLA-A, -B, -C and -DRB1 allele frequencies show surprisingly wide-ranging A-SM:P-SA ancestral fractions (55:45 to 90:10) in Māori and other Polynesians (e.g. see Figure 2). This phenomenon might appear to reflect different proportions of genetic inputs from their ancestors, but is more likely associated with population histories, which include founder effects and local selective forces.

Overall, our findings are entirely consistent with the expectation that both Polynesians and Māori are intermediate between A-SM and P-SA populations (Kimura et al. 2008, Wollstein et al. 2010). In particular, Polynesian sub-populations share a common Taiwanese ancestor and have experienced gender-biased admixture with P-SA populations. These results complement previous mtDNA and Y-chromosome haplotype scores frequencies for the VUW DNA Bank samples (Friedlaender et al. 2008, Kimura et al. 2008, Underhill et al. 2001) and thus correspond to the accepted archaeolinguistic reconstruction of Pacific settlement (Bellwood et al. 2011, Gray et al. 2011, Kirch 2010).

Genetic data collected for Polynesians over the years contribute significant support for key elements of the Synthetic Total Evidence Model of Pacific Settlement. This review brings them together for the first time since its first formulation (Chambers 2006) and extends the later accounts (Bellwood et al. 2011, Chambers 2013, Chambers & Edinur 2015) which are focussed on support for the Out of Taiwan component. Today, the gene pools of Polynesians and Māori are expanding through opportunities for intermarriage with members of other ethnic groups (i.e. principally European). We have demonstrated (e.g. see Figure 3) the significant effects of this admixture in the genepool of present day Māori and Polynesians, and its medical importance is discussed in the following section.

New insights into health in Māori and Polynesians

It is important to recognise New Zealand as a genetically heterogeneous multi-ethnic country, and all medical consequences of this composition have to be explored. We have reported extensively on the unique genetic repertoire in the transfusion and transplantation antigens and genes in Māori and Polynesians as compared with other well characterised populations such as Europeans and North Americans. Currently, there is a general shortage of studies in Māori and Polynesians and their genetic associations, drug responses, and medical conditions, including various autoimmune diseases. These include many such as subcutaneous T cell panniculitis and diabetes, which are common to Polynesians, or like Sydenham’s chorea, which is disproportionately common to them (Jackson & Lennon 2015, Woo et al. 2003). However, Māori and Polynesians are often unaffected by autoimmune diseases which afflict Europeans.
Figure 2. HLA class I and II allele frequencies were used to estimate ancestral fraction in Māori and Polynesian sub-populations based on simulated proportions of Taiwan Aborigines (TA: Taiwan Ami, Atayal, Paiwan, Rukai, Puyuma and Tsou) and Papuan-speaking Australoids (P-SA: Papua New Guinea Goroko and West Schrader Ranges) metadatasets (MD). Reference populations obtained from Edinur et al. (2009), Gonzalez-Galarza et al. (2011), Lin et al. (2000), Main et al. (2001), Velickovic (2001), Velickovic et al. (2002). Abbreviations: A-SM = Austronesian-speaking Mongoloid, P-SA = Papuan-speaking Australoid, MFA = Māori with Full Ancestry, PFA = Polynesians with Full Ancestry and PNG = Papua New Guinea.

Figure 3. PCO plot constructed using HLA class I and II allele frequencies shows Polynesians falling as intermediate between cumulative A-SM and P-SA genepools. Māori with full ancestry (MFA) lie close to the general Polynesian cluster, and admixed Māori groups (TM, MAH and TTM) are displaced towards the European reference, ELAN. Reference populations obtained from Edinur et al. (2009), Gao et al. (1992a, 1992b), Gonzalez-Galarza et al. (2011), Hagelberg et al. (1999), Lin et al. (2000), Mack et al. (2000), Main et al. (2001), Tracey & Carter (2006), Tracey (2007), Velickovic (2001), Velickovic et al. (2002). Abbreviations: TM = Total Māori, MFA = Māori with Full Ancestry, MAH = Māori with Admixed History, TP = Total Polynesians, PFA = Polynesians with Full Ancestry, PAH = Polynesians with Admixed History, P = Velickovic Total Polynesians (Cook Islands + Tokelau + Tonga + Samoa), PMD = Polynesians Meta Dataset (VTP + PFA), TTM = Tracey Total Māori, A-SM = Austronesian-speaking Mongoloids, P-SA = Papuan-speaking Australoids.
such as ankylosing spondylitis, uveitis, psoriasis, rheumatoid vasculitis, and coeliac disease. These disorders are all associated with particular types of HLA, MICA and KIR alleles that are common in Europeans. Indeed, the complex issue of admixture fraction and methods for its determination present a serious potential barrier to the wider introduction of genetic-based medicine (Callister et al. 2015).

