Restriction fragment length polymorphisms associated with the factor VIII and factor IX genes in Polynesians

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Abstract

New Zealand Maoris (72 X chromosomes) have been compared with Pacific Island Polynesians (121 X chromosomes) and Caucasian New Zealanders (51 X chromosomes) as a control group to determine the allelic frequency of six RFLPs associated with the genes for two X linked diseases (haemophilia A and haemophilia B). RFLPs examined were *BclI*, *XbaI*, and *BglII* within the factor VIII gene, the factor VIII extragenic *TaqI* system, and the factor IX intragenic *TaqI* and *XmnI* sites. The information obtained facilitates the design of strategies for both carrier detection and prenatal diagnosis of haemophilia A within these groups. Strong linkage disequilibrium was observed between the factor VIII *BclI* and *XbaI* sites in Polynesians. Genetic counselling for Polynesians with haemophilia B continues, however, to rely on phenotypic diagnosis. The RFLP data from the two separate loci on the X chromosome in Polynesians show similarities with Chinese and Japanese populations, reinforcing theories of an early Polynesian ancestry originating in east Asia.

The incidence of haemophilia A averages 1 to 2 per 10 000 males throughout the world and haemophilia B has an incidence of one fifth that of haemophilia A. The incidence in New Zealand is similar.¹ Accurate carrier diagnosis and early prenatal detection, both important components of comprehensive haemophilia care, have been greatly improved by the development

of DNA genotyping. Restriction fragment length polymorphisms (RFLPs) can be used as linkage markers to determine the inheritance of normal or mutant haemophilia genes. When these RFLP markers are intragenic and informative in a kindred of interest, carrier status can be assigned with a theoretical accuracy exceeding 99%.² A further benefit of using this technique is the early prenatal diagnosis (at 8 to 10 weeks) of abnormal males by chorionic villus sampling. Recent developments using the polymerase chain reaction (PCR) have been applied to RFLP analysis in haemophilia.³ These techniques have both shortened the time required to obtain a result and allowed small quantities of DNA to be analysed, an important factor when using DNA from chorionic villi for prenatal diagnosis. RFLP analysis is limited, however, by the number of polymorphic markers identified which are sufficiently common to be of use in general populations. This restricts the number of potentially informative cases.

The allelic frequency of any polymorphism within a population determines the usefulness of that RFLP for genetic counselling. For two allele systems, the most informative allelic frequency is 50%, which results in a 50% rate of heterozygosity (and hence informativeness). The combination of two or more independent RFLPs will increase the rate of informativeness proportionately. In reality this is often complicated by linkage disequilibrium between polymorphic sites within the same gene. Among Caucasians the most useful RFLPs within the factor VIII gene are the *XbaI* site in intron 22, the *BclI* site in intron 18, and the *BglII* site in intron 25. Previous studies in Caucasian populations suggest that the use of this combination of RFLPs will result in 70% of women being informative.⁴⁻⁵ An additional 20 to 25% of women in these populations are informative when the closely linked extragenic *TaqI* site, with its 5% rate of error, is analysed.⁵⁻⁶ Several useful intragenic factor IX RFLPs have been reported.²⁻⁷⁻¹⁰ Three frequently used factor IX RFLPs, a *TaqI* site in intron IV, an *XmnI* site in intron III, and a *DdeI* site in intron I are reported to be informative in approximately 70% of Caucasian women when used in combination.²

Before DNA based genetic counselling can be implemented it is essential to establish the allelic

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frequencies of commonly used RFLPs in the local population. This is particularly important in populations of non-Caucasian origin because RFLPs may differ significantly among different ethnic groups.¹¹⁻¹⁵ The Polynesian race is a distinct ethnic group and although New Zealand's population is predominantly Caucasian there is a significant Polynesian or part-Polynesian community. At least 10% of the total New Zealand population, and approximately 10% of the haemophilia population, is Polynesian. We report here the results of a survey of six haemophilia related RFLP/probe systems in a sample of New Zealand resident Polynesians representing five different Pacific Island groups.

