



Ethnic differences in nicotine metabolic rate among New Zealanders

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Abstract

Aims To estimate (a) the prevalence of gene variants associated with slow nicotine metabolism in the general Maori population and (b) nicotine intake and metabolic rate in Maori and European smokers.

Methods The procedure involved (a) genotyping 85 Maori participants for cytochrome P-450 2A6 (CYP2A6) gene variants, which are associated with reduced nicotine metabolic rate (ie CYP2A6*9 and *4); and (b) measuring salivary cotinine (COT) and *trans*-3'-hydroxycotinine (3-HC) as biomarkers of nicotine intake and metabolic rate in 12 female smokers from the Hawke's Bay Region (6 Maori and 6 European).

Results (a) The frequencies of the slow nicotine metabolising variants, CYP2A6*9 and *4, were significantly higher in Maori compared to European ($p < 0.01$). Indeed, the prevalence of the CYP2A6*9 variant in these Maori was among the highest in the world (~20%). (b) In smokers, the Maori group had ~35% lower 3-HC:COT ratios indicating a reduced metabolic rate, as well as 2-fold lower cotinine levels per cigarette smoked, indicating reduced nicotine intake ($p < 0.05$). The CYP2A6*9 allele was significantly more frequent in Maori smokers (70%) compared to Europeans (30%), $p = 0.03$.

Conclusions The findings of this study provide evidence that Maori are genetically slower nicotine metabolisers compared to Europeans. Although more research is required, this study may help explain ethnic differences in smoking initiation and may also have important implications for smoking cessation programs—since metabolic differences between groups with varying ancestry implies that different optimal dosages of nicotine replacement therapy may be required for successful quitting.

In New Zealand (NZ), the prevalence of cigarette smoking is around 22% for the general population. However the smoking rates are markedly higher for Maori (~46%) compared to Europeans (20%) and, for reasons that are unclear, the rates for female Maori are among the highest in the world—52% nationally and up to 60% in some regions.¹ Extensive targeted campaigns for smoking cessation in the 1990s has led to a reduction in tobacco consumption in NZ, yet the high prevalence of smoking for Maori has not decreased.¹

High rates of smoking are associated with elevated rates of smoking-related diseases, and it has been estimated that smoking is responsible for around 30% of Maori deaths compared to about 17% nationally.² As these statistics emphasise, identifying the determinants of the high smoking prevalence in Maori and using this information to develop new targeted cessation strategies is of major public health importance in NZ.

Whilst cultural and economic factors contribute to ethnic differences, these do not explain all of the prevalence disparity between Maori and Europeans. Data from the 2001 NZ Census show that ethnic differences for smoking prevalence exist across all socioeconomic strata.³ Identifying the underlying metabolic and/or genetic differences between groups with different ancestral backgrounds may be important, since this knowledge could provide new insights into the most effective ways of reducing tobacco-related disease in Maori.

Nicotine is the primary, although not the sole, compound for initiation and maintenance of sustained smoking behaviour.⁴ Smokers tend to consume a regular number of cigarettes per day—presumably to maintain the desired pharmacological effects of nicotine.⁵ The sustained daily levels of nicotine ingested by smokers are partly determined by the rate at which nicotine is metabolised in the liver.⁶ Variation in the rate of nicotine metabolism is also thought to influence an individual's initial risk of becoming a smoker as well as their degree of dependence on tobacco.

Benowitz and colleagues have shown that nicotine metabolic rate varies widely among individuals, and among the major ethnic/racial groups in the United States (i.e. Asian, African, and Caucasians/Europeans).^{7,8} In particular, these researchers showed that Chinese-American smokers exhibit (on average) a 35% reduction in nicotine metabolic rate and take in less nicotine per cigarette compared to European-American smokers.⁷

Information on ethnic differences in nicotine metabolism may have important implications for smoking cessation programs—as a slower metabolic rate implies that lower optimal dosages of nicotine replacement therapy (NRT) may be required for certain populations of Asian origin.⁷

