

A high incidence of polymorphic *CYP2C19* variants in archival blood samples from Papua New Guinea

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Date received (in revised form): 7th March, 2008

Abstract

There is considerable inter-ethnic variability in the incidence of *CYP2C19* genetic poor metabolisers (*var/var*). About 3 per cent of Caucasians are *CYP2C19 var/var*. By contrast, an extremely high incidence (70 per cent) is observed in the Melanesian island of Vanuatu. The colonisation of the Pacific Islands is believed to have involved migration through Papua New Guinea (PNG), and hence a high incidence may also be expected in this population. The reported incidence in PNG was only 36 per cent, however. PNG is a country of extensive ethnic diversity, and the incidence of the *CYP2C19 var/var* in other regional populations of PNG is currently not established. In this study, restriction fragment length polymorphism–polymerase chain reaction analysis of archival blood serum samples was used to determine the prevalence of the *CYP2C19**2 and *3 variant alleles in three different ethnic and geographically isolated populations of PNG. In the largest population studied (Iruna), the frequency of both variant *CYP2C19* alleles was high (0.37 and 0.34, respectively). Specifically, the frequency of the *CYP2C19**3 allele was significantly higher than in the PNG (East Sepik) population reported previously (0.34 vs 0.16; $p < 0.0001$). In the Iruna population, 48.9 per cent of the samples were homozygous variants for *CYP2C19**2 or *3, which although higher was not statistically different from the East Sepik population (36 per cent). The results of this study indicated that other regional populations of PNG also have a relatively high incidence of the *CYP2C19* genetic polymorphism compared with Caucasian populations. The high incidence reported in Vanuatu, however, may be due to genetic drift rather than a PNG founder population, as the Vanuatu population is dominated by the *CYP2C19**2 allele, with a lower contribution from the *3 allelic variant.

Keywords: *CYP2C19* genetic variants, ethnic variability, Papua New Guinea

Introduction

Poor metabolism of drugs, such as the anti-malarial agent proguanil¹ and the anti-ulcer agent omeprazole² is associated with genetic variants of the *CYP2C19* enzyme (*CYP2C19**2 and *CYP2C19**3).³ The *CYP2C19**2 (c.681G > A) variant results in a splice site mutation⁴ and the *CYP2C19**3 (c.636G > A) variant results in a premature stop codon⁴ and both result in a loss of functional enzyme. There is a gene dose-dependent

decrease in drug metabolism, and individuals who are homozygous wild-type, heterozygous or homozygous variant for these null alleles are termed extensive (EM), intermediate (IM) or poor (PM) metabolisers.

The restriction fragment length polymorphism–polymerase chain reaction (RFLP-PCR) analysis of these variant alleles is widely used and well validated. It is also clearly established that the *2 and *3 null alleles account for the majority of

phenotypic PMs⁵ and that the *3 null allele is specific to Asian populations and is extremely rare in Caucasian populations.³

There is considerable inter-ethnic variation in the distribution of the *CYP2C19* genetic polymorphism, with an incidence of the homozygous variant of ~3 per cent in Caucasians⁵ and ~20 per cent in Asians.⁶ An extremely high frequency (70.6 per cent) has been reported in the Melanesian island of Vanuatu.⁷ Relatively little is known about the incidence in other ethnic populations, however, such as the Melanesians and Polynesians. Anthropological evidence suggests that the people of Vanuatu may have migrated from Papua New Guinea (PNG) about 3,500 years ago.^{8–11} The incidence of the *CYP2C19* genetic polymorphism in the East Sepik (northern coast) region of PNG is reported to be 36 per cent,¹² which, although higher than in Caucasian populations, is considerably lower than that observed in Vanuatu. PNG is one of the most ethnically diverse regions of the world, however, and the incidence of the *CYP2C19* genetic polymorphism in other regional populations of PNG is not currently established. An alternative PNG population may be the source of the high incidence of *CYP2C19* variants in Vanuatu.

We report the use of archival serum samples to determine the prevalence of the *CYP2C19* *2 and *3 variant alleles in three different ethnic and geographically isolated regional populations of PNG. The results of this study may provide further information about the source of the high incidence of the *CYP2C19* genetic polymorphism in Vanuatu and may help to determine whether a high incidence can be predicted for other Pacific Island populations.