Materials and methods

SUBJECTS

Peripheral blood was drawn from 125 unrelated volunteer subjects after written informed consent was obtained in accordance with the requirements of the Auckland Hospital Research Ethics Committee. All subjects were residents of New Zealand and stated their race on the consent form. Subjects were included in the study if they were of 100% European heritage (Caucasians without Maori or Polynesian ancestors), were of 100% Pacific Island Polynesian heritage, or declared themselves to be Maori and to have $\geq 50\%$ Maori heritage. Twenty-seven subjects (three males) were Caucasian, 37 subjects (two males) were New Zealand Maori, and 61 subjects (one male) were Pacific Island Polynesian. Among the non-Maori Polynesians, ten were Niue Islanders, 19 were Cook Islanders, four were Tongan, and 28 were Samoan.

METHODS

Blood was drawn directly into tubes containing CPD anticoagulant, and total genomic DNA was extracted by the method of Kunkel *et al.*¹⁶ DNA (2.5 to 5 μ g) was digested with restriction endonucleases according

to the manufacturers' recommendations. Restriction fragments were separated on 0.8 to 1.0% agarose gels and blotted onto Gene Screen Plus membranes (DuPont) by Southern transfer.¹⁷ Membranes were hybridised for approximately 24 hours at 42°C in formamide (50%), 4 $\times$ SSC, SDS (1%), and 0.5% non-fat milk powder with DNA probe labelled with [³²P]dCTP by the random primer labelling system.¹⁸ After hybridisation, membranes were washed for five minutes in 2 $\times$ SSC at room temperature, then for 30 minutes in 2 $\times$ SSC with SDS (1%) at 65°C, and then for 10 minutes in 0.1 $\times$ SSC with SDS (0.1%) at 65°C. The membranes were left wet and exposed to x ray film (X-Omat AR, Kodak) with intensifying screens at -70°C for one to four days. The DNA probes used for the factor VIII gene included: (1) p114.12, supplied by the American Type Culture Collection (ATCC), which detects a *Bcl*I polymorphism in intron 18¹⁹; (2) p482.6, supplied by ATCC, which detects an *Xba*I polymorphism in intron 22²⁰; (3) cDNA-C, kindly supplied by the Genetics Institute (Cambridge, MA), which detects a *Bgl*II polymorphism in intron 25¹¹; and (4) St14.1, kindly supplied by Dr J-L Mandel of Strasbourg, which detects a multiallelic *Taq*I RFLP system in an extragenic site.²¹ The DNA probe used for both the *Taq*I and *Xmn*I RFLPs within the factor IX gene was probe VIII, kindly supplied by Professor G Brownlee of Oxford.²

Results

FACTOR VIII REGION

Allele frequencies, intragenic RFLPs

Table 1 shows the allelic frequencies for the three factor VIII intragenic RFLPs for each racial group. The frequencies for all three RFLPs were similar (within 95% confidence interval) for Caucasian residents of New Zealand and other reported Caucasian populations,^{5 6 19 20} although the New Zealand frequency for the *Bcl*I polymorphism was relatively high (83%). Polynesian allelic frequencies at the *Bcl*I

Table 1 Allelic frequencies for restriction sites in the factor VIII gene.

	<i>Bcl</i> I		<i>Xba</i> I		<i>Bgl</i> II	
	No positive	% positive	No positive	% positive	No positive	% positive
Caucasians (NZ)	39/47	83 (69-90)*	26/47	55 (40-70)	42/51	82 (69-91)
Caucasians (other)†	294/425	69	105/187	56	118/139	85
Maori (NZ)	46/72	66 (52-76)	41/72	57 (45-69)	68/72	94 (87-98)
Pacific Island						
Polynesian totals	67/121	55 (46-64)	58/115	50 (41-60)	119/120	99 (95-99)
Cook Islanders	19/38		15/34		37/38	
Tongans	5/8		4/8		8/8	
Samoans	37/56		35/56		56/56	
Niueans	6/19		4/17		18/18	
Chinese‡	79/96	82	28/49	57	54/54	100

*% positive (95% confidence interval).

