LINKING THE PHARMACOKINETICS OF SULFADIMIDINE TO N-ACETYLTRANSFERASE 2 (NAT2) GENE POLYMORPHISMS IN BIOMEDICAL SCIENCES STUDENTS; A PHENOTYPE-GENOTYPE CORRELATION

Lars A.A. Damen[1], Thom K. van der Made[1]

Corresponding author: Lars A.A. Damen (lars.damen@student.ru.nl)

[1] Master Student Biomedical Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

ABSTRACT:

INTRODUCTION: Prediction of clinical effects after drug administration is of paramount importance for the improvement of beneficial effects and minimization of adverse drug reactions in daily practice. Clinical effects can differ between individuals as a result of variation in expression of metabolizing enzymes. The NAT2 protein is a metabolizing enzyme responsible for bioactivation and detoxification of commonly prescribed drugs such as isoniazid, which is used to treat tuberculosis. This protein is encoded by the NAT2 gene, a gene which is highly polymorphic. Two polymorphisms common in the Caucasian population are studied, NAT2*5B and NAT2*7. We expect that NAT2*7 leads to a slow acetylating status, whereas NAT2*5B does not.
OBJECTIVE: The aim of this study was to link NAT2 activity to a specific genotype pattern.
METHODS: 9 Students of Biomedical Sciences at the Radboud University ingested a 1500 mg dose of sulfadimidine. Sulfadimidine concentrations were measured in a blood sample and several saliva and urine samples were obtained from the students to determine their metabolism phenotype. Genotype was determined by restriction fragment length polymorphism using the bamH1 and kpn1 restriction enzymes.
RESULTS: Of the 9 students, 7 showed a slow phenotype with a half-life of 10.2 hours (SD=6.61 hours) and a metabolic rate of 45% (SD=6.61%). Two of the slow phenotype students had a mutation in both alleles, one showed a mutation in NAT2*7, 2 students in NAT2*5B and one student had no mutations. The 2 students with a fast acetylating phenotype had a half-life of 3.0 hours (SD = 2.47 hours) and a metabolic rate of 83% (SD = 14.7%). These students had either a mutation in NAT2*5B or NAT2*7.
CONCLUSION: There might not be a relation between the genotype of two common polymorphisms in the NAT2 gene and acetylating phenotype, although the small sample size limits our ability to draw firm conclusions.

WHAT'S KNOWN: There is evidence that phenotypic activity correlates to the NAT2 genotype. Different ethnic populations, with a different genetic make-up, show a different phenotypic status.

WHAT'S NEW: In this study, we specifically used a cohort of Biomedical students to investigate the relationship between NAT2 phenotype and genotype, because differences in lifestyle pattern may influence metabolic rate. However, due to the small sample size, no definitive conclusion could be drawn.

KEYWORDS: NAT2, polymorphisms, genetic association studies, pharmacokinetics

Introduction

Prediction of the clinical effects after drug administration is of paramount importance for the improvement of beneficial effects and minimization of adverse drug reactions in daily clinical practice. However, this is difficult due to large inter-individual variation in drug-responses. Genetic heterogeneity in enzymes responsible for metabolism and transport of drugs, drug targets and disease etiology, but also environmental factors, may determine the response to drugs. In most cases biomarkers or genetic screening are used to improve clinical practice. As for genetic screening, Single Nucleotide Polymorphisms (SNPs) are used to determine which genetic profile a patient expresses and whether this profile can result in the desired response to a particular drug.

This 'personalized medicine' approach could lead to avoidance of unwanted side effects or drug plasma concentrations outside of the therapeutic window. Besides the advantages at an individual level, prediction of the clinical effects will also reduce health care costs. Davies et al. showed that adverse drug reactions (ADR) account for one fifth of hospital readmissions in the UK [11]. A study conducted in France indicated an incidence of 3.6 % of hospital admissions due to ADRs [12].

