Arylamine *N*-acetyltransferase-2 genotypes in the Thai population

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Aims To determine the frequencies of the major arylamine- *N*-acetyltransferase-2 (*NAT2*) alleles in the Thai population.

Methods DNA samples from 235 Thai individuals were analysed by polymerase chain reaction with restriction fragment length polymorphism assays.

Results The frequency distribution of major *NAT2* alleles, including *NAT2*4*, *NAT2*5, NAT2*6* and *NAT2*7* were 0.381 (95% CI 0.337, 0.426), 0.038 (0.023, 0.060), 0.326 (0.283, 0.370) and 0.204 (0.169, 0.244), respectively. When converted to phenotypes, the study population comprised 63.8% rapid acetylators and 36.2% slow acetylators.

Conclusions The pattern of *NAT2* alleles of Thais is similar to those of many Asian populations, although the frequency of *NAT2*4* is significantly lower and *NAT2*7* is higher than that of Oriental populations.

Keywords: acetylation, arylamine-*N*-acetyltransferase, genetic polymorphism, *NAT2*

Introduction

Genetic polymorphism of arylamine *N*-acetyltransferase-2 (NAT2) is responsible for some of the observed interindividual variation in the metabolism of several therapeutic drugs, carcinogens and other xenobiotics [1]. The *NAT2* gene locus maps to human chromosome 8, and more than 29 allelic variants have been described [2]. Population studies using NAT2 selective probe drugs and, subsequently to the advent of molecular techniques, analysis of *NAT2* alleles has led to the classification of individuals as rapid and slow acetylators. The latter are apparently more prone to adverse reactions to some drugs [1] and probably more susceptible to certain types of diseases and cancer [3]. The frequencies of acetylator phenotypes/genotypes in various ethnic groups have been investigated and found to vary widely [4]. The *NAT2*4, *6* and **7* alleles are found in many Asian populations [5]. *NAT2*7* is a unique allele for Asians, while *NAT2*5* is the most commonly found in Caucasians and Africans [4, 6]. Among Asian populations, Chinese and Japanese have a lower prevalence of NAT2 slow-acetylator phenotypes and genotypes than Filipinos, Malays and Khmer, and a much lower frequency than Indians and Arabs, where the latter genotypes tend more to resemble African and Caucasian populations, i.e. lower allele frequencies of *NAT2*4* and **7* and a higher allele frequency for *NAT2*5* [4, 7–9]. Acetylator phenotypes in the Thai population were previously reported to have a higher frequency of phenotypic slow acetylators than Japanese or Chinese [10]. The aim of our study was to determine the frequencies of the main *NAT2* alleles in a Thai population of the northeast region of Thailand, which will be the basis for an epidemiological study of certain diseases found to have a high prevalence in this region and potentially associated with acetylation polymorphism.

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Methods

Subjects

Subjects in the study were unrelated Thai individuals who were native-born from several provinces in the northeast region of Thailand. They were interviewed for ethnic background and only subjects of Thai origin for at least two generations were recruited for the study. They comprised 172 male and 63 female subjects with age ranges between 30 and 71 years. All individuals were considered healthy by physical examination and had no history of cancer or other chronic diseases. The study protocol was

approved by the ethics research committee of the Khon Kaen University and all subjects gave their written informed consent to participate.

Genotyping protocol

Venous blood (10 ml) was drawn in ethylenediamine-tetra-acetic (EDTA) acid tubes. Genomic DNA was extracted and resuspended in Tris-EDTA buffer. Samples were stored at −20 °C until analysis. Genotyping was performed using previously published methods, with some modification [6]. A 1091 base-pair fragment consisting of all polymorphic sites in this study was generated by the polymerase chain reaction (PCR) using reported primers [6]. The polymorphic nucleotides at position 282, 481, 590 and 857 of the *NAT2* coding region were analysed by digesting the PCR products with the endonucleases *Fok*I, *Kpn*I, *Taq*I and *Bam*HI, respectively.

