

## Genetic analysis of *N*-acetyltransferase polymorphism in a Chinese population<sup>1</sup>

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**KEY WORDS** *N*-acetyltransferases; polymorphism (genetics); genotype; Chinese; alleles; polymerase chain reaction

**AIM:** To study the genetic basis of *N*-acetyltransferase polymorphism in Chinese. **METHODS:** Genotypes in 120 healthy Han volunteers from 19 provinces of China were assayed. The 3 common mutant alleles (M1, M2, M3) and one normal wild-type (WT) allele of the *N*-acetyltransferase (NAT2) gene were detected by allele-specific polymerase chain reaction technique. **RESULTS:** The NAT2 allele frequencies in 120 Chinese (WT = 0.625, M1 = 0.0458, M2 = 0.188, M3 = 0.142) were different ( $P < 0.01$ ). The NAT2 genotype distribution for all detected combinations of NAT2 alleles in 120 Chinese subjects was consistent with Hardy-Weinberg equilibrium ( $\chi^2 = 7.27$ ,  $\nu = 8$ ,  $0.7 > P > 0.5$ ). Fifty subjects (41.7%) were homozygous wildtypes, 50 subjects (41.7%) were heterozygous mutants, and 20 subjects (16.7%) were homozygous mutants. **CONCLUSION:** The lower frequency of mutant M1 allele compared with that of Caucasians explains the low frequency of slow acetylators in Chinese.

Human polymorphic *N*-acetyltransferase (NAT2) catalyzes the *N*-acetylation of numerous drugs and carcinogens<sup>[1]</sup>. The rapid and slow acetylator phenotype involves the NAT2 gene, which is a polymorphic acetyltransferase gene locus that segregates individuals into homozygous rapid, heterozygous intermediate, and homozygous slow acetylators<sup>[2,3]</sup>. The "slow acetyla-

tion" in human is due to mutations in the single coding exon of the NAT2 gene, 3 major slow acetylator alleles (2 common in Caucasians, 1 common in Asians) have independently been identified in several human populations. Nucleotide 341T → C (Ile → Thr) and 481C → T (silent) mutations were found together and named the M1 allele<sup>[3]</sup>. Another allele containing both the 341 and 481 mutations with an additional 803A → G (Lys → Arg) mutation has also been identified<sup>[5]</sup>, and will be considered as M1 in present study. An allele termed M2 carries 282C → T (silence) and 590G → A (Arg → Gln) mutations<sup>[3,5,6]</sup>. The M3 allele contains 2 point mutations [282C → T (silence) and 857G → A (Gly → Glu)]<sup>[6]</sup>. These discoveries of molecular mechanism of NAT2 polymorphism led to the development of allele-specific polymerase chain reaction (PCR) and amplification-restriction tests for genotyping<sup>[7]</sup>.

In China, the proportion of slow acetylators reported by independent studies concerning Chinese population range from 10% to 40% approximately. This variation may be a result of different probes used in these studies. The accuracy of PCR might overcome these shortcomings and give a more confident frequency of slow acetylator in Chinese.

## METHODS

**Subjects** With personal written consent, 120 unrelated healthy Han volunteers aged  $34 \pm s 12$  a (18 - 67 a), weighing  $60 \pm s 23$  kg, participated in the study. All volunteers (M 59, F 61) worked in Jinling Hospital and were from 19 provinces of China. The study was approved by Ethics Committee at Jinling Hospital.

**DNA sources and isolation** Venous blood (about 2 mL) was collected in heparinized centrifugal tubes (with edetic acid as anticoagulant). Genomic DNA was extracted from 1 mL blood by the method reported<sup>[7]</sup>. Briefly, the