Genetic screening of the Arylamine N-acetyltransferase (NAT2) gene may provide important information about the response to commonly prescribed drugs such as isoniazid, hydralazine, and sulfamethoxazole [1]. Isoniazid, for instance, is a common first-line anti-tuberculosis drug. If the concentrations of this drug become too high, isoniazid has the potential to induce liver damage [18]. Isoniazid is metabolized by the genetically polymorphic arylamine N-acetyltransferase type 2 (NAT2). An increased activity of NAT2 is related to increased acetylation capacity for drugs that are metabolized by the NAT2 enzyme.

The NAT2 gene encodes the NAT2 protein, which is a phase II metabolizing enzyme responsible for metabolism by N-acetylation or O-acetylation of drug compounds containing aromatic amines and hydrazines [1]. In effect, this enzyme catalyzes the bioactivation and/or detoxification of drugs as isoniazid, hydralazine, and

sulfamethoxazole [1].

NAT2 is a single, intronless gene, which is located at 8p22 [1]. It is considered to be highly polymorphic, composing seven general nucleotide changes that are associated with its acetylating phenotype [1]. 191 G > A, 341 T > C, 590 G > A, and 857 G > A, which reduce the acetylation activity of NAT2; and 282 C > T, 481 C > T, and 803 A > G, which did not [1]. This study will focus on the NAT2*5B (C481T) and NAT2*7 (G857A) polymorphisms, because these polymorphisms are not yet evaluated in students, whereas these are common polymorphisms in the Caucasian population. NAT2*4 is considered to be the wildtype allele.

Sulfadimidine is one of the drugs that is metabolized by the NAT2 protein [16]. Sulfadimidine is an antibacterial agent, which has been used to treat urinary tract infections since 1942. Nowadays this compound is rarely used in the clinic, because there are more effective compounds on the market to treat urinary tract infections. However, this drug offers a great possibility to study the acetylator phenotype in humans. This drug is not harmful when given once in an amount of 1500 mg. Sulfadimidine is predominately metabolized to N4-acetylsulfadimidine [16]. In this paper, we will further distinguish between rapid and slow acetylators. In the literature, participants were classified as slow or fast acetylators based on the acetylated / unchanged sulfadimidine plasma level ratio, using a threshold value of 70% [1]. The prevalence of slow and fast acetylators differs between ethnic populations [1,4-6].

The aim of this study was to link NAT2 activity to a specific genotype pattern. NAT2*7 leads to a slower acetylating status, whereas NAT2*5B will not [1]. Therefore we expect that students without mutations, so called wild types, are fast acetylators. One 'slow' allele will lead to a slow acetylation phenotype, those with two mutations in NAT2*7'alleles will show a more extreme phenotype [19]. We expect students with SNPs in NAT2*5B not to have a slower acetylating status. We used PCR to determine the genotype of the students. Urine and saliva samples were analyzed at specific time points to produce time-concentration curves and to calculate pharmacokinetic parameters, which include the metabolic ratio, metabolic clearance, half-life and the Area Under the Curve (AUC).

Methods

Study design

Biomedical students enrolled in the course Drugs Development at the Radboud University were included in our study on a voluntary basis. This population consisted of seven Caucasian and two non-Caucasian students. Students were excluded in case of liver or kidney disorders, pregnancy, G6PDH-deficiency or after prior use of sulfa-compounds. First, the participants gave their informed consent after being informed by a physician, due to admission of medication and extraction of one blood sample and several saliva and urine samples. Because participants were required to be sober at the start of the experiment, they were only allowed to drink water in the 9 hours prior to the start of experiment. Half an hour before administering sulfadimidine, saliva and urine samples were collected for baseline measurements. Subsequently, all students ingested 1500mg of sulfadimidine. During the 24 hours after administration of the drug, urine and saliva samples were collected at specific time points (table 1). These samples were subsequently analyzed for sulfadimidine and the N-acetylsulfadimidne in plasma and urine, while saliva samples were only analyzed for sulfadimidine. Four and half hours after administration of the drug a blood sample (approximately 6 mL) was collected. This sample was analyzed for bound and unbound sulfadimidine and N-acetylsulfadimidine concentrations and for the presence of polymorphisms in NAT2*5B or NAT2*7 alleles.