Materials and methods

Archival serum samples

Serum samples were collected as a part of a blood group phenotype survey in PNG over 30 years ago.^{13,14} Samples were from three geographically isolated areas of PNG: Tufi, Iruna and Fly River. PNG has a large ethnic diversity owing to the geographical isolation of the regional populations, and this is

associated with a large number of languages.¹⁵ The languages spoken by people from Tufi, Iruna and Fly River are mutually exclusive. The Fly River samples were collected from the inland Kiunga region in Western province, Iruna samples were collected at the United Church Hospital (Iruna) Magarida in the Central province on the south-east coast of PNG and the Tufi samples were collected from small isolated villages in the Oro (formally, Northern) province on the north-east coast of PNG. The participants gave oral consent to donate their blood voluntarily, as a gift to be used for the welfare of other Papua New Guineans. The samples were transported to Port Moresby, centrifuged (2500 g) for serum collection and then stored at -20°C . The serum samples were stored in insulated boxes with dry ice for transportation by air to New Zealand and were then stored at -80°C at the University of Auckland. The use of these samples for analysis of the *CYP2C19* genetic polymorphism was approved by the University of Auckland Human Participants Ethics Committee (UoA HPEC 2005/004).

DNA extraction

Genomic DNA was extracted from serum as previously described,¹⁶ with minor modifications. Serum (200 μl) was incubated with 1 per cent (w/v) sodium dodecyl sulphate and proteinase K (100 μg) in a 200 μl reaction overnight at 55°C to denature the protein complexes. DNA was then prepared by phenol/chloroform extraction, followed by ethanol precipitation in the presence of glycogen (50 μg) as a co-precipitant. The DNA was air dried (<20 minutes) and resuspended in elution buffer (10 mM Tris-HCl, pH 8.5; 50 μl ; Roche Diagnostics, GmbH, Mannheim, Germany).

Genotyping procedures: PCR

The *CYP2C19**2 and *CYP2C19**3 primers used in this study were as previously described,¹⁷ with minor modification of the *CYP2C19**2 reverse primer to 5'-GTAAACACAAAACTAGTCAATG3' (altered base pair [bp] is underlined), as the published primer sequence did not correspond to the *CYP2C19* sequence (NT030059.12; Genbank).⁴

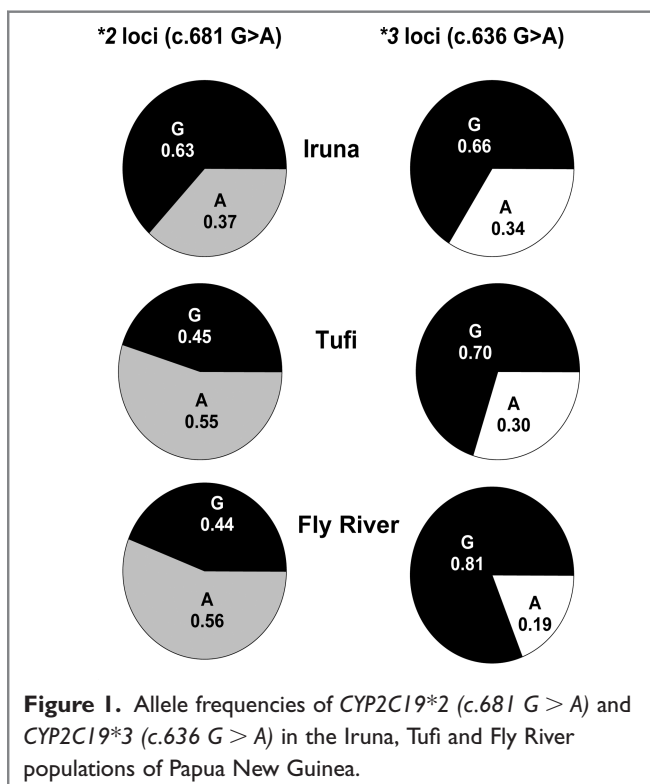
The PCR Master Mix (Qiagen, New Zealand) used in the amplification reaction contained *Taq* DNA polymerase (2.5 units), 10X Qiagen PCR buffer containing 1.5 mM magnesium chloride (MgCl_2), and 200 μM of each deoxynucleoside triphosphate (dNTP). The PCR amplification conditions were as previously described,¹⁷ with minor modifications. The PCR reaction was performed in a 50 μl experiment containing ~ 80 ng of DNA template, 0.5 mM of each dNTP, 0.5 μM of each primer and 1.5 mM MgCl_2 in 1X PCR buffer (Qiagen). The amplification conditions were: one cycle of 94°C for five minutes (initial denaturation); 37 cycles of 94°C for 20 seconds (denaturation), one cycle of 53°C for ten seconds (annealing); one cycle of 72°C for ten seconds (extension); and one cycle of 72°C for five minutes (final extension).