Some ambiguous genotype assignments of certain genotypes arose, due to the fact that the mutation C282T may be present either alone, or in combination with mutations G590A, G857A, A803G and others [11]. As a result, we were unable to distinguish genotype *NAT2⋆7B/⋆4* from *NAT2⋆7A/⋆13 (⋆12B); NAT2⋆6A/⋆4* from *NAT2⋆6B/⋆13 (⋆12B)*; and *NAT2⋆6A/⋆7A* from *NAT2⋆6B/7B*, as each genotype generates an identical pattern of restriction fragments. We extended the work to allow unambiguous genotyping, particularly for *NAT2⋆6A, ⋆6B, ⋆7A* and *⋆7B*. An allele-specific amplification to detect mutation C282T was carried out using two primer pairs, i.e. 282C/N4 and 282T/N4 (282C; 5′-TGT TAG GAG GGT ATT TTT A*C*-3′, 282T; 5′-TGT TAG GAG GGT ATT TTT A*T*-3′, and N4; 5′-TCT AGC ATG AAT CAC TCT GC-3′). A Robocycler Gradient 40 (Stratagene) was used for 33 cycles each including denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and elongation at 72 °C for 1 min. The resulting PCR products of 882 bp were subjected to *Taq*I or *Bam* HI digestion, to detect whether the mutation 282T was present with mutations G590A or G857A, respectively. The digestion products with fragments of 106, 170, 226 and 380-bp or 106, 380 and 396 bp in the presence or absence of restriction site at nucleotide 590, respectively, were separated using a 4% Metaphor gel. Similarly, fragments of 282 and 600-bp or 882-bp (no digestion) in the presence or absence of a restriction site at nucleotide 857 were separated on a 2% agarose gel.

Statistical analysis

The equality of proportions of allele frequencies and acetylator phenotypes between Thai and other populations were tested by the *Z*-test. Allele frequencies and 95% confidence limits (CI) were calculated using the statistical software package, Stata (version 6; Stata Corp., TX, USA).

Results

In this study seven different allelic variants of the *NAT2* gene were identified. Table 1 shows the distribution of mutations described by these allelic variants. Mutation at nucleotide 282 may be *NAT2⋆13* (C282T) or *NAT2⋆12B* (C282T + A803G). A total of seven rapid acetylation genotypes, comprising 63.8% (95% CI 57.3, 70.0) of all individuals was observed, while slow acetylators (36.2% of the population; 95% CI 30.0, 42.7) were distributed among 14 different genotypes. The phenotype frequencies predicted by the Hardy Weinberg equilibrium [1], given R (rapid allele) = 0.381, then r^2 (expected phenotype) = 0.383 (38.3%) which is in agreement with the observed genotype frequency (36.2%, $P \leq 0.05$). *NAT2* allele frequencies of Thais compared with Asians and other populations are shown in Table 2. The frequency of *NAT2⋆4* was significantly higher than that of Caucasians and Arabs, but still lower than Chinese, Japanese and Koreans ($P < 0.05$). Prevalences of *NAT2⋆5* were low: 0.038 (95% CI 2.3, 6.0), and similar to other Asian populations. However, *NAT2⋆7* frequency was significantly higher than most populations reported previously (i.e. 0.204; 95% CI 0.168, 0.244).

In cases of ambiguous assignment of genotypes, we did not find any individuals carrying the *NAT2⋆7A/⋆13* and *NAT2⋆6B/⋆13* genotypes. In fact, we observed only *NAT2⋆7B/⋆4* and *NAT2⋆6A/⋆4*, the alternative genotypes. These findings allowed to correctly assign the phenotypes for individuals who carry those alleles.

Discussion

There is wide interethnic variation of arylamine-*N*-acetyltransferase-2 phenotype, with rapid acetylators ranging from nearly 100% in New Guineans to as low as 10% in Moroccans [1]. Data presented here show that

Table 1 Allele frequencies in healthy Thais.

Mutation	NAT2 *alleles*	n	*%*	*95% CI⋆*
Wild type	*⋆4*	179	38.1	33.7, 42.6
481T	*⋆5*	18	3.8	2.3, 6.0
282T + 590 A	*⋆6A*	131	27.9	23.9, 32.2
590 A	*⋆6B*	22	4.7	3.0, 7.0
857 A	*⋆7A*	28	6.0	4.0, 8.5
282T + 857 A	*⋆7B*	68	14.5	11.4, 18.0
282T	*⋆13A* or *⋆12B*	24	5.1	3.3, 7.5

n Total number of alleles = 470. ⋆ 95% confidence interval.