DNA extraction

DNA was isolated from 1 ml peripheral blood using the Puregene DNA Isolation kit. First, 200 µl whole blood was added to a microtube containing 600 µl RBC lysis solution. The solution was vortexed and the supernatant removed. After that, 200 µl of a cell lysis solution was added. A protein precipitation solution (PP4) was used to precipitate the proteins. Subsequently 200 µl 100% isopropanol (IP5) and 200 µl 70% ethanol (ET6) were added, respectively, to precipitate DNA. Lastly, 50 µl DNA Hydration Solution (DH7) was added for DNA hydration.

PCR-sequencing

The primers were designed to amplify the human NAT2 gene sequence from position 761 bp to 1861 bp. The length of the whole PCR product was 1101 bp, which contained the NAT2*5B and NAT2*7 polymorphisms at bp 481 and bp 857 respectively in this fragment The primer sequences were NAT2-For2 5'-aactctaggaacaaattggac-3'and NAT2-Rev2 5'-tttctagcatgaatcactctg-3'. PCR reactions were performed with 1 µl DNA and 49 µl of the PCR mix. This mix consisted of 1.0 µl 10 µM NAT2-For2 primer, 1.0 µl 10 µM NAT2-Rev2 primer, 1.0 µl2.5 mM dNTP's, 5.0 µl 10x PCR buffer (Invitrogen), 2.0 µl 50 mM MgCl2 (Invitrogen), 38.75 µl mQ and 0.25 µl Taq DNA polymerase (Invitrogen). Besides the DNA samples we took mQ as negative control. The amplifications were done using the following PCR program. Initial denaturation was done at 94 ^{0}C for 5 minutes (step 1), another denaturation step at 94 ^{0}C for 30 s (step 2), 30 s at 58 ^{0}C (annealing, step 3), 1.5 minute at 72 ^{0}C (elongation, step 4). Repeat step [2-4] 39 times, followed by a step at 72 ^{0}C of 5 minutes. Amplification was verified by gel electrophoresis (1.5% agarose gel, at 125 Volt).

Digestion of PCR products with restriction enzymes

The restriction enzymes Kpn1 and BamH1 were used to determine mutations in the NAT2*5B and NAT2*7 gene sequence, respectively. Kpn1 cuts between G'GATCC and BamH1 cuts between GGTAC'C. First the mixes were made containing 1.0 µl 10x NEB buffer 3, 0.1 µl 100x BSA, 3.4 µl mQ and 0.5 µl BamH1 or Kpn1 restriction enzyme. Subsequently, 5 µl PCR product and 5 µl of the corresponding mix were added. The samples were incubated at 37 ^{0}C for 2 hours to digest the PCR product. A gel, containing 1.5 % agarose, was run for 15-20 minutes.

Determination of acetylator phenotype

Concentrations of sulfadimidine and N-acetylsulfadimidine in plasma, urine and saliva

Plasma, urine and saliva concentrations were measured according to standard procedures. (supplementary 1)

Free protein concentration in the blood

For determination of the free protein plasma concentration Amicon ultra-filtration membranes from the Centricon system were used. These filters, with a cut-off of 30000 Dalton, allow small proteins like sulfadimidine to pass the filter membrane, whereas plasma proteins are not able to pass.

Renal Clearance
The renal clearance was calculated by dividing the total quantity of the drug excreted in the urine by the AUC.

Statistical analysis
Regression lines, standard deviations and t-tests were performed using graphpad prism 4.03 for Windows, GraphPad Software, San Diego California USA. Only an effect can be determined when the difference of a variable between groups is significantly different (P<0,05)

Results

After collecting urine, saliva and blood sample measurements, time-concentration curves were plotted using the measured concentrations of drug and metabolite. In our data set, differences between fast and slow metabolizers were observed in AUC, half-life, metabolic rate and plasma clearance. Figure 1 shows the concentration curves of a slow and a fast metabolizer. Calculated data from the subjects (n=9) and two historical data sets are depicted in table 2. Acetylating phenotype was based on the metabolic ratio <70% (NacS/ total dose ingested, as shown in the cumulative excretion- NacS column). In subject two, acetylating phenotype was not based on metabolic ratio but on half-life. The obtained values are summarized and shown in table 2. One of the factors that show whether someone is most likely a slow