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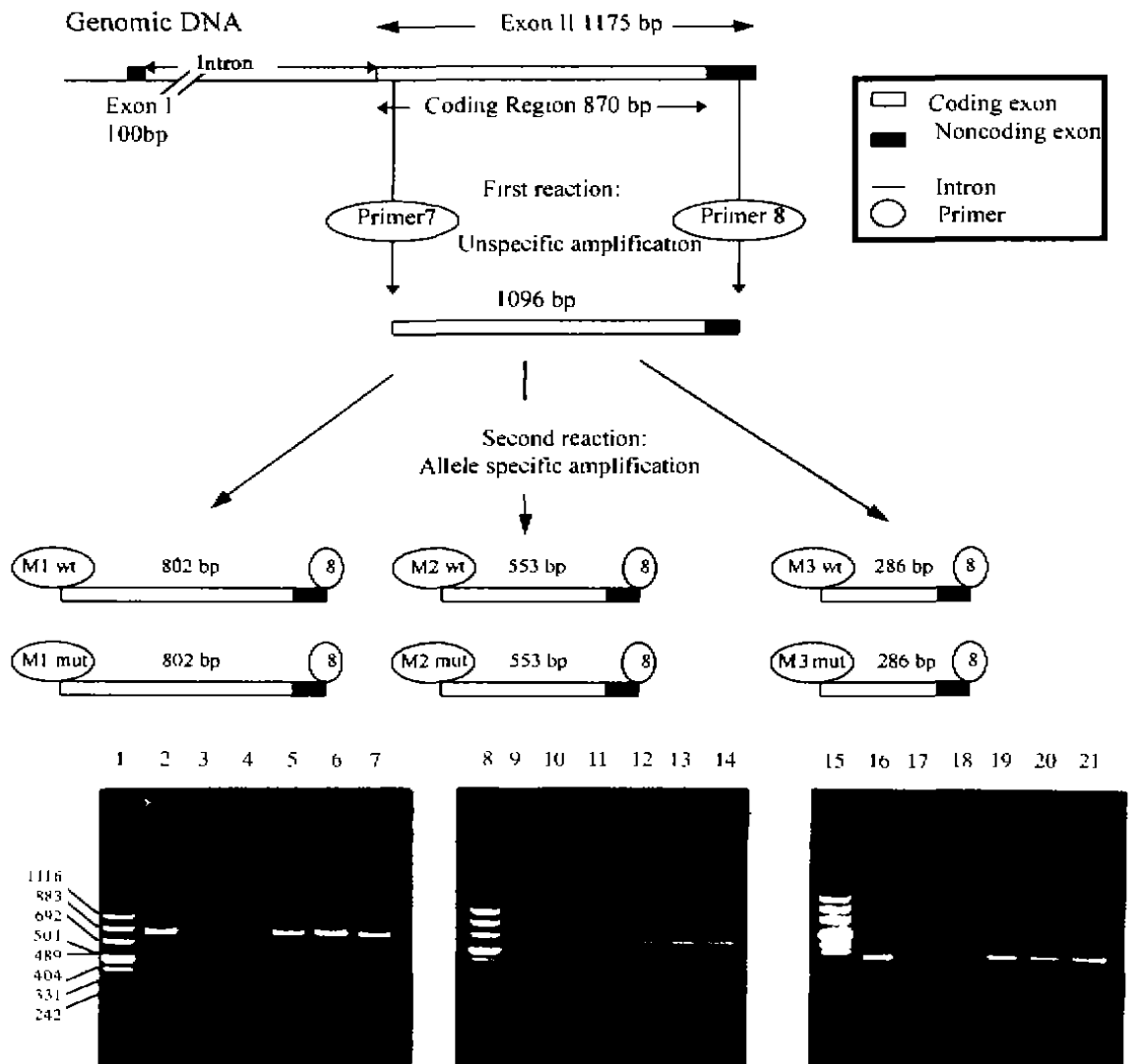
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white and red blood cells were destroyed with Triton X-100, to obtain nuclei of leukocytes. Nuclear protein was disassembled with NaClO<sub>4</sub> and SDS to liberate DNA, which was extracted with PCI (phenol : chloroform : isoamylalcohol, 25:24:1). DNA was precipitated with ethanol.

**Genomic DNA amplification** Primer 7 and primer 8 amplified the whole NAT2 gene (1096 bp), including the 870 bp coding sequence from genomic DNA in the first step. The amplification of genomic DNA was carried out by PCR in a 50 μL reaction of Tris-HCl 10

mmol·L<sup>-1</sup>, pH 8.3, KCl 50 mmol·L<sup>-1</sup>, MgCl<sub>2</sub> 1.5 mmol·L<sup>-1</sup>, each dNTP 0.2 mmol·L<sup>-1</sup>, each primer (primer 7 and primer 8) 0.2 mmol·L<sup>-1</sup>, and Taq DNA polymerase (Sino-American Biotechnology Company, Shanghai, China) 2.5 units. This mixture was subjected to 7 min at 94 °C, followed by 25 cycles at 94 °C for 45 s to denature DNA, at 54 °C for 45 s to anneal primers, and at 72 °C for 45 s to synthesize DNA. A 7-min incubation at 72 °C followed to ensure complete extension (Perkin-Elmer Model 9600 DNA Thermal Cycle) (Fig 1).