RFLP analysis

*CYP2C19*3* PCR products were subjected to an additional purification step (High Pure PCR product purification kit; Roche Diagnostics GmbH) before restriction enzyme digestion to eliminate suspected incomplete *Bam*HI digestion observed in preliminary experiments. The *CYP2C19*2* and *CYP2C19*3* PCR products were subjected to *Sma*I and *Bam*HI restriction enzyme digestion, respectively, as previously described,¹⁷ with the minor modification that the *CYP2C19*2* PCR product (20 μl) was incubated with 20 U of *Sma*I. The RFLP products were analysed by gel electrophoresis on a 3 per cent (w/v) agarose gel and visualised by staining with ethidium bromide and ultraviolet illumination.

Direct DNA sequencing

Random samples were subjected to DNA sequencing to confirm that the *CYP2C19*2* and *CYP2C19*3* genotypes were assigned correctly. Prior to sequencing, the PCR products were purified using the High Pure PCR product purification kit. The ABI PRISM™ BIG DYE Terminator (BDT) Sequencing Kit, version 3.1 (ABI, Forster City, CA, USA) was used for the DNA sequencing reaction and the product was analysed at the DNA



sequencing facility of the Centre of Genomics and Proteomics (University of Auckland, New Zealand) on an ABI PRISM™ 3100 Genetic Analyzer.

Statistical analysis

Genotype and allele frequencies were calculated from the counts. Expected genotype numbers were calculated using the Hardy–Weinberg equation ($p^2 + 2pq + q^2 = 1$) using the allele numbers, where q is the number of the variant alleles (*CYP2C19*2* and *CYP2C19*3*) and p is the number of wild-type alleles at the corresponding *CYP2C19*2* and *CYP2C19*3* loci. The chi-squared test was used to determine if the data were in agreement with the Hardy–Weinberg equilibrium. As the *CYP2C19* genotype is closely correlated with the phenotype, the numbers of PMs in the largest population studied (Iruna) was compared with the PM incidence in the previously published East Sepik data,¹² using the continuity-corrected chi-squared test with 95 per cent confidence interval.

Table 1. The incidence of the *CYP2C19* genotype and predicted phenotype in the Iruna, Tufi and Fly River regions of Papua New Guinea

Genotype			Frequency (n)			Predicted phenotype	Incidence (%)		
c.681	c.636		Iruna	Tufi	Fly River		Iruna	Tufi	Fly River
G/G	G/G	*1/*1	3	0	1	Extensive metaboliser	6.4	0	12.5
G/A	G/G	*1/*2	9	2	2	Intermediate metaboliser	44.7	30	25
G/G	G/A	*1/*3	12	1	0				
G/A	G/A	*2/*3	10	5	3	Poor metaboliser	48.9	70	62.5
A/A	G/G	*2/*2	8	2	2				
G/G	A/A	*3/*3	5	0	0				

Results

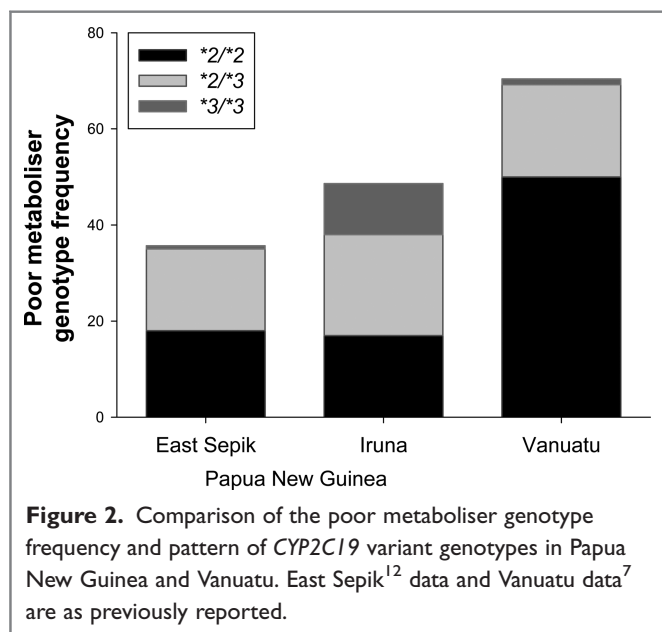
Genomic DNA of sufficient quality and quantity was extracted from 65 serum samples across the three regions of PNG (Iruna, $n = 47$; Tufi, $n = 10$; Fly River, $n = 8$). Amplification of the *CYP2C19**2 and *CYP2C19**3 target sequence from these archival serum samples resulted in 321 and 271 bp products, respectively. Digestion of the *CYP2C19**2 amplicon with *Sma*I resulted in products of 212 and 109 bp (homozygous wild type; c.681 G/G); 321, 212 and 109 bp (heterozygote; G/A); and a single undigested product of 321 bp (homozygous *2; A/A). Digestion of the *CYP2C19**3 amplicon with *Bam*HI resulted in products of 175 and 96 bp (homozygous wild-type; c.636 G/G); products of 271, 175 and 96 bp (heterozygote *3; G/A); and a single undigested product of 271 bp (homozygous *3; A/A). A total of 45 samples were randomly selected for direct DNA sequencing and there was 100 per cent agreement with the RFLP assignments.