After nicotine is absorbed through the lungs by cigarette smoking it is primarily (~80%) metabolised to cotinine (COT) by the liver enzyme—Cytochrome P-450 2A6 (CYP2A6).⁴ COT is subsequently metabolised by CYP2A6 to *trans*-3'-hydroxycotinine (3-HC). The ratio of the 3-HC and COT concentration (3-HC:COT ratio) in saliva is highly correlated with oral clearance of COT in smokers ($r=0.9$), which in turn reflects intrinsic metabolic clearance of nicotine by the liver via the CYP2A6 enzyme.⁹

Therefore, a single 3-HC:COT ratio derived from a saliva sample taken first thing in the morning can be considered a reliable index of CYP2A6 activity and hence the rate of hepatic metabolism of nicotine.⁹ COT concentration on its own is highly correlated with plasma cotinine ($r=0.99$) and is a widely used biomarker for the dose of inhaled or ingested nicotine (i.e. nicotine intake).^{5,10}

Variation in CYP2A6 enzyme activity (i.e. nicotine metabolic rate) is strongly influenced by genetics with a heritability of ~60% in Caucasians.¹¹ Several DNA sequence polymorphisms in the CYP2A6 gene have been associated with nicotine metabolic rate, degree of tobacco dependence, and susceptibility to smoking-related disease.¹² Large variation in CYP2A6 allele frequencies has been observed between ethnic groups worldwide.¹³ Thus, CYP2A6 gene variants are potentially useful biomarkers for ethnic differences in nicotine metabolism and tobacco dependence.

Two variants of the CYP2A6 gene (CYP2A6*9 and *4 alleles), which have been associated with slow nicotine metabolism, are far more prevalent in Asian populations

(Chinese, Japanese, Koreans) compared to Europeans.¹³ Specifically, individuals possessing 1 or 2 copies of CYP2A6 *9 or *4 exhibit significantly reduced, or complete absence of, nicotine metabolism via the CYP2A6 enzymatic pathway.^{13,14} Given the putative ancestral (genetic) links between the NZ Maori population and South East Asia we suspected similar frequencies might exist for these slow nicotine metabolising gene variants in Maori.

The present study investigated:

- (a) The prevalence of the CYP2A6*9 and *4 alleles in the general Maori population, and
- (b) Nicotine intake and metabolic rate in a sample of Maori and European smokers using salivary metabolites as markers of CYP2A6 enzyme activity.

Materials and Methods

Participants—(a) We estimated the population prevalence of two functionally important CYP2A6 gene variants known to be common in Asians. This was achieved by screening a pre-existing bank of Maori DNA samples (n=44), which was considered to be fairly representative of the general Maori population in terms of age, sex.¹⁵ For this sample, the term “Maori” was defined by (i) self-report using the 2001 census definition for ethnicity and (2) an ancestral definition—i.e. having four Maori grandparents. Smoking status of these participants was not determined. Renewed ethics approval for this aspect of the research was granted by the Wellington Ethics Committee in 2004.

(b) We also recruited 12 female smokers from the Hawke’s Bay region. Six participants were classified as “Maori” defined as described above. Because of the heritable (genetic) nature of nicotine metabolic rate, it was important to control for genetic admixture as much as possible. The Iwi (tribes) represented in the Maori group included Ngati Rakaipaaka, Ngati Kahungunu, Nga Puhī, Tainui, and Tuhoe.

A comparison group of six European female smokers with no reported Maori ancestry were also recruited from the Hawke’s Bay region and were matched to the Maori group for age. All participants were fully informed about the nature of the research and were required to sign a consent form before commencement. Ethics approval for the study was obtained from the Hawke’s Bay Ethics Committee. All participants were above 18 years of age.

Questionnaire—A questionnaire, designed to fit the NZ context, was used to obtain the relevant smoking information from the participants. The measures included in the questionnaire were cigarette consumption (i.e. number of cigarettes smoked per day), brand, strength and type of cigarettes/tobacco smoked as well as the time to first cigarette and Fagerstrom Test for Nicotine Dependence (FTND). (The FTND scale is commonly used to assess levels of nicotine addiction whereby a value of 0 represents low dependence and 10 is very highly dependent).¹⁶

Genotyping of CYP2A6 variants—Participants provided buccal cell swabs for DNA analysis. The DNA was extracted and purified using commercially available BuccalAmp™ DNA Extraction Kits (Epicentre). We obtained good DNA yield and quality using this non-invasive sampling method.

