The Differential Effect of NAT2 Variant Alleles Permits Refinement in Phenotype Inference and Identifies a Very Slow Acetylation Genotype

Jhon D. Ruiz1, Carmen Martínez1, Kristin Anderson2, Myron Gross3, Nicholas P. Lang4, Elena García-Martín5, José A. G. Agúndez1*

1 Department of Pharmacology, Medical School, University of Extremadura, Badajoz & Cáceres, Spain, 2 Division of Epidemiology & Community Health, School of Public Health, University of Minnesota, Minneapolis, Minnesota, United States of America, 3 Department of Laboratory Medicine and Pathology, School of Medicine, University of Minnesota, Minneapolis, Minnesota, United States of America, 4 Department of Surgery, University of Arkansas Medical Sciences, Little Rock, Arkansas, United States of America, 5 Department of Biochemistry & Molecular Biology, School of Biological Sciences, University of Extremadura, Cáceres, Spain

Abstract

Indirect evidences suggest that acetylation phenotype categories are heterogeneous and that subcategories, related to specific NAT2 variant alleles might exist. We analyzed the in vivo acetylation phenotype and genotype in 504 north-American subjects of Caucasian origin. The analyses of the SNPs rs1801280 and rs1799930 allowed the discrimination of five categories with different acetylation status within the study population. These categories are related to the distinct effect of NAT2 alleles on the acetylation status in vivo and to the occurrence of a gene-dose effect. These five phenotype categories, from higher to lower acetylation capacity, correspond to the genotypes NAT2*4/*4, NAT2*4/*5 or *4/*6, NAT2*5/*5, NAT2*5/*6 and NAT2*6/*6 (p<0.001 for all comparisons). The NAT2*6/*6 genotype correspond to a phenotype category of very-slow acetylators. The refinement in phenotype prediction may help to identify risks associated to phenotype subcategories, and warrants the re-analysis of previous studies that may have overlooked phenotype subcategory-specific risks.

Citation: Ruiz JD, Martínez C, Anderson K, Gross M, Lang NP, et al. (2012) The Differential Effect of NAT2 Variant Alleles Permits Refinement in Phenotype Inference and Identifies a Very Slow Acetylation Genotype. PLoS ONE 7(9): e44629. doi:10.1371/journal.pone.0044629

Introduction

The widespread use of genetic biomarkers as surrogate endpoints aiming to describe risks, exposures, intermediate effects of treatments, and biologic mechanisms is a goal that scientists have long been pursuing. The adoption of any genetic test as a surrogate biomarker requires previous demonstration of its analytical and clinical validity as well as its clinical utility, and increasing the predictive capacity of genetic biomarkers is one of the major problems that we have to solve in order to transfer advances in pharmacogenomics to routine clinical practice. Determination of the polymorphic acetylation (NAT2 genotype or phenotype) was initially proposed to predict adverse reactions in patients with tuberculosis receiving isoniazid, prior to the concomitant administration of procainamide and phenytoin, and to analyze the role of NAT2 in drug interactions. These effects, together with the role of NAT2 in cancer risk, in non-malignant spontaneous disorders and in drug response and toxicity, make NAT2 a relevant target for pharmacogenomic testing in clinical practice [1,2].

Nearly fifty years ago, Evans et al. demonstrated that acetylation of isoniazid was bimodally distributed and that the in vivo acetylation status was inheritable [3,4]. Since then, traditional phenotype determination by inference from genetic analyses has classified the population in three groups: rapid, intermediate and slow acetylators. Although this classification of individuals into three phenotype categories is widely accepted, it would be desirable to refine further the predictive capacity of acetylation pharmacogenomic testing [5]. Heterologous expression of NAT2 allozymes provided indirect evidence suggesting a differential effect of NAT2 variant alleles and hence heterogeneity within the slow acetylation phenotype (reviewed in [6]).

This study aims to analyze whether this evidence of heterogeneity within rapid and slow acetylators exists in vivo, whether commonly used pharmacogenomic tests are adequate for the inference of phenotype subcategories, and to measure the activities for such phenotype subcategories. Because acetyl metabolites may be pharmacologically active, or function as intermediates in toxic metabolic pathways, further refinement in phenotype prediction may help to identify risks associated to one or more of such phenotype subcategories.