CYP2C19 variant alleles (*2 and *3) were detected in all three populations studied (Figure 1), with the *2 variant allele (c.681 G > A) ranging in frequency from 0.37 to 0.56 and the *3 variant allele (c.636 G > A) frequency from 0.19 to 0.34 across the three populations. This confirms the high frequency of *2 and *3 alleles observed by Masta *et al.*¹² in the East Sepik region of PNG. The distributions of both the *CYP2C19**2 and *CYP2C19**3 alleles were in agreement with the

Hardy–Weinberg equilibrium across all three regions (data not shown). Due to the very small sample size for the Tufi ($n = 10$) and Fly River ($n = 8$) populations, however, we have precluded any further data analysis of these regional populations in case these allele frequencies are not representative of the true population frequencies. Therefore, only the data for the Iruna population ($n = 47$) was used for comparison with the published data for the East Sepik region.¹²

There was a significantly higher incidence (0.34; $p < 0.0001$) of the *CYP2C19**3 (c.636 G > A) variant allele in the Iruna population compared with the East Sepik population (0.14–0.19). The combined frequency of the *2 and *3 alleles results in a very high total frequency of variants (0.71) and results in a high incidence of the *CYP2C19* PM genotype (homozygous and compound heterozygote variant) in the Iruna region of PNG of 48.9 per cent (Table 1). In addition, a high incidence (62.5 and 70 per cent) of the *CYP2C19* PM genotype was also observed in the Fly River and Tufi samples, respectively (Table 1). This incidence was not significantly different between the three regional populations. Importantly, due to the high frequency of the *CYP2C19* variants in the Iruna population, wild-type (EM) individuals (*1/*1; c.681 G/G and c.636 G/G) were in the minority, at 6.4 per cent (Table 1).

The Iruna population had a lower *CYP2C19**2 frequency compared with East Sepik (0.37 vs 0.45)



but had a significantly higher *CYP2C19**3 frequency (0.34 vs 0.16; $p < 0.0001$). Due to the differences in sample size between the two populations ($n = 47$ vs $n = 401$), however, the overall incidence of the PM genotype was not statistically different between the Iruana and the East Sepik populations (48.9 per cent vs 36 per cent).

Discussion

The RFLP-PCR analysis of *CYP2C19* variant alleles in whole blood is a widely used and well validated method.¹⁷ We have adapted this assay for determination of the *CYP2C19* genotype from archival serum using published methods for DNA extraction from serum.¹⁶ Direct DNA sequencing of random samples was also undertaken, as a rare variant, which overlaps with the *Bam*HI recognition sequence in exon 4, can result in a misclassification of the *CYP2C19**3 variant when using RFLP analysis.¹⁸ There was agreement between the DNA sequence and the RFLP assignment for all the samples tested.

A high incidence (36 per cent) of the *CYP2C19* genetic polymorphism has been reported previously in the East Sepik province of PNG.¹² This is lower than the incidence predicted by Kaneko *et al.*

(>74 per cent), however, based on the observed high frequency of *CYP2C19* variants in Vanuatu.^{7,19,20}

Colonisation of the Pacific Islands is believed to have involved migration from South-east Asia, via PNG, through Melanesia and Micronesia into Polynesia.^{8–11} The first migration wave is believed to have been from South-east Asia to PNG about 40,000 years ago, with a second wave of migration that extended into the Pacific (Remote Oceania) during the past 3,500 years. Thus, the high prevalence of variant *CYP2C19* in Vanuatu may be due to genetic drift rather than a high incidence of the polymorphism in the migrating founder population. Alternatively, as PNG is a country of large ethnic diversity and comprises many geographically isolated populations, another PNG population may be the source of the high incidence of *CYP2C19* variants in Vanuatu.