**Fig 1.** PCR test used for identification of mutations at 341C, 590A, and 857A. Lanes 1, 8, and 15, DNA molecular weight markers (pUC mix). The presence of mutations was investigated by pairs of reactions that differed in mutant-sensitive primer: lanes 2 and 3 (341T/341T), 6 and 7 (341T/341C), 9 and 10 (481C/481C), 11 and 12 (481T/481T), 13 and 14 (481C/481T), and 17 (857G/857G), 18 and 19 (857A/857A), 20 and 21 (857G/857A). Lanes 4 and 5, no subject homozygous for the mutation was identified in this population because of the low frequency of the 341C mutation.

**Allele-specific amplification**<sup>[4]</sup> The resulting 1096-bp PCR product was subjected to allele-specific amplification in the second step. The presence or absence of the 341(T→C), 590(G→A) and 857(G→A) nucleotide substitutions was detected by 3 pairs of allele-specific PCR. The primers were different for each mutation tested (Tab 1, Fig 2): for the 341T mutation (corresponding to NAT2 haplotypes 341C + 481T + 803G and 341C + 481T), a pair of primers named M1 WT (Primer 1) and M1 Mut (primer 2) complementary to the wild-type and 341 C sequences, respectively, were separately used in combination with the

primer 8. The 590A mutation (corresponding to 590A alone and 282T + 590A allelic variants) was detected by the pair of primers, M2 WT (primer 3) and M2 Mut (primer 4) in combination with the primer 8. The 857A mutation was detected by using primers M3 WT (primer 5) and M3 Mut (primer 6) in combination with the primer 8. The amplification for the 3 mutations were carried out at the same condition in a 25 μL reaction containing Tris-HCl 10 mmol · L<sup>-1</sup>, pH 8.3, KCl 50 mmol · L<sup>-1</sup>, MgCl<sub>2</sub> 1.5 mmol · L<sup>-1</sup>, each dNTP 0.2 mmol · L<sup>-1</sup>, each primer 0.5 μg (upstream primers were used paired with the common downstream primer P8), and Taq DNA polymerase 1.25 units. Thirteen cycles of amplification were performed (94 °C, 30 s; 57 °C, 25 s; 72 °C, 25 s) followed by a 5-min extension at 72 °C. PCR products were loaded onto a 1.2 % agarose gel containing ethidium bromide, run at 50 V for 20 min, visualized and photographed under ultraviolet light. The presence or absence of mutations were judged by the existence of allele-specific amplification band (Fig 1).

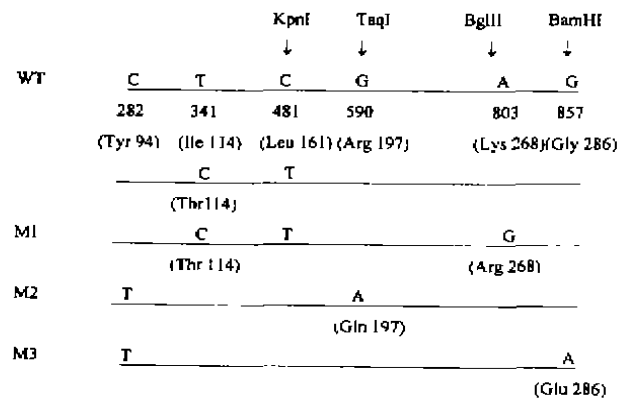
**Calculations** The distribution of genotypes expected assuming a Hardy-Weinberg equilibrium was calculated from the allele frequencies, using the equation  $p^2 + 2pq + 2pr + 2ps + q^2 + 2qr + 2qs + r^2 + 2rs + s^2$ , where p is M1, q is M2, r is M3 and s is WT, corresponding to the 10 possible NAT genotypes. The  $\chi^2$  test was used to compare the observed genotype incidence ( $O_n$ ) with the expected genotype incidence ( $E_n$ ) calculated above using the equation  $\chi^2 = \sum (O_n - E_n)^2 / E_n$ . The number of possible genotypes ( $n$ ) was 10, and the number of degrees of freedom ( $\nu$ ) was defined as one less than the number of genotypes found.