Methods

The subjects were drawn from a study previously described [7,8,9]. Brieﬂy, cases (n=93), of newly diagnosed cancer of the exocrine pancreas were recruited from all hospitals in the 7-county metropolitan area of the Twin Cities, Minnesota and the
Mayo Clinic (from the latter, only cases residing in the Upper Midwest of the US were recruited). Controls (n = 411) were randomly selected from the general population and frequency matched to cases by age and sex (Table 1). All were Caucasian. Each participant provided written, informed consent prior to interview and blood draw. The study was approved by the Institutional Review Boards of the University of Minnesota and The Mayo Clinic, USA and by the Ethics Committee of the University of Extremadura, Spain.

In vivo NAT2 activity was measured with a widely used caffeine-based assay, as described by Butler et al. [10] with minor modifications as described elsewhere [8,11]. The caffeine assay is highly accurate and reproducible, and it is considered as a gold standard for acetylation phenotyping. Details on accuracy and reproducibility were published elsewhere [10,12,13,14,15]. In brief, subjects ingested 200 mg of caffeine, following an overnight fast. Subjects refrained from the consumption of caffeine- and methylxanthine-containing foods and beverages from midnight until 5 h after the dose of caffeine. A urine specimen was collected 5 h after the administration of caffeine and samples were acidified and stored as described elsewhere [11]. Regarding HPLC analysis, 200 μl of urine were saturated with 125 mg of ammonium sulfate, and 6.0 ml of chloroform:isopropanol (95:5) were added. Each sample was vortexed and centrifuged, and the organic phase was removed and evaporated to dryness. The residue was resuspended in 250 μl of 0.05% acetic acid, filtered, and frozen until analysis.

Acetylation phenotype was assigned on the basis of a molar AFMU/1X ratio, which served as quantitative determinant of acetylation capacity with a cut-off value = 0.66 (log AFMU/1X = -0.18) in agreement with previous studies [11].

NAT2 genotyping aimed to identify the signature SNPs for alleles corresponding to the NAT2*5, NAT2*6, NAT2*7 and NAT2*14 clusters, that is, rs1801280 (I114T), rs1799930 (R197Q), rs1799931 (G286E) and rs1001279 (R64Q), respectively. Although several NAT2 alleles have been described (for an updated list of NAT2 alleles and haplotypes see the website http://louisville.edu/medschool/pharmacology/consensus-human-arlyamine-n-acetyltransferase-gene-nomenclature/nat_pdf_files/Human_NAT2_alleles.pdf), the SNPs analyzed in this study identify the vast majority of slow NAT2 variant allele clusters [16,17]. Genotyping was carried out by the use of TaqMan® probes (details available in Table S1). For every SNP analyzed, twenty samples with heterozygous genotypes and up to twenty samples with homozygous genotypes (homozygous non-mutated and homozygous mutated when available), were determined as blind duplicates. In all samples with genotype/phenotype discordance (n = 32) the genotypes were confirmed by the use of PCR-based mutation-specific amplification as described elsewhere [8] or by direct sequencing of the amplified fragments. In all cases the genotypes fully corresponded to those obtained with TaqMan probes. Haplotype assignation and phenotype inference: All possible haplotypes combining the four SNPs analyzed were constructed and their frequencies were analyzed by using PHASE and the NAT2 haplotype table described elsewhere [16]. Phenotype inference was carried out as described elsewhere [16]. Putative departures of Hardy-Weinberg Equilibrium were calculated by using the software Haplovieq 4.1. Continuous variables (acetylation ratios), expressed as mean (SD), were compared with the Student’s T test, and tests for trend were calculated with the Spearman’s rank correlation by using the statistical software SPSS 15.0 for Windows (SPSS Inc. Chicago, Illinois, USA). A p value <0.05 was considered significant. When multiple comparisons were made, adjustments for multiple comparisons were carried out according to Bonferroni’s procedure.

Results

The SNP frequencies and the genotypes observed in the 504 participants are summarized in Table 2. The degree of phenotype-genotype concordance by using the traditional phenotype classification (i.e. rapid/slow phenotypes), where NAT2*4 containing genotypes are considered a rapid phenotype, and other genotypes a slow phenotype, was equal to 93.7%. We selected 435 individuals with genotypes NAT2*4/*4, *4/*5, *4/*6, *5/*5, *5/*6 and *6/*6 and phenotype/genotype concordance for further analyses. These corresponded to 73 cases and 362 control subjects. Carriers of variant alleles NAT2*14 were not included in the analyses, because these alleles were not present in the study population (Table 2). In addition, carriers of the variant alleles NAT2*7 were not included in the comparisons because these alleles were rare in the population study (Table 2).