Table 2 *NAT2* allele frequencies compared with Asian and other populations.

Ethnic groups	n	NAT2⋆4	NAT2⋆5	NAT2⋆6	NAT2⋆7	*Others*†	*SA*‡
Caucasian [6]	744	0.250§	0.450§	0.280§	0.020§	–	0.556§
African [6]	256	0.360	0.300§	0.220§	0.020§	0.09	0.414
Emirati Arabs [7]	212	0.180§	0.540§	0.210§	0.040§	0.020	0.651§
Spanish [15]	1008	0.216§	0.441§	0.256§	0.012§	0.071	0.615§
Indians [5]	278	0.440	0.200§	0.320	0.040§	–	0.314
Malays [5]	292	0.410	0.120§	0.380	0.090§	–	0.348
Khmer [9]	64	0.484	0.156§	0.297	0.063§	–	0.266
Chinese [16]	374	0.510§	0.07§	0.320	0.100§	–	0.283§
Japanese [8]	316	0.640§	0.019	0.230§	0.110§	–	0.130§
Koreans [8]	170	0.681§	0.018	0.180§	0.110§	0.011	0.102§
Taiwanese [8]	200	0.520§	0.025	0.310	0.150	–	0.230§
Filipino [8]	200	0.395	0.065	0.360	0.180	–	0.366
Aborigines Australia [17]	98	0.410	0.02	0.170§	0.400§	–	0.348
Thais (this study)	470	0.381	0.038	0.325	0.204	0.051	0.362

n, total number of alleles determined in the studies. †Only major alleles are considered, while other alleles include *NAT2⋆12, NAT2⋆13* or *NAT2⋆14*. ‡SA, slow acetylator phenotype, derived from genotypes. §Significantly different compared with Thai population, $P < 0.05$.

the Thai population has a relatively high prevalence of rapid acetylators. When *NAT2* genotypes were converted into acetylation phenotypes, the frequency of rapid acetylators was in agreement with previous observations [10]. The pattern of allelic variants of Thais resembles that of East Asian (Oriental) and some southeast Asian populations in that frequency distribution of the wild type allele *NAT2⋆4* is high, while that of *NAT2⋆5* is very low and *NAT2⋆7* is relatively high. This is distinct from those observed in Caucasian, African and Arab populations, where the frequency distribution of the above alleles is in the opposite direction [4, 5, 8]. Compared with Oriental populations, Thais exhibited a relatively lower frequency of the *NAT2⋆4* (0.381 *vs* 0.51–0.68; Table 2) and hence rapid acetylator phenotype, although the frequency of this allele was similar in Thais to other southeast Asian populations (e.g. Filipinos, Malays) and to Indians. However, the pattern of allele frequencies of slow acetylators in Thais is different from that of Indians and Malays who are otherwise similar to European and Arab populations [4, 5]. Apart from the *NAT2* gene, a recent study showed that the frequency distribution of some other alleles, for instance, *CYP2C19* [12] and *MICA* alleles, polymorphic major histocompatibility complex gene in Thais [13], are different from those in Oriental and Caucasian populations. Overall, these observations suggest that the Thai population is more closely related to the southeast Asian populations than to Orientals.

NAT2 catalyses the *N*-acetylation (usually deactivation) and *O*-acetylation (usually activation) of aromatic and heterocyclic amines [14]. Polymorphic expression of the enzyme may modify the risk for bladder cancer, colon cancer and mammary cancer [3]. Moreover, polymorphism of *NAT2* has been found to be associated with certain diseases, for instance, chronic discoid lupus erythematosus, food allergy and adverse drug reactions to antituberculosis drugs and others [1]. Because our understanding of the roles of NAT2 are still incomplete, this study provides a background for further epidemiological studies to evaluate the impact of NAT2 genotype/phenotype polymorphism, environmental exposure and other genetic susceptibility genes on cancer risk and some other diseases.