**RESULTS**

All the 3 mutations (341C, 590A, 857A) were detected with PCR assay in the population studied. The frequencies of distinct mutant alleles and the NAT2 genotype distribution for all detected combinations of NAT2 alleles in 120 Chinese subjects were summarized in Tab 2, and was consistent with Hardy-Weinberg equilibrium ( $\chi^2 = 7.27, \nu = 8, 0.7 > P > 0.5$ ). Fifty subjects (41.7 %) were homozygous wildtypes,

**Tab 1. Oligonucleotide allele specific primers used for NAT2 genotyping; upstream primers were used paired with the common downstream primer P8.**

Primer	Nucleotide sequence	Primer position /nt	Amplification length/bp	Specificity
<b>Upstream</b>				
P1	5'-CTOCTGCAGGTGACCAT	325 - 341	802	341T (Wild-type)
P2	5'-CTOCTGCAGGTGACCCAC	325 - 341	802	341C (Mutation, M1)
P3	5'-TITACGCTTGAACCTCG	574 - 590	553	590G (Wild-type)
P4	5'-TITACGCTTGAACCTCA	574 - 590	553	590A (Mutation, M2)
P5	5'-CCAAAACCTGGTGATCG	841 - 857	286	857G (Wild-type)
P6	5'-CCAAAACCTGGTGATGA	841 - 857	286	857A (Mutation, M3)
P7	5'-GCCTATAAGAACTCTAGGAAC	31 - 51	1096	Whole NAT2 gene
<b>Downstream</b>				
P8	5'-CACTCTGCTTCCCAAGAT	1109 - 1126		



**Fig 2. Polymorphic N-acetyltransferase alleles.**

50 subjects (41.7 %) were heterozygous and 20 subjects (16.7 %) were homozygous mutants. All homozygous wildtype and heterozygous subjects were classified as rapid acetylators. All homozygous mutants were slow acetylators.

Tab 2. Observed and expected frequencies of NAT2 genes in the Chinese population.

Genotype	n	Observed/%	Expected/%
WT/WT	50	41.7 (32.8-50.5)	39.1
WT/M1	8	6.7 (2.2-11.1)	5.7
WT/M2	21	17.5 (10.7-24.3)	23.4
WT/M3	21	17.5 (10.7-24.3)	17.7
M1/M1	0	0	0.21
M2/M2	9	7.5 (2.8-12.2)	3.5
M3/M3	3	2.5 (0-5.3)	2.0
M1/M2	1	0.8 (0-2.4)	1.7
M1/M3	2	1.7 (0-4.0)	1.3
M2/M3	5	4.2 (0.6-7.7)	5.3

**DISCUSSION**

Mutation 341C rarely occurred without 481T and 803G, so the 341C mutation is sufficient for detecting the slow acetylator alleles previously designated M1<sup>[11]</sup>. Mutation 590A was found with mutation 282T 94 % of the time<sup>[11]</sup>. Mutation 282T was also found alone, but it did not cause slow acetylation in COS cells<sup>[6]</sup>. Thus 590A is the more suitable mutation for detecting the M2 allele (282T/590A). A recent study in 844 Caucasian<sup>[12]</sup> revealed that if detecting 481T, 590A, and 857T, 5.7 % rare mutation alleles could not be detected, while detecting 341C, 590A, and 857T instead, only 1.7 % would be disregarded. So our detection is sufficient to detect the slow mutations in Chinese.

Our genotyping procedure is based on previous elucidation of NAT2 DNA sequence of wild-type and three mutated gene<sup>[3]</sup>. To enhance specificity, PCR-directed preamplification of the whole NAT2 gene has been introduced, thus circumventing false amplification of highly homozygous NAT1 and of a NAT pseudogene. Conditions for each PCR assay were optimized and DNA marker was used to avoid false negative or positive signals.