The CYP2A6*9 single nucleotide polymorphism (SNP) was genotyped using an allele-specific PCR technique. The primers for this assay, 2A6*9S and 2A6*9AS, have been previously described by Yoshida et al¹⁴ and correspond to nucleotide positions -395 to -376 and -48 to -28 on the CYP2A6 gene (Accession number AC008537) respectively.

The PCR mixture consisted of approximately 50ng of genomic DNA, 1 x PCR Buffer [67-mmol/L Tris-hydrochloric acid (pH 8.8), 16.6-mmol/L ammonium sulfate, 0.45% Triton X-100, 0.2 mg/mL gelatine and 1.5mmol/L MgCl₂], 0.25-mmol/L deoxyribonucleoside triphosphate (dNTP), 0.4 μmol/L of each primer and 1U of *Taq* DNA polymerase in a final reaction volume of 25 μL.

Thermal cycling and agarose gel electrophoresis were performed as stated by Yoshida et al.¹⁴ Genotyping of the CYP2A6*4 variant was conducted using the exact primers and protocols as published in the paper by Nakajima et al.¹⁷

Measurement of nicotine intake and metabolism—We utilised a non-invasive method to assess nicotine intake and metabolic rate in New Zealand smokers. Participants were asked to provide

approximately 1–2 ml of oral fluid (saliva) in a sterile, airtight plastic collection tube upon waking in the morning and *before* consuming a cigarette, coffee, or food.

Samples were kept at 4°C in the participant's home until collection. We have found that the concentration of COT and 3-HC in saliva samples does not change significantly even when stored at room temperature for 7 days (coefficient of variation < 5%) (unpublished data). The 3-HC:COT ratio determined from saliva was used as an index for nicotine metabolic rate as described by Dempsey et al.⁹

For smokers with fairly constant smoking habits, cotinine levels vary only by about 15% over the course of the day. COT has an *in vivo* half-life of approximately 24 hours. Thus, measurements of salivary COT taken upon waking in the morning were considered an indication of the previous days total ingested nicotine (intake).

Metabolite analyses of the saliva samples were performed at ESR's accredited analytical chemistry laboratory using LC-MSMS instrumentation.

Statistical analyses—The primary test variables included in the statistical analysis were CYP2A6 genotypes, salivary COT concentration, 3-HC:COT ratio, number of cigarettes smoked per day, COT/cigarette, and FTND score. Gene frequencies between general population groups were compared statistically using chi-squared analysis.

To compare means between smoking groups, independent samples T tests were performed. Where appropriate the significance of the T Test was confirmed using an analogous non-parametric test (i.e. Mann-Whitney U). This overcomes problems associated with asymmetrically distributed data. Fisher's Exact Test was used to compare gene frequencies between smoking groups. A p value of ≤0.05 was considered statistically significant. Statistical analyses were performed using SPSS version 12 software.

Results

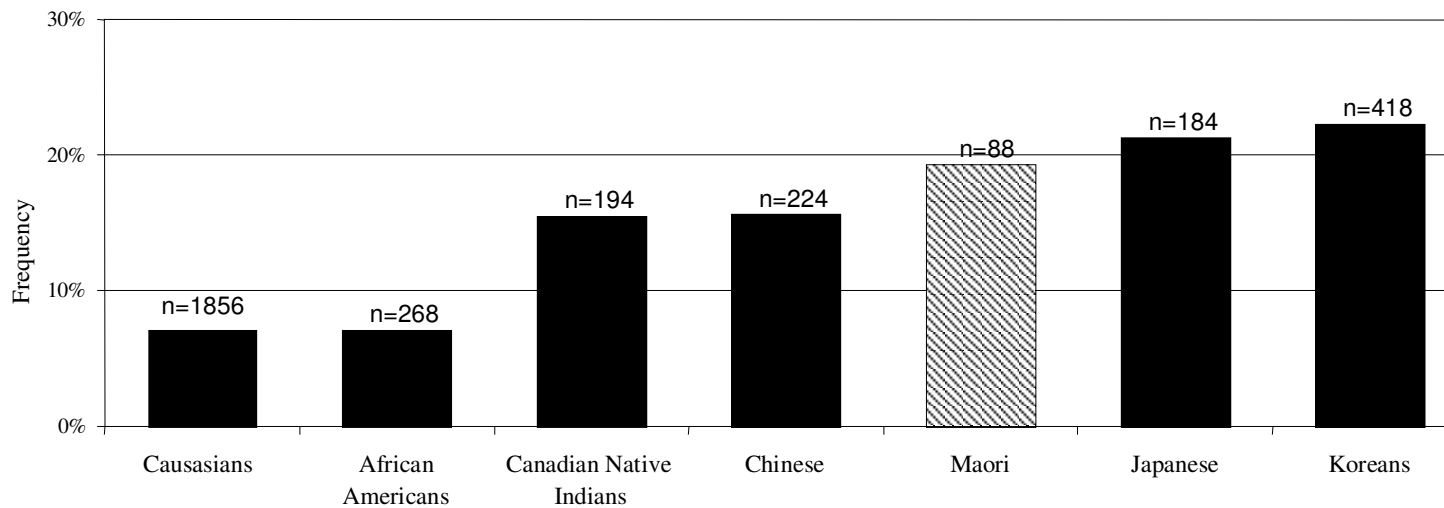
(a) Estimating prevalence of slow nicotine metabolising alleles in the general Maori population