Table 1. Characteristics of the individuals included in the study.

|                     | Overall study group | Overall study group | Individuals selected | Individuals selected |
|---------------------|---------------------|---------------------|----------------------|----------------------|
|                     | Male (n = 312)      | Female (n = 192)    | Male (n = 274)       | Female (n = 161)     |
| Age years (mean ± SD) | 65.3 ± 11.3        | 65.7 ± 13.0        | 65.6 ± 11.2          | 66.0 ± 12.4          |
| Never smokers (n;% ) | 104 (33.3%)         | 113 (58.9%)        | 89 (32.5%)           | 98 (60.1%)           |
| Past smokers (n;% ) | 167 (53.5%)         | 59 (30.7%)         | 147 (53.6%)          | 47 (28.8%)           |
| Current smokers (n;% ) | 41 (13.1%)         | 20 (10.4%)         | 38 (13.9%)           | 18 (11.0%)           |
| Pack-years (mean ± SD) | 37.4 ± 30.6        | 23.7 ± 21.2        | 37.8 ± 30.7          | 23.2 ± 19.9          |
| Non-drinkers/drinkers | 112/200             | 95/97              | 96/178               | 80/83                |
| Servings of alcohol per week (mean ± SD) | 9.1 ± 11.2 | 4.9 ± 5.2 | 9.4 ± 11.6 | 5.2 ± 5.4 |
| Cases/Controls (n)        | 63/249              | 30/162             | 53/221               | 20/141               |

Individuals selected for phenotype inference refinement correspond to 435 individuals with genotypes NAT2*4/*4, *4/*5, *4/*6, *5/*5, *5/*6 or *6/*6 and phenotype-genotype concordance.

Pack-years calculation includes smokers and ex-smokers. Servings of alcohol per week include drinkers only.

doi:10.1371/journal.pone.0044629.t001
Table 2. NAT2 SNP frequencies observed in the present study.

| SNP identifier | Amino Acid | No. | Observed frequency (%) | Expected frequency (%) | Hardy Weingberg's P |
|----------------|------------|-----|------------------------|------------------------|---------------------|
| rs1801280 (NAT2*5) | T/T | 114 | 32.14 | 31.64 | 0.647 |
| | T/C | 114 | 48.22 | 49.22 | |
| | C/C | 114 | 19.64 | 19.14 | |
| rs1799930 (NAT2*6) | G/G | 197 | 52.18 | 52.88 | |
| | G/A | 197 | 41.07 | 39.68 | 0.430 |
| | A/A | 197 | 6.75 | 7.44 | |
| rs1799931 (NAT2*7) | G/G | 286 | 92.06 | 91.84 | |
| | G/A | 286 | 7.54 | 7.99 | 0.209 |
| | A/A | 286 | 0.40 | 0.17 | |
| rs1801279 (NAT2*14) | G/G | 64 | 100.0 | 100.0 | |
| | G/A | 64 | 0.00 | 0.00 | |
| | A/A | 64 | 0.00 | 0.00 | |

Expected frequencies are calculated from observed allele frequency.

doi:10.1371/journal.pone.0044629.t002

Table 3 shows the acetylation capacity of the six genotype categories analyzed in the study. The individuals with the genotype categories NAT2*4/*5 and NAT2*4/*6 showed similar acetylation values. However, for the rest of individuals, each genotype category corresponded to a distinct phenotype category, with non-overlapping 95% confidence intervals for the activity, and in all cases the differences between these categories were statistically significant. This provides in vivo evidence that in the absence of NAT2*4 alleles, variant alleles NAT2*5 and NAT2*6 confer different acetylation capacity. In addition, a gene-dose for these variant alleles can be observed within the slow acetylator phenotype, as there is a statistically significant trend to slower acetylation capacity among individuals with the genotypes as follows: NAT2*5/*5 > NAT2*5/*6 > NAT2*6/*6 (Spearman’s rank correlation with the number of NAT2*6 alleles, log AFMU/1X = −0.359; P<0.001). These findings were not influenced by the sex of participants, age, smoking status, pack-years, drinking status, servings of alcohol per week as stated by multivariable linear regression, or by the case-control status (Table 4, Table S2). The effect of NAT2*7 in vivo could not be elucidated because of the low allele frequency in the study population. We identified only two carriers of the alleles NAT2*7 in homozygosity, with metabolic ratios equal to −0.51 and −0.96. The mean value (−0.74), is close to the mean value for carriers of the NAT2*6/*6 genotypes, thus suggesting that the NAT2*7 alleles in homozygosity may confer a very slow acetylation phenotype; although due to the sample size the comparisons of the acetylation phenotype were not statistically significant. Table S3 includes details of the log AFMU/1X ratios of carriers of NAT2*7.