†From Antonarakis *et al.*,¹¹ Gitschier *et al.*,¹⁹ Moodie *et al.*,⁵ and Wion *et al.*²⁰

‡From Chan *et al.*¹⁵

intron 18 site were slightly lower than those reported for both Caucasian and Chinese populations.^{5 15 20} The frequency of the *XbaI*/intron 22 site in the Polynesian group was similar to Caucasian and Chinese values, but there was a virtual absence of polymorphism at the *BglII*/intron 25 site. New Zealand Maoris had allelic frequencies intermediate between Caucasians and Polynesians at the *BglII* site.

For each RFLP, the expected and observed percentage of informative women, as determined by the Hardy-Weinberg principle, was similar. For the *BclII*/intron 18 RFLP, 28% of Caucasians ($n=22$), 46% of Maoris ($n=35$), and 49% of Pacific Island Polynesians ($n=66$) were informative. For the *XbaI*/intron 22 RFLP, 50% of Caucasians ($n=22$), 48% of Maoris ($n=35$), and 50% of Pacific Island Polynesians ($n=66$) were informative. For the *BglII*/intron 25 RFLP, the values were 19% of Caucasians ($n=24$), 10% of Maoris ($n=35$), and 1% of Pacific Island Polynesians ($n=69$).

For comparative purposes, previously reported values in a Chinese population¹⁵ for these three intragenic RFLPs are also included in table 1.

Linkage disequilibrium, intragenic RFLPs

Linkage disequilibrium between the *BclII*/intron 18 and the *XbaI*/intron 22 RFLP sites was not detected in the control population of 20 Caucasian females. Examination of the *BglII*/intron 25 site did not improve on the 60% informativeness of the first two RFLPs in this group (figure). In contrast, among 35 Maori females and 57 Pacific Island Polynesian females, for whom all three intragenic RFLPs were studied, the increase in informativeness obtained by two or three RFLPs in the same female was less than

expected, owing to strong linkage disequilibrium between the *BclII* and *XbaI* sites in introns 18 and 22. Precise figures for the association of the alleles at the *BclII* and *XbaI* sites could not be determined in this study since the majority of the subjects were female, and for those who were doubly heterozygous the phase of the alleles remained in doubt.

The estimated frequency for each allelic pair was based on male subjects and non-doubly heterozygous females. A strong association between *BclII* fragment 0.9 and *XbaI* fragment 1.4 and a corresponding strong association between *BclII* fragment 1.2 and *XbaI* fragment 6.2 was observed among both groups of Polynesian subjects.

Allele frequencies, extragenic RFLPs

At the factor VIII linked extragenic *TaqI* site, striking differences in allelic frequencies were observed between Polynesian and Caucasian subjects. As shown in table 2, the frequencies of system 1 alleles 5-6 and 8 were higher among both groups of Polynesians, and the frequencies of alleles 3 and 4 were lower. Within system 2, the β fragment, which was not found in Caucasians, occurred in 41% of Pacific Island Polynesians and 11% of Maoris. The combined informativeness of the system 1 and system 2 *TaqI* RFLPs was between 60% and 70% for each ethnic group. The improvement in informativeness of RFLP analysis provided by the addition of the extragenic *TaqI* study to the three intragenic studies was examined in each population. Among six Caucasian females, uninformative after full intragenic study, three (50%) were informative by *TaqI* analysis. Among 12 Maori females uninformative after full intragenic study, seven (58%) were informative after

The expected (e) and observed (o) informativeness among females studied with all three intragenic factor VIII RFLPs.

Table 2 Frequencies of the allelic *TaqI* fragments and informativeness shown by the *St14.1* probe.

	New Zealand Caucasians (%)	New Zealand Maoris (%)	Pacific Is Polynesians (%)
System 1	(n=32)*	(n=43)	(n=100)
Allele 1	0	0	0
2	6	7	6
3	41	7	6
4	25	2	4
5-6	16	38	53
7	3	11	7
8	9	35	24
System 2	(n=32)	(n=44)	(n=110)
α	100	89	59
β	0	11	41
Informativeness	(16 females)	(21 females)	(49 females)
Sys 1	69	62	45
Sys 2	0	5	41
Combined	69	67	61

*n=number of X chromosomes analysed.