To estimate the general population frequencies for CYP2A6*9 and *4 alleles, we generated genotype data for a group of Maori with no European grandparents. Figure 1 shows the allele frequencies for these Maori as well as other ethnic groups from around the World (data previously published^{13,14}). The CYP2A6*9 allele ranged in frequency from around 8% in Caucasians to 15–22% in Asian groups. The variant was found to be prevalent in the Maori sample (about 20%) and was >2 times more common compared to Caucasians (p<0.001).

The CYP2A6*4 allele on average is less prevalent than CYP2A6*9 but also tended to be more frequent in Asian subgroups compared to non-Asian groups. In our Maori sample we observed a frequency of around 9% for the CYP2A6*4, which is >4 times higher than in Caucasians (p<0.001).

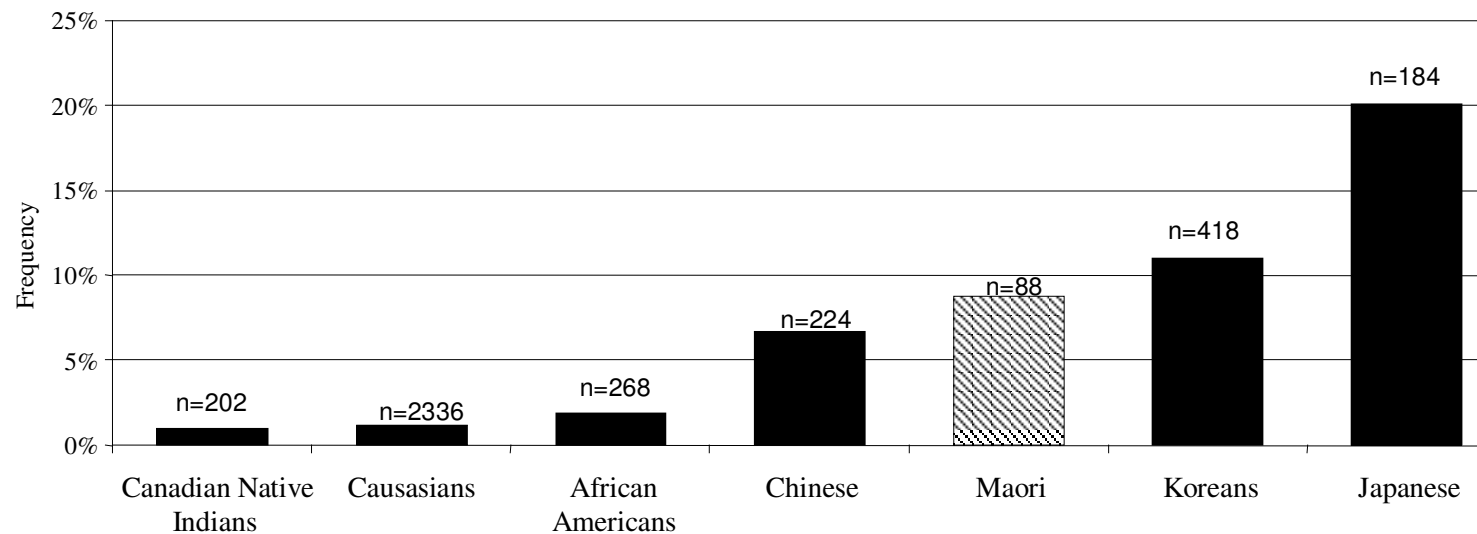
Figure 1. Frequency of slow nicotine metabolising alleles CYP2A6*9 (A) and CYP2A6*4 (B) among different worldwide ethnic groups (x-axis). Data for non-Maori groups are from Schoedel et al, 2002 (Caucasians, AA CNI, Chinese), and Yoshida et al, 2003 (Japanese and Korean)^{13,14}