Discussion

Differential effects of acetylation status by different slow acetylation alleles have been suggested previously, but to our knowledge they have not been formally evaluated in vivo. Indirect evidence from in vitro studies and from clinical association studies suggest that NAT2 variant alleles produce different functional effects, implying heterogeneity within the “slow” acetylator phenotype [6]. Antituberculosis drug-induced hepatotoxicity risk is particularly high in carriers of the NAT2*6/*6 allele, thus suggesting that these individuals may constitute a subcategory of “very slow” acetylators [18,19]. These and other clinical association studies (reviewed in [6]) suggest that the NAT2 slow acetylator phenotype is heterogeneous, and that multiple slow acetylator phenotypes exist [20]. However, no clear association between NAT2 variant alleles and in vivo phenotype categories among slow acetylator individuals has been proved so far. Our findings indicate that the NAT2*6 allele cluster is related with the slowest acetylation capacity in vivo with a gene-dose effect, thus demonstrating the occurrence of a category of “very slow acetylators” with the genotype NAT2*6 homozygosity. Because of the ethnic origin of the population study, we were unable to dissect the effect of the allele clusters NAT2*7 and NAT2*14; it should, however, be emphasized that these clusters are rare in caucasian populations [21] and that the allele frequencies observed in this study are consistent with those reported for other Caucasian individuals [21,22].

The effect of NAT2 variant alleles may vary by substrate or with substrate concentration [6]. For instance, it has been shown that the NAT2*7 allele cause a different effect in the N-acetytransferase activity towards 2-aminofluorene and to sulfamethazine [23]. Therefore the findings obtained in this study should not be extrapolated to other NAT2 substrates without confirmation with every specific substrate. Nevertheless, our findings in vivo agree with findings obtained in vitro which suggests that the protein level expressed by common NAT2 alleles is NAT2*4>NAT2*5>NAT2*6 [6], thus suggesting that the differential effect of NAT2 alleles observed with the probe drug caffeine is likely to be relevant to other NAT2 substrates.

The aims of this study are to refine the phenotype inference of NAT2 genotyping and the identification of clinically relevant associations of the new genotype categories with cancer risk,
differential treatment response or clinical outcome are beyond the aims of the study. Although this study included patients with cancer of the exocrine pancreas and control subjects, no association of NAT2 genotype categories with pancreatic cancer risk was observed, in agreement with previous studies [24,25].

The findings reported in this study show that acetylation capacity in vivo is related to different NAT2 genotypes among slow acetylators, and indicate that variations in the acetylation NAT2 status among slow acetylator individuals result from the co-dominant expression of the NAT2*5 and NAT2*6 alleles or haplotypes, whose diplotypes are related to distinct slow acetylation phenotypes. Additional studies are required to go further in the refinement in phenotype inference, particularly in other human populations with different NAT2 allele frequencies. It may be argued that the difference in function between the variants NAT2*5 and NAT2*6, although statistically significant, is a minor difference compared to the function of any genotype containing at least one NAT2*4 allele and therefore that the clinical relevance of this difference may be limited. However, NAT2*6/*6 homozygotes show roughly a 30% reduction on enzyme activity compared to NAT2*5/*5 homozygotes. For comparison, the reduction on enzyme activity between NAT2*4 heterozygotes (intermediate acetylators) and NAT2*4/*4 homozygotes (rapid acetylators) in this study is 28%. A 30% reduction in activity among individuals who have a very impaired acetylation capacity may have a higher clinical relevance than a comparable reduction.

### Table 3. Acetylation ratios (log AFMU/1X) in subjects with different NAT2 genotypes.

| Phenotype   | Genotype       | Number | Mean Ratio | SD  | 95% CI min | 95% CI max |
|-------------|----------------|--------|------------|-----|------------|------------|
| Overall rapid | NAT2*4/any    | 197    | 0.209      | 0.155 | 0.182      | 0.226      |
| Rapid       | NAT2*4/*4      | 36     | 0.327      | 0.169 | 0.270      | 0.385      |
| Rapid-Intermediate | NAT2*4/*5 | 95     | 0.170      | 0.139 | 0.142      | 0.199      |
| Rapid-Intermediate | NAT2*4/*6 | 66     | 0.186      | 0.141 | 0.151      | 0.220      |
| Overall Slow | Slow/Slow     | 238    | -0.537     | 0.147 | -0.556     | -0.518     |
| Slow        | NAT2*5/*5      | 91     | -0.480     | 0.140 | -0.509     | -0.451     |
| Slow        | NAT2*5/*6      | 115    | -0.551     | 0.131 | -0.575     | -0.527     |
| Slow        | NAT2*6/*6      | 32     | -0.646     | 0.149 | -0.698     | -0.592     |