Increasing admixture with Europeans may mean that ‘European autoimmune diseases’ such as those above may become more common in self-declared Māori and Polynesian patients. We stress that simply pointing out that such differences exist as real phenomena does not in any way imply making value judgements about them or suggest that any one group may be genetically superior to another one. Nor should it be taken to imply fatalism in the sense that one might be doomed by ancestry to develop a particular set of conditions or in the sense that genes alone determine an individual’s fate. So these observations should not be interpreted as evidence to reinforce negative stereotypes of Māori, Pacific Islanders or Europeans. All these ideas are examples of outmoded ‘Biological Determinism’ thinking and ignore the important interactions between genotypes and environments. It is clear that changes in one’s environment or behaviour can often be positive influences on disease risk schedules.

Intermarriage between Polynesians and other ethnicities is not uncommon in New Zealand. Our statistical inferences showed a possibility of alloimmunisation during pregnancy (i.e. neonatal alloimmune thrombocytopenia: NAIT) in Europeans and Polynesians and the risk is increased for mixed marriage. These are due to the highly polymorphic nature of loci encoded for blood group and human platelet antigens and the presence of less frequent genotypes (HPA-1bb, HPA-3bb, and HPA-5bb among Europeans and HPA-6bb in PFA) in both population groups (see Bennett et al. (2002) and Edinur et al. (2013b)) and for HPA genotypes in Europeans and Polynesians. Several cases of HLA antibody-mediated NAIT have been reported, including HLA types (HLA-B*27 and HLA-B*56, respectively) that are common to Europeans and Polynesians (Hutchinson et al. 2015, Thude et al. 2006). The tissue type HLA-B*56 is already common in Polynesians (gene frequency: 0.03–0.34) and intermarriage with Europeans may increase the frequency of the tissue type HLA-B*27 (present gene frequency: 0.00–0.02) in Polynesians (Whyte et al. 2005). All these might lead to an increase in NAIT cases in Polynesians in New Zealand. Thus, prenatal diagnostics and genetic counselling may become the future preventive healthcare for some couples in New Zealand.

Several markers tested in Māori and Polynesians are characteristic of tissue identity and important in determining compatibility in transplantation (ABO, HLA, MICA and KIR) and transfusion (blood groups including ABO, Rhesus and Kell and HPA). Incompatibility between donor and recipient may lead to adverse effects including sensitisation, rejection, graft versus host disease and haemolytic transfusion reaction (Anstee 2009, Wise & Carter 2002). Therefore, knowledge on the prevalence and expression of these markers at the population level will help to design an efficient donor recruitment strategy for blood, stem cells and organ donation and will facilitate donor centres searching for donors with unique profiles. In general, feasibility of finding suitable donors for Polynesian and Māori patients is higher within their own ethnic groups, as they share common and unique gene pools. In this context, family donation is most likely to be an efficient way to maintain sufficient blood/organ supply in transfusion and transplantation and is acceptable in the Polynesian extended family culture (Wurtzburg 2003). However, patients with rarer genotypes and haplotypes present a great challenge for recruitment centres in searching for a compatible donor. The highly polymorphic loci encoding the clinically relevant transplantation and transfusion antigens together with past episodes of natural selection and admixture have equally introduced what are rare genotypes in Māori and Polynesians, but which may be common in other populations.