A. Slow Nicotine Metabolising Allele (CYP2A6*9)



n = number of alleles tested in each group.

B. Slow Nicotine Metabolising Allele (CYP2A6*4)



(b) Estimating nicotine intake and metabolic rate in smokers

To examine the hypothesis that differences exist for nicotine intake and metabolic rate between Maori and European smokers, we employed a non-invasive method to test smokers for both metabolic and genetic variants of CYP2A6 activity.

Descriptive analyses of the questionnaire data showed that there was variation in the strength, brand, and type of cigarettes/tobacco smoked among the participants. Of the 6 Maori smokers, 5 reported that they smoked *Port Royal* brand cigarettes whilst 1 Maori participant smoked *Horizon* brand. Of the European group, 2 participants smoked *Park Drive* brand, 2 smoked *Benson and Hedges* brand, 1 smoked *Holiday* brand, and 1 smoked *Rothmans* brand. Of the Maori smokers, 5/6 typically rolled their own cigarettes compared to only 1/6 of the European smokers.

Comparative analyses of the primary test variables are shown in Table 1. The Maori group had ~50% lower salivary COT compared to the European group ($p=0.03$). According to cotinine levels normalised for number of cigarettes smoked, the Maori smokers took in less nicotine per cigarette on average compared to the European smokers ($p=0.002$).

The 3-HC:COT ratio (nicotine metabolic rate) was significantly lower (~35%) in the Maori smokers compared to European smokers ($p=0.04$). Both the Maori and European group smoked an equal number of cigarettes per day on average ($n=16$). The FTND score was slightly higher on average in Maori smokers but not significantly different from the European group. The CYP2A6*9 allele was significantly over-represented in the female Maori smokers compared to the European smokers (70% vs 30%, respectively). The CYP2A6*4 allele was not observed in this group of smokers.

Table 1. Estimates of nicotine intake and metabolic rate in female smokers

| Smoking measure | Maori (n=6) | European (n=6) | P value* |
|-----------------------------------|---------------|----------------|----------|
| 3-HC/COT ratio [†] | 0.36 ± 0.06 | 0.54 ± 0.06 | 0.04 |
| Salivary COT (ng/ml) [†] | 123.06 ± 21.9 | 261.9 ± 48.3 | 0.03 |
| No. of cigarettes per day | 16 | 16 | ns |
| COT/cigarette [†] | 8.5±1.7 | 18.8±1.9 | 0.002 |
| FTND score [♦] | 4 | 3 | ns |
| CYP2A6*9 allele ^Ω | 70% | 30% | 0.03 |

[†]Values are means ± SE; *P values are 2-sided and were determined by independent T test. P values ≤0.05 are statistically significant. Where appropriate the significance was confirmed using non-parametric Mann-Whitney U test. [♦]FTND is Fagerstrom Test for Nicotine Dependence; ^ΩSlow metabolising allele of the Cytochrome P450-2A6 gene.

Discussion

The high smoking prevalence in Maori is one of the greatest health concerns facing NZ. Characterising the nicotine metabolic profiles that are unique to Maori may help (a) explain why young Maori people seem to be more susceptible to establishing tobacco dependence and becoming long-term smokers; and (b) inform targeted

smoking cessation programs perhaps allowing more tailored NRT to be prescribed for people with Maori ancestry.

We have conducted a study to assess nicotine metabolism in New Zealand. Specifically, we determined frequencies of the CYP2A6 gene slow-metabolising variants, *9 and *4, and showed both to be significantly more prevalent in the general Maori population compared to Caucasians (see Figure 1). To estimate levels of nicotine intake and metabolic rate, we also measured nicotine metabolites in saliva from smokers belonging to the most at-risk societal group in NZ—female Maori.