### Table 4. Effect of the case-control status on the Acetylation ratios (log AFMU/1X) in subjects with different NAT2 genotypes.

| Genotype | Status | Mean Log ratio (SD) | 95% CI min | 95% CI max | Inter-group comparison |
|----------|--------|---------------------|------------|------------|------------------------|
| NAT2*4/*4 | Case (n = 7) | 0.273 (0.181) | 0.105 | 0.441 | p = 0.373 |
|          | Control (n = 29) | 0.341 (0.167) | 0.277 | 0.404 | p = 0.605 |
| NAT2*4/*5 | Case (n = 20) | 0.157 (0.197) | 0.065 | 0.249 | p = 0.474 |
|          | Control (n = 75) | 0.181 (0.117) | 0.154 | 0.209 | p = 0.605 |
| NAT2*4/*6 | Case (n = 8) | 0.166 (0.116) | 0.077 | 0.254 | p = 0.173 |
|          | Control (n = 58) | 0.197 (0.140) | 0.159 | 0.235 | p = 0.705 |
| NAT2*5/*5 | Case (n = 11) | -0.496 (0.134) | -0.405 | -0.586 | p = 0.103 |
|          | Control (n = 80) | -0.479 (0.141) | -0.447 | -0.510 | p = 0.173 |
| NAT2*5/*6 | Case (n = 20) | -0.595 (0.122) | -0.536 | -0.653 | p = 0.010 |
|          | Control (n = 95) | -0.543 (0.132) | -0.516 | -0.569 | p = 0.010 |
| NAT2*6/*6 | Case (n = 7) | -0.714 (0.087) | -0.633 | -0.795 | p = 0.010 |
|          | Control (n = 25) | -0.627 (0.158) | -0.563 | -0.691 | p = 0.010 |

The 435 individuals (73 cases and 362 control subjects) with genotypes NAT2*4/*4, *4/*5, *4/*6, *5/*5, *5/*6 and *6/*6 and phenotype/genotype concordance were included in the comparison.

According to multiple comparison adjustment of the 15 genotype pairs according Bonferroni’s procedure, a P value ≤0.0033 is considered as significant. Individual number for p values <0.0001 are rounded as “p<0.0001”.

doi:10.1371/journal.pone.0044629.t003
doi:10.1371/journal.pone.0044629.t004
among individuals who have a high acetylation capacity. These findings provide a novel framework for evaluating interactions between NAT2 genotype and adverse drug reactions or cancer risk.

Supporting Information

Table S1 Details of the genotyping procedures used in the present study.

Table S2 Comparison of the Acetylation ratios (log AFMU/1X) in healthy subjects with different NAT2 genotypes.