Table 3 Allelic frequencies for restriction sites in the factor IX gene.

	TaqI		XmnI	
	No positive	% positive	No positive	% positive
Caucasians (NZ)	17/51	33 (19-47)*	11/51	22 (12-36)
Caucasians (other)†	61/192	32	21/72	29
Maori (NZ)	5/72	7 (3-16)	4/72	6 (2-15)
Pacific Island				
Polynesian totals	1/120	0.8 (0.1-5)	0/116	0 (0-4)
Cook Islanders	0/38		0/36	
Tongans	0/8		0/8	
Samoans	1/56		0/56	
Niueans	0/18		0/16	
Chinese‡	2/68	3	2/56	4
Japanese§	0/81	0	0/81	0

*% positive (95% confidence interval).

†From Winship *et al.*² Camarino *et al.*⁹ and Hassan *et al.*²²‡From Lubahn *et al.*¹³§From Kojima *et al.*¹²

TaqI analysis. Among 28 Pacific Island Polynesian females uninformative after full intragenic study, 16 (57%) were informative after *TaqI* analysis. Informativeness for the factor VIII gene based on examination of all four RFLPs was, respectively, 77%, 76%, and 78% for the three ethnic groups.

FACTOR IX REGION

Table 3 shows the allelic frequencies for the *TaqI* RFLP in intron IV and the *XmnI* RFLP in intron III of the factor IX gene for each ethnic group, including previously reported values for Chinese¹³ and Japanese.¹² The RFLP frequencies for New Zealand resident Caucasians are similar to those of Caucasian populations in previous reports.^{2,7,22} In contrast, the *XmnI* site and the *TaqI* site were virtually absent in the Pacific Island Polynesians. The frequencies of both sites in New Zealand Maoris was intermediate between Caucasian and Pacific Island Polynesian values.

Discussion

The results of New Zealand Caucasian controls for all six RFLP systems used were consistent with previously reported Caucasian communities.^{2,5-7,11,19,20} The relatively high (83%) frequency of the *BclI* site in the New Zealand Caucasians was unexpected, but could be the result of the small number of chromosomes analysed (47 X chromosomes). It is worth noting that the 95% confidence interval for this site overlaps with other reported frequencies. Important differences were observed between the Polynesians and Caucasians, and similarities between the Polynesian and Oriental populations. The factor VIII *BglI* polymorphism frequency in Caucasians is approximately 80 to 85%, whereas in a total of 120 X chromosomes in Polynesians only a single subject was found in

whom the *BglI* site was absent. The nearly total lack of this polymorphism is also a characteristic of Chinese factor VIII genes.¹⁵ The intermediate frequency of the *BglI* site in 72 chromosomes from persons stating themselves to be Maori reflects the mixed racial composition of this New Zealand group. A New Zealand Maori was defined as $\geq 50\%$ Maori heritage and allows up to 50% non-Polynesian (predominantly Caucasian) ancestry. The New Zealand Caucasian controls showed very little linkage disequilibrium between the *BclI* and *XbaI* sites, consistent with other reports.^{5,20} In contrast there was almost complete linkage disequilibrium between these two sites in the Polynesians. The strong linkage disequilibrium shown in the Polynesian subjects is probably the result of a founder effect during the colonisation of the Pacific Islands. The short time period during which this occurred is insufficient for equilibrium to have developed between the *BclI* site and the *XbaI* site. The linkage disequilibrium displayed by the Polynesians makes the combined use of both the *BclI* and *XbaI* RFLPs of minimal benefit in improving informativeness. We found only a very small increase in informativeness when *XbaI*, *BglI*, or both were added to the *BclI* study in this ethnic group.