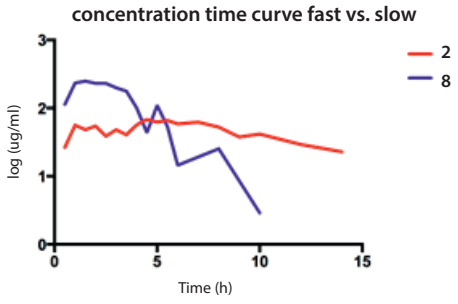

Figure 1 *Time-concentration curve of a fast and a slow acetylators. The log(concentration sulfadimidine) was plotted against the time. Student #2 is a slow metabolizer, this student has an higher AUC and t1/2. Student #8 is a fast metabolizer and shows a higher peak concentration, while the clearance of the drug is much faster.*

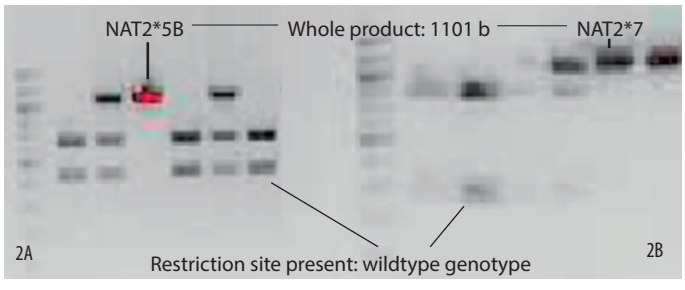

Figure 2*: Genotype patterns in fast and slow acetylators. Mutations in bamH1 (right), kpn1 (left) or both are depicted in the diagrams. Two wildtype alleles are present when one fragment at 1101 bp is visible, if there is one mutation present (heterozygous),3 bonds will be visible, two bonds will be visible if two mutations are present (homozygous).*

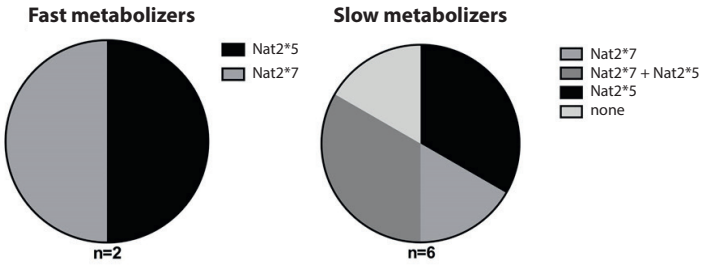

Figure 3 The genotype of fast and slow metabolizers. *The fast metabolizers have a mutation in the Nat2*5 or Nat2*7. Slow metabolizers may have a genotype which has no, one or two mutations.*

or fast metabolizer is the AUC. A high AUC means that the drug is present in the body for a longer period at a higher concentration. However, no significant difference can be observed in the AUC between both groups (784 µg.h/mL 95%CI -1835-3403) although subject 3 and 4 had a very high AUC. In these two subjects also a high half-life was observed. All slow acetylators showed a higher mean half-life, with a mean increase of 7,1 hours compared to the fast acetylators, although this difference is not significant (95%CI -7,5-21,7)