References

1. Agundez JA (2008) N-acetyltransferases: lessons learned from eighty years of research. Curr Drug Metab 9: 663–668.
2. Andrade RJ, Agundez JA, Lucena ML, Martinez C, Cueto R, et al. (2009) Pharmacogenomics in drug-induced liver injury. Curr Drug Metab 10: 956–970.
3. Evans DA, Manley KA, Mc RV (1960) Genetic control of isoniazid metabolism in man. Br Med J 2: 485–491.
4. Evans DA, Storey PB, Wittstadt FB, Manley KA (1960) The determination of the isoniazid inactivator phenotype. Am Rev Respir Dis 82: 853–861.
5. Agundez JA, del Barrio J, Padro T, Stephens C, Fare M, et al. (2012) Trends in qualifying biomarkers in drug safety. Consensus of the 2011 meeting of the Spanish Society of Clinical Pharmacology. Frontiers in Pharmacology 3: 1–6.
6. Hein DW (2009) N-acetyltransferase SNPs: emerging concepts serve as a paradigm for understanding complexities of personalized medicine. Expert Opin Drug Metab Toxicol 5: 353–366.
7. Anderson KE, Sinha R, Kullendorff M, Gross M, Lang NP, et al. (2002) Meat intake and cooking techniques: associations with pancreatic cancer. Mutat Res 506:507: 225–231.
8. Anderson KE, Kadlubar FF, Kullendorff M, Harnack L, Gross M, et al. (2005) Dietary intake of heterocyclic amines and benzo(a)pyrene: associations with pancreatic cancer. Cancer Epidemiol Biomarkers Prev 14: 2261–2265.
9. Zhang J, Zhang X, Dhalal EB, Gross MD, Kadlubar FF, et al. (2011) Sequence variants in antioxidant defense and DNA repair genes, dietary antioxidants, and pancreatic cancer risk. Int J Mol Epidemiol Genet 2: 236–244.
10. Butler MA, Lang NP, Young JF, Caporaso NE, Vines P, et al. (1992) Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. Pharmacogenetics 2: 116–127.
11. Gross M, Kruseselbrink T, Anderson K, Lang N, McGovern P, et al. (1999) Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population. Cancer Epidemiol Biomarkers Prev 8: 683–692.
12. Miners JO, Birkett DJ (1996) The use of caffeine as a metabolic probe for human drug metabolizing enzymes. Gen Pharmacol 27: 245–249.
13. McQuillan SH, Nierenberg DW, Bresnick E (1995) Analysis of within-subject variation of caffeine metabolism when used to determine cytochrome P450IA2 and N-acetyltransferase-2 activities. Cancer Epidemiol Biomarkers Prev 4: 139–146.
14. Bolt HM, Selinska S, Dannappel D, Blaszkewicz M, Golka K (2005) Re-investigation of the concordance of human NAT2 phenotypes and genotypes. Arch Toxicol 79: 196–200.
15. Tang BK, Kadar D, Kalow W (1987) An alternative test for acetylator phenotype with caffeine. Clin Pharmacol Ther 42: 509–513.
16. Agundez JA, Golka K, Martinez C, Selinska S, Blaszkewicz M, et al. (2008) Ururavng ambiguous NAT2 genotyping data. Clin Chem 54: 1390–1394.
17. Selinska S, Blaszkewicz M, Lehmann ML, Osianniokov D, Mosermann O, et al. (2011) Genotyping NAT2 with only two SNPs (rs1041983 and rs1801280) outperforms the tagging SNP rs1495741 and is equivalent to the conventional 7-SNP NAT2 genotype. Pharmacogenet Genomics 21: 673–678.
18. Huang YS, Chern HD, Su WJ, Wu JC, Lai ML, et al. (2002) Polymorphism of the N-acetyltransferase 2 gene as a susceptibility risk factor for antituberculosis drug-induced hepatitis. Hepatology 33: 883–889.
19. Leiro-Fernandez V, Valetorre D, Vazquez-Gallardo R, Botana-Rial M, Constenla L, et al. (2011) N-acetyltransferase 2 polymorphisms and risk of anti-tuberculosis drug-induced hepatotoxicity in Caucasians. International Journal of Tuberculosis and Lung Disease 15: 1403–1408.
20. Cascoiri I, Brockmoller J, Mrozikiewicz PM, Muller A, Roots I (1999) Arylamine N-acetyltransferase activity in man. Drug Metab Rev 31: 409–502.
21. Garcia-Martin E (2008) Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. Curr Drug Metab 9: 487–497.
22. Modelli R, Chatterjee N, Church TR, Chen J, Yager M, et al. (2006) Cigarette smoking, N-acetyltransferase genes and the risk of advanced colorectal adenoma. Pharmacogenomics 7: 819–829.
23. Wachtman JM, Zang Y, Trent JO, Hein DW (2008) Structure/function evaluations of single nucleotide polymorphisms in human N-acetyltransferase 2. Curr Drug Metab 9: 471–486.
24. Agundez JA (2008) Polymorphisms of human N-acetyltransferases and cancer risk. Curr Drug Metab 9: 520–531.
25. Bartos H, Malaveille C, Lowenthal AB, Maisonneuve P, Hautefeuille A, et al. (1998) Genetic polymorphism of N-acetyltransferases, glutathione S-transferase M1 and NAD(P)H:quinone oxidoreductase in relation to malignant and benign pancreatic disease risk. The International Pancreatic Disease Study Group. Eur J Cancer Prev 7: 215–223.

Table S3 Details of the acetylation ratios of individuals carrying NAT2*7.

Acknowledgments

We are grateful to Prof. James McCue for assistance in language editing, and to Ms. Gara Esguevillas, for technical assistance.

Author Contributions

Conceived and designed the experiments: JAGA EGM. Performed the experiments: JDR CM KA MG NL. Analyzed the data: JAGA EGM. Contributed reagents/materials/analysis tools: KA MG CM NL JDR. Wrote the paper: JAGA.