Low frequency of slow acetylator genotype in the Chinese population (16.7 %) conforms to findings among Asians: 27 % of slow acetylators

have been genotyped by testing 6 mutations among 70 Chinese from Hong Kong<sup>[11]</sup> and 20 % among 84 Chinese male from Shanghai and Tianjin<sup>[10]</sup>. In Japanese, the frequency is much lower, 8 % among 51 Japanese<sup>[8]</sup> and 7 % among 145 Japanese<sup>[9]</sup>. This study confirmed that low frequency of slow acetylator in the Far East population was mainly due to the decrease in prevalence of M1 alleles again. In the Chinese population, not as in other white subjects, the majority of NAT2 alleles are composed of WT, M2, M3.

Genetic variations in drug-metabolizing enzyme have been shown to correlate with risk of toxicity or cancer<sup>[1]</sup>. Noninvasive genotyping methods represent a potentially powerful tool for these epidemiology studies, because large populations can be rapidly screened for specific genotypes by PCR methods and minute amount of DNA collected from dried blood spots, hairbulbs or sections from paraffin-embedded tissues can be used.

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### 中国人群 *N*-乙酰转移酶多态性的基因分析<sup>1</sup>

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**关键词** *N*-乙酰转移酶; 多态性(遗传学); 基因型; 中国人; 等位基因; 聚合酶链反应

**目的:** 研究中国人群 *N*-乙酰转移酶(NAT2)多态性的基因基础。 **方法:** 对 120 名汉族健康志愿者进行基因型分析。 NAT2 基因型的检测应用等位基因 PCR 技术。 **结果:** NAT2 基因的野生型及 M1, M2, M3 突变等位基因在 120 人中的发生率(WT = 0.625, M1 = 0.0458, M2 = 0.188, M3 = 0.142)存在显著差异( $P < 0.01$ ), 由它们组合而成的各种基因型在人群中的分布符合 Hardy-Weinberg 平衡( $\chi^2 = 7.27, \nu = 8, 0.7 > P > 0.5$ )。 经过基因型分析可将受试者分为野生型纯合子、杂合子及突变纯合子, 其比例为 50:50:20。 **结论:** 与高加索人比较, 中国人的 M1 突变基因的发生率较低, 说明了中国人慢乙酰化者比例较低的原因。

## Effects of vitamin C on myocardial mitochondrial function and ATP content in hypoxic rats

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**KEY WORDS** ascorbic acid; anoxia; heart mitochondria; adenosine triphosphate; blood flow velocity; blood gas analysis; blood pressure; membrane fluidity; Na<sup>+</sup>-K<sup>+</sup>-exchanging ATPase; myocardium

**AIM:** To observe the effects of large dose of vitamin C (V<sub>c</sub>) on myocardial mitochondrial function, ATP content, and myocardial structure in acute and chronic hypoxic rats.

**METHODS:** Rats were exposed to a simulated altitude 4000 m (barometric pressure = 43 kPa) for 3 and 30 d. V<sub>c</sub> (0.75 g·kg<sup>-1</sup>·d<sup>-1</sup>) was injected ip. The heart mitochondrial respiratory function were determined by Clark-type O<sub>2</sub>

electrode; mitochondrial membrane fluidity (MMF) were assayed through fluorescence polarizative method; the contents of ATP, ADP, and AMP in myocardial tissue were measured with HPLC. **RESULTS:** After administration of V<sub>c</sub>, the ATP content was increased from 35 ± 3 mg·g<sup>-1</sup> to 53 ± 3 mg·g<sup>-1</sup> in acute hypoxic rats ( $P < 0.01$ ), from 42 ± 4 mg·g<sup>-1</sup> to 48 ± 3 mg·g<sup>-1</sup> in chronic hypoxic rats ( $P < 0.01$ ); p<sub>a,O<sub>2</sub></sub> was increased from 7.2 ± 1.4 kPa to 9.5 ± 1.2 kPa in acute hypoxic rats ( $P < 0.01$ ); mitochondrial respiratory control rate (RCR) was increased from 2.1 ± 0.6 to 4.7 ± 0.5 in acute hypoxic rats ( $P < 0.01$ ), and from 3.3 ± 0.7 to 4.5 ± 0.6 in chronic hypoxic rats ( $P < 0.01$ ); MMF was increased in acute and chronic hypoxic rats ( $P < 0.05$ ); the degree of myocardial necrosis in vitamin C preventive rats was attenuated as compared with those of acute

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