The present study has confirmed the high *CYP2C19**2 and *CYP2C19**3 allele frequency observed by Masta *et al.*¹² in an additional population that is geographically distant (Central province, south-east coast) from East Sepik (Northern Province). There is a significantly higher incidence of the *CYP2C19**3 (c.636 G > A) variant allele in the Iruana population compared with the East Sepik population (0.34 vs 0.16).¹² The frequency of this variant allele was also originally reported to be low (0.133) in Vanuatu.^{7,19} A more detailed analysis of 24 island populations of Vanuatu,²⁰ however, demonstrated considerable variability in the incidence of the *CYP2C19**3 (c.636 G > A) variant allele (0.02–0.33). The *3 allele is absent in Caucasian populations⁵ but is found in Asian populations at a frequency of 0.045–0.156.²¹ To our knowledge, the highest incidence of the *CYP2C19**3 allele reported is in Indonesian populations of south-east Asia (0.37);²² thus, the frequency of the *3 allelic variant in the Iruana population of PNG is one of the highest reported to date.

Although the incidence of homozygous *2 and *3 variants in this study was very high, no individuals homozygous for both allelic variants (ie c.681 A/A & c.636 A/A) were observed in any of the samples analysed. This supports the independent segregation of the two variant alleles, as reported previously.⁴

Moreover, the *2 allele frequency is consistently higher than the *3 allele in Asian populations,²¹ East Sepik,¹² Vanuatu²⁰ and also the Iruna region of PNG in the present study. The combination of a high *2 and *3 allele frequency in the Iruna population of PNG, however, results in a high incidence of genotypic PMs (48.9 per cent). This PM incidence is intermediate between that reported for Indonesia (34 per cent²²) and for Vanuatu (61.0–70.5 per cent^{7,20}) but higher than that reported previously for PNG (36 per cent¹²). 10.6 per cent of the Iruna population were homozygous *3/*3, whereas these individuals are absent or have a low incidence in East Sepik (0–2 per cent¹²) and Vanuatu (1.2 per cent;⁷ Figure 2). Throughout the Vanuatu archipelago, there is considerable variability in the incidence of *3/*3 individuals (0.0–16.0 per cent), with the majority of islands having a *3/*3 incidence of <0.5 per cent.²⁰ The incidence of the *2/*2 genotype is also highly variable within the archipelago (16–63 per cent). This large variability may be the consequence of the relative isolation of these island populations. Similarly, the high incidence of *3/*3 individuals in Iruna compared with the East Sepik population may also be the consequence of genetic drift of a relatively isolated population. Importantly, the differences in the incidence of not only the *CYP2C19* PM genotype, but also the *CYP2C19**3 allelic variant in the Iruna and East Sepik populations of PNG indicate that neither population is a likely 'source' of a founder population responsible for the high incidence of *CYP2C19* variants in Vanuatu.

An increased incidence of genetic variants of another drug metabolising enzyme, CYP2B6, has also been reported in PNG. Compared with Caucasian populations, the incidence of the *CYP2B6**6 variant is increased in PNG (0.28 versus 0.62, respectively), with a number of other, rarer variants observed at a low frequency, similar to that found in Caucasians.²³ By contrast, the incidence of the *CYP2C8**1 variant is reported to be low or non-existent in 285 individuals so far studied.²³

The results of the present study confirm the relatively high incidence of *CYP2C19* variants in

PNG reported previously.¹² The relatively high *CYP2C19* PM incidence in PNG could have profound consequences for the clearance of many drugs which are metabolised by this enzyme, including the anti-malarial drug proguanil.^{1,24}

If the increased frequency of the *CYP2C19**2 variant allele observed in Vanuatu occurred early in the colonisation of Remote Oceania, the other island populations of Polynesia and Micronesia may also have high PM frequencies. Relatively little is known about the *CYP2C19* genetic status of Pacific Island populations. Phenotypic studies in New Zealand Maori of 'mixed racial background with Caucasian parentage'²⁵ and South Pacific Islanders who 'considered themselves to be >75 per cent Polynesian',²⁶ however, indicate a PM incidence of 7 per cent and 13.6 per cent, respectively. Further studies to determine the incidence of the *CYP2C19* PM genotype in these populations is warranted, as this enzyme is involved in the metabolism of an increasing list of drugs and the medical consequences in these populations may be important.

Acknowledgments

We would like to thank Dr Brian Browning for advice and help with the statistical analysis, Dr Peter Strang for assistance in obtaining samples from Iruna and Dr Nick Thompson, who provided linguistic information for the regional populations. Funding for this work was provided by the University of Auckland New Staff Research Fund.

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