One good example is the presence of the tissue type haplotype (e.g. HLA*02:12-C*07:02-B*39:05-DRB1*08:02), which is common in Amerindians but not in Polynesians (Edinur et al. 2013a, Lie et al. 2007). This haplotype, and only this Amerindian-specific haplotype, has been observed in a number of New Zealand patients and donors tested by the Tissue Typing Laboratory, New Zealand Blood Service (P Dunn, personal communication). The very limited genetic contribution from Amerindians to the Polynesian gene pool is associated with the South American slave trade in the 18th century and this factor has also contributed towards a reduced genetic variability in several Polynesian sub-populations (Maude 1981). However, all these datasets confirm that those claiming full Māori ancestry (i.e. those with four Māori grandparents) do make up a significant fraction of the contemporary population (perhaps as high as 30–50%). Nonetheless, Polynesian and Māori gene pools are becoming more diverse, largely due to inter-group marriage with those from the European lineages. As a consequence, the numbers of available donors for un-admixed Polynesian and Māori patients become smaller, but increase the number of potential donor and recipient pairs that belong to the admixed group. This consideration may be critical in some situations. For instance, progeny of first generation admixture are most likely to find donors among similar individuals, because they have an equal mix of Polynesian and European HLA genes. Recruitment of blood and stem cell donors in Māori and Polynesians in New Zealand still needs to increase even further in order to capture enough donors with mixed ethnicity.

Recently, Cornwall et al. (2015) conducted a survey to study New Zealanders’ knowledge and attitudes to organ and tissue donation. Their findings showed limited knowledge, but positive support among the younger generation of New Zealand. We have also reported on the characteristics of donors recruited by the New Zealand Bone Marrow Donor Registry and demonstrated that an average of 80% of newly recruited donors have a unique HLA (i.e. individual specific) phenotype not found elsewhere. A search of Bone Marrow Donors Worldwide (BMDW; www.BMDW.org), a co-operation of 75 stem cell registries with 25 million donors, shows that Polynesians are under-represented among worldwide donors (Edinur et al. 2015). Together these present a great challenge to find compatible donors, especially for Polynesian patients. In this context, the heterogeneous and admixed society of New Zealand requires a combination of more efficient and holistic approaches to recruitment of larger numbers of Polynesian donors, a high-throughput molecular platform for donor- and recipient-matching plus increasing public awareness and knowledge on organ and stem cell donation (Cornwall et al. 2015, Edinur et al. 2015).

Our research has also been directed to assess the new molecular blood group and HPA typing methods in Polynesian and Māori populations. Scores for other loci are based on the application of the pre-existing and established genotyping technologies (e.g. Lumexin v. sequencing for HLA and KIR typing). Diagnostic kits or molecular techniques have been tested for other populations which may or may not cover genetic variants
in Māori and Polynesians. Our validation using three different blood group and HPA genotyping methods (i.e. PCR-SSP, SBT and SNP assay) gave 100% concordance and thus reflects the accuracy of the DNA-based methods. Each molecular platform should detect all known polymorphisms associated with cellular expression, or otherwise they would lead to false prediction of phenotypes. For example, commercial PCR-SSP kits used in our survey do not include Jk and HPA-6 variants which are common or polymorphic in A-SM populations including Polynesians and Māori. Thus, routine uncritical application of these kits would lead to false predictions and will subsequently increase risk of alloimmunisation. Overall, there is compelling evidence for the wider application of molecular approaches for HLA, KIR, MICA, blood group and HPA typing. Molecular methods show exact variations in particular genes and have been shown to improve resolution of polymorphic loci to a precision that could not previously be achieved using serological approaches (Dunn 2011, 2015, Patnaik et al. 2012, Robinson et al. 2013).