The frequency distribution of the *TaqI*/St14.1 system I in Maoris and Pacific Island Polynesians is more like the pattern observed in the Chinese¹⁵ than that found in persons of Caucasian origin. In system II, the β allele was present in 41% of the Pacific Island Polynesians. The β fragment is rare in Caucasians but is found in 43% of Chinese.¹⁵ Intragenic RFLPs were informative in 50 to 55% of Polynesian women, which increased to 78% when combined with the extragenic *TaqI* system. For Caucasians in this study, 60% were informative when all three intragenic sites were used, and this was increased to 77% when coupled with the extragenic *TaqI* system. Thus, when using DNA

marker analysis, there will still be approximately 20 to 25% of women who will be uninformative and for these women accurate phenotypic data will remain essential. We propose a diagnostic strategy for haemophilia A affected families in Polynesian populations as follows. Technically the *BclI* results from both Southern blotting and PCR are easier to interpret. The *BclI* RFLP is therefore preferred in the initial assessment for haemophilia A carrier detection and prenatal diagnosis. Owing to linkage disequilibrium the *XbaI* and *BglI* RFLPs will not increase the informativeness of the *BclI* results in most cases. The extragenic *TaqI*/St14.1 system is very informative in Polynesian populations and could be used in uninformative *BclI* cases. However, caution must be exercised because of error owing to recombination when using extragenic systems.

Reports suggest that the factor IX *TaqI* and *XmnI* polymorphic sites are the most useful RFLPs for haemophilia B studies in Caucasians.² The frequencies of these sites in New Zealand Caucasians support this observation. In contrast, we found only one chromosome with the polymorphic *TaqI* site out of a total of 120 Pacific Island Polynesian chromosomes and no polymorphic *XmnI* sites out of a total of 116 Pacific Island Polynesian chromosomes. The allelic frequencies for these sites in the Maori group was intermediate between the full Polynesians and Caucasian frequencies, presumably reflecting mixed Polynesian and Caucasian ancestry. The *TaqI* and *XmnI* sites also appear to be absent or of very low frequency in East Asians,^{12 13} who in this respect resemble the Polynesians. For haemophilia B affected families the *TaqI* and *XmnI* RFLPs commonly used in Caucasian studies are of minimal value in Polynesian populations. As with the Japanese¹² and Chinese,¹³ alternative intragenic RFLPs will have to be identified before a suitable diagnostic strategy of DNA analysis can be determined for Polynesian families. Alternatively, direct determination of the factor IX mutation using PCR coupled to a variety of methods, such as direct sequencing and mismatch analysis of heteroduplexes either on denaturing gradient gel electrophoresis or by chemical cleavage, should provide a diagnosis in most cases.²³⁻²⁸ These new strategies for the rapid detection of mutations may become the methods of choice in ethnic groups for which RFLP analysis is inadequate.

The Polynesian race is a distinct ethnic group which consists of approximately one million persons widely dispersed throughout the Islands of the central and south Pacific Ocean. More than one third of all Polynesians currently reside in New Zealand, including 300 000 indigenous New Zealand Maoris and about 60 000 recent immigrants from other South Pacific Island populations. The Polynesian race, a relatively new ethnic group, is thought to have been derived from the Lapita culture more than 3500 years

ago.²⁹ The colonisation of the Pacific Islands by the Polynesians is considered to have been a stepwise progression from the Melanesian Islands north of New Guinea eastwards through Fiji, Samoa, and Tonga to islands as remote as Hawaii, Easter Island, and New Zealand over a period of approximately 2500 years. The new RFLP data presented here from two separate loci on the X chromosome in Polynesians show similarities with the reported studies on populations of east Asian origin. The linkage disequilibrium between the *BclI* and *XbaI* sites in the factor VIII gene is consistent with a theory of the Polynesian race originating from a small founder population less than 8500 year ago. This figure, derived from the equation $a=b[1-\phi]n^*$, is based on a recombination frequency equal to 0.00018 and assumes that the small founder population had complete linkage disequilibrium. Our genomic data support current concepts^{29 30} of a rapid and recent Polynesian colonisation of the Pacific Islands east of the Bismarcks by Lapita people, who were in turn derived from an east Asian population.

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*a=final frequency, b=initial frequency, ϕ =recombination frequency, n=time in generations.³¹

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