Comparison of ethnic (ancestral) groups indicated that the Maori smokers had approximately 35% slower nicotine metabolic rate through the CYP2A6 liver pathway compared to Europeans. The amount of nicotine ingested per cigarette was also lower in the Maori group.

These findings suggesting that Maori metabolise nicotine more slowly are consistent with cigarette consumption data showing that Maori tend to smoke fewer cigarettes per day.¹ Interestingly, these trends are similar to those reported in Asian smokers, who have on average a 35% slower nicotine metabolic rate and ingest less nicotine per cigarette and smoke fewer cigarettes per day compared to Caucasians.⁷

We also demonstrated a marked difference in CYP2A6*9 allele frequencies between the Maori and European smokers, which is consistent with the significant differences in general population prevalence of this variant shown in Figure 1. The genetic differences we have found support the argument that DNA variants, which translate into functional metabolic changes, should be considered when attempting to explain differences in smoking-related traits among groups with different ancestral backgrounds.

It is important to address the limitations and future directions of this work. Firstly, further analysis of a much larger sample of smokers (including males and being representative of the general population) is required to confirm the findings of the present study and to accurately estimate the difference in metabolic rate among Maori and European.

Other NZ subgroups such as Pacific Islanders should also be investigated. Larger studies will also allow statistical assessment of the ethnic variation in brand and type of cigarettes smoked. In addition, future research should include data from non-genetic modifiers of nicotine metabolic rate (e.g. caffeine and alcohol consumption) to adjust for potential confounding effects.

Nevertheless, our findings raise some interesting questions about the role of nicotine metabolism in tobacco dependence in relation to Maori. A recent prospective study of adolescent smokers has provided compelling evidence that genetically slow metabolisers have an *increased* risk of tobacco dependence.¹⁸ It was hypothesised in this report that slower nicotine inactivation may lead to prolonged and/or higher brain exposure which might enhance the initial neurophysiological processes that lead to dependence.¹⁸

Therefore, our data suggesting that female Maori smokers are more likely to be genetically slower nicotine metabolisers might partially explain why young Maori females are the most likely ethnic subgroup in NZ to establish and maintain an addiction to tobacco smoking.¹⁹ If more rigorous genetic research of Maori smokers

supports this notion, then there may be a case for designing future-targeted prevention campaigns to include information about genetic/metabolic predispositions.

The results of our study might also help explain why Maori find it more difficult to quit smoking using NRT. NRT is currently the main pharmacological smoking cessation treatment in NZ and is largely subsidised by the Government.

A recent evaluation of the NZ Quitline, a telephone smoking-cessation counselling service, revealed that significantly fewer Maori (10%) were able to quit smoking using NRT after 12 months compared to European (14%) ($P = 0.026$).²⁰

It is plausible that genetically reduced nicotine metabolic rate may influence a group's response to NRT and likelihood of quitting. Specifically, slower metabolism may mean that nicotine replacement levels, attained through patches and/or gum, are far greater than the personalised levels attained through cigarette smoking causing people to relapse to smoking due to the onset of adverse events (e.g. insomnia, nausea, and/or headache).

It is important to note, that being a slow metaboliser is not unique to Maori people and a significant proportion of individuals with no Maori ancestry are also slow nicotine metabolisers. Thus, the ultimate aim of this line of research is to utilise genetic and metabolic information for *individualization* of NRT. Whilst there have been several studies aimed at customising NRT through questionnaire-based methods, none so far have investigated the utility of measuring nicotine metabolic rate as a predictor of smoking cessation.

Being able to accurately predict the rate of nicotine metabolism based on CYP2A6 enzyme activity and/or genotype could facilitate personalised dosing of NRT to ensure optimal nicotine levels are met and side effects are avoided or reduced. In turn, this should improve effectiveness of NRT for the individual and help increase the overall smoking quit rates. The implementation of new targeted cessation strategies integrating knowledge from genetics, clinical medicine, population health programmes, and tobacco legislation looks to be our best strategy for driving down the smoking prevalence in NZ and subsequently reducing the burden of smoking-related disease.

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