In addition, genotyping is the preferred technique when there are no reliable antisera, poor cell expression and cross reactivity. At present, several molecular methods as simple as PCR-SSP to rapid and high-throughput PCR-SSOP based platforms such as Luminex and SNP genotyping assay have been tested and used by others for HLA, MICA, KIR, blood group and HPA typing, individually (Dunn 2011, 2015, Middleton 2005, Veldhuisen et al. 2009, Wu & Csako 2006). Certain clinical cases require matching not only for ABO, but also for HPA and HLA loci (e.g. refractoriness to platelet transfusion and transfusion-related acute lung injury). It is expected that these loci could be genotyped simultaneously in the near future, using a single high-throughput platform. The application of molecular techniques has a promising future in clinical settings and the thrust of our intended message is that serological methods have served well for a prolonged period, but are now seriously challenged by newer DNA-based technologies and a lack of serological reagents. We have tested some of the molecular techniques on Māori and Polynesian DNA samples and examined various aspects of their performance including their economics (Edinur et al. 2013c). Overall, we find them to be relatively expensive, but very reliable especially for the genetically heterogeneous population of New Zealand.

Finally, we point to the fact that our new databases are of interpretive, predictive and prospective value. For instance, some studies are presently forced to speculate that their findings are most likely due to admixture between Māori and Europeans. They can now validate their conclusions, by asking subjects the key diagnostic questions about their grandparents by retrospective interview. Our experience reported here also shows that this would now be possible by screening the samples for the ancestry informative HLA markers reported in our studies (Edinur et al. 2012, 2013a, 2013b, 2013c, Nemat-Gorgani et al. 2014, Riccio et al. 2013). The predictive value of our new data is such that as novel potentially autoimmune disorders, such as Sydenham’s chorea, are found to have differing incidence between Māori and European, the most likely candidate immune system markers will be those showing greatest differences in allele frequency between the two groups. Further, when new HLA associations are eventually found for diseases, the relative incidence of the disease in the two groups will be predictable in part from the relative occurrence of the associated marker in the two groups. The data are of prospective value to all other populations with Austronesian ancestry (these presently number in excess of 350 million people, living mainly in SE Asia). In complementary fashion, our studies show that data obtained overseas may give valuable pointers to candidate systems and markers of value to New Zealand medicine. We are currently conducting a series of studies with Malaysian subjects (Abd Gani et al. 2015, NurWaliyuddin et al. 2014, Wan Syafawati et al. 2015) which will help to test this proposition further. The genetic screening programme in Malaysia, especially on the predominantly A-SM sub-populations (i.e. Malay subethnic groups), also provides a reference standard for genetic markers (i.e. human neutrophil antigens and cytokines) that have never previously been tested in Māori and Polynesians (Manaf et al. 2015, Norhalifah et al. 2015).

In our view extended investigation of the KIR system has particular value and urgency. These systems have been implicated in many biological and medical processes (Riccio et al. 2013). Māori and Polynesians have an unusual frequency (59–65% - 31 allelic types) of the reduced KIR A haplotype (held to be significant in combating disease) compared with the extended KIR B haplotype (only 35–41% - 18 allelic types and held to be of more significance in reproduction). Their KIR haplotypes are also unusual with respect to the range of HLA Class I signals that they recognise; namely HLA-A > HLA-B and HLA-C. Thus it is clear that the performance of the KIR system may be radically different in Māori and Polynesian patients compared with Europeans. For instance, these differences could include the roles and associations involved in the vital recognition of ‘missing self’ (i.e. down-regulated cell surface markers) in virus infected cells and tumours. This might, therefore, include an important role in ‘graft versus leukaemia’ in stem cell therapy (Ruggeri et al. 2002). Unfortunately, however, despite enormous research efforts, the clinical relevance of KIR and its interaction with HLA remains elusive.

**Conclusion**

The central theme of our research programme is the development and application of molecular methods to measure genetic variability in Māori and Polynesian populations. Each experimental work was designed to study ancestry and improve health outcomes, e.g. as presented here for transplantation and transfusion medicine. Our new experimental works on blood groups, HPA, HLA and MICA and most recently on KIR showed differences between Polynesian and European gene pools and demonstrated the reliability of current genotyping platforms that would suit well the needs of transfusion and transplantation centres, an important clinical aid in matching organs/stem cells and providing safe blood. These datasets were used for dual analyses of ancestry and health and can also be used as the reference standard for future study of diseases in Māori and Polynesians.

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