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References

1 Evans DAP. *Genetic Factors in Drug Therapy: Clinical and Molecular Pharmacogenetics*. Cambridge: Cambridge University Press, 1993.

2 Hein DW, Grant DM, Sim E. Update on consensus arylamine *N*-acetyltransferase gene nomenclature. *Pharmacogenetics* 2000; **10**: 291–292.

3 Hein DW, Doll MA, Fretland AJ, *et al*. Molecular genetics and epidemiology of the NAT1 and NAT2 acetylation polymorphisms. *Cancer Epidemiol Biomarkers Prev* 2000; **9**: 29–42.

4 Lin HJ, Han CY, Lin BK, Hardy S. Slow acetylator mutations in the human polymorphic *N*-acetyltransferase gene in 786 Asians, blacks, Hispanics, and whites: application to metabolic epidemiology. *Am J Hum Genet* 1993; **52**: 827–834.

5 Zhao B, Lee EJ, Wong JY, Yeoh PN, Gong NH. Frequency of mutant CYP1A1, NAT2 and GSTM1 alleles in normal Indians and Malays. *Pharmacogenetics* 1995; **5**: 275–280.

6 Bell DA, Taylor JA, Butler MA, *et al*. Genotype/phenotype discordance for human arylamine *N*-acetyltransferase (NAT2) reveals a new slow-acetylator allele common in African-Americans. *Carcinogenesis* 1993; **14**: 1689–1692.

7 Woolhouse NM, Qureshi MM, Bastaki SM, Patel M, Abdulrazzaq Y, Bayoumi RA. Polymorphic *N*-acetyltransferase (NAT2) genotyping of Emiratis. *Pharmacogenetics* 1997; **7**: 73–82.

8 Lin HJ, Han CY, Lin BK, Hardy S. Ethnic distribution of slow acetylator mutations in the polymorphic *N*-acetyltransferase (NAT2) gene. *Pharmacogenetics* 1994; **4**: 125–134.

9 Bechtel YC, Bechtel PR, Lelouet H, Choisy H, Dy NR. The acetylator polymorphism in a Khmer population: clinical consequences. *Therapie* 2001; **56**: 409–413.

10 Kukongviriyapan V, Lulitanond V, Areejitranusorn C, Kongyingyose B, Laupattarakasem P. *N*-acetyltransferase polymorphism in Thailand. *Hum Hered* 1984; **34**: 246–249.

11 Cascorbi I, Roots I. Pitfalls in *N*-acetyltransferase 2 genotyping. *Pharmacogenetics* 1999; **9**: 123–127.

12 Tassaneeyakul W, Tawalee A, Kukongviriyapan V, Blaisdell J, Goldstein JA, Gaysornsiri D. Analysis of the CYP2C19 polymorphism in a North-eastern Thai population. *Pharmacogenetics* 2002; **12**: 221–225.

13 Romphruk AV, Naruse TK, Romphruk A, *et al*. Diversity of MICA (PERB 11.1) and HLA haplotypes in Northeastern Thais. *Tissue Antigens* 2001; **58**: 83–89.

14 Hein DW, Doll MA, Rustan TD, *et al*. Metabolic activation and deactivation of arylamine carcinogens by recombinant human NAT1 and polymorphic NAT2 acetyltransferases. *Carcinogenesis* 1993; **14**: 1633–1638.

15 Agundez JA, Olivera M, Martinez C, Ladero JM, Benitez J. Identification and prevalence study of 17 allelic variants of the human NAT2 gene in a white population. *Pharmacogenetics* 1996; **6**: 423–428.

16 Lee EJ, Zhao B, Seow-Choen F. Relationship between polymorphism of *N*-acetyltransferase gene and susceptibility to colorectal carcinoma in a Chinese population. *Pharmacogenetics* 1998; **8**: 513–517.

17 Ilett KF, Chiswell GM, Spargo RM, Platt E, Minchin RF. Acetylation phenotype and genotype in aboriginal leprosy patients from the north-west region of Western Australia. *Pharmacogenetics* 1993; **3**: 264–269.