Plasma clearance and metabolic ratio were decreased in slow acetylators with 6,8 L/h (95% CI -3,3 ; 16,9) and 35,4% (95% CI 17,2 ; 53,6), respectively. Typical plasma concentration versus time curves for the two phenotypes are depicted in Figure 1, in which a higher AUC and an increase in half-life were observed for the slow acetylator. These subjects also showed differences in the amount of NacSulfadimidine and Sulfadimidine excreted in the urine, which appeared to be 50% and 30%, respectively, of the total amount of sulfadimidine ingested. Of the nine students included in the study, only eight were genotyped (two fast and six slow, based on metabolic ratio), due to refusal of one participant to extract a blood sample. Two separate alleles were analyzed for genotyping, NAT2*5B by kpn1 restriction and the NAT2*7 by BamH1 restriction. After restriction in the NAT2*5B allele, fragments should be 441bp and 660 bp in case the SNP is not present, while the fragment should be intact when the SNP is present (see figure 2A). A combination of both fragments and the whole PCR product indicates a heterozygous genotype. In the NAT2*7 allele the fragments should normally be found at 384bp and 817 bp, while presence of a SNP leads to an intact fragment (figure 2B).Two of the students who were genotyped had a mutation in both alleles, while one had no mutations. Three students had one mutation in NAT2*5B, two of them had a mutation in NAT2*7. These genotypes were coupled to the acetylating status of students based on metabolic ratio (table 1). The two fast metabolizers both had one mutation, but in a different allele. Of the six slow metabolizers, two had a mutation in both alleles, two had a mutation in NAT2*5B, one student had a mutation in the NAT2*7 and one had no mutations (figure 3).

Discussion

Variation in the activity of the NAT2 enzyme has important implications regarding therapeutic responses to commonly prescribed drugs such as isoniazid, hydralazine, and sulfamethoxazole. In order to predict different therapeutic responses between individuals, we performed a study to correlate phenotypic activity to NAT2 genotype in a cohort of Biomedical Sciences students. Our data suggest that there is no correlation between phenotypic activity and genotype.

A combination of polymorphisms in the NAT2*5B and NAT2*7 genes results in a slow acetylating phenotype. However, mutations in either NAT2*5B or NAT2*7 could not predict the phenotype. We expected that the NAT2*7 would lead to a slow phenotype based on the available literature, however, this mutation was also found in a fast acetylator [1]. Other study groups had already reported a good overall correlation between phenotypic activity and genotype [4,7,13]. In contrast, one study showed discordance between phenotype and genotype of 86% in a Hmong population in the USA [14]. The researchers concluded that environmental and genetic abnormalities may have contributed to the discordance.

In our study 22% of the students were fast acetylators, while 78% were slow. Literature showed that discrepancies between different studies regarding correlation between phenotype and genotype can be observed. Other studies already demonstrated concordance between phenotypic activity and genotype in different ethnic populations. In a study by Gross et al., 59.5% of the study population (American-Caucasian) was classified as slow acetylators [4]. Lucia Taja-Chayeba et al. showed that 59.8% of their population were slow acetylators [1]. Another study by Fuselli S. et al. demonstrated that 18%, 56% and 25% of their target population (Native Americans) were, respectively, fast, intermediate or slow acetylators [5]. Garcia Martin E. showed that inter-individual differences exist, for example individuals with Chinese descent are more often slow acetylators than in those with Japanese descent [6].

In this study, seven students were classified as slow acetylators, whereas two students were classified as fast acetylators. The mean half-life in slow and fast acetylators was 10.2 and 3.0 hours, respectively. This does correlate with existing literature, wherein the mean half-life of sulfadimidine in fast acetylators (n=6) was 1.70 +- 0.50 hours, the mean half-life in slow acetylators was 7.55 +- 0.90 hours [16]. The mean metabolic ratio in slow and fast acetylators was 45 and 83 %, respectively. In the literature also a difference in metabolic ratio was found between slow and fast acetylators, respectively 63 and 77% [16].

We have shown that there is no direct relation between phenotype and genotype. However, our study has several limitations. First, the number of persons participating in this study was low. Secondly, calculated 95% confidence intervals for the half-life were very large, these resulted in non-significant differences between the slow and fast acetylator groups. This may be due to practical errors. Thirdly, sulfadimidine plasma concentrations were calculated based on a saliva/plasma ratio, which was measured at one specific time point. This was less invasive for the students and it was assumed that this ratio could be extrapolated to all the other time points. Although this gives a relatively accurate concentration-time relation, the saliva/plasma ratio might not be the same over the whole period. At time of measurement concentrations of sulfadimidine can be altered due to the production of saliva, while the same amount of drug is still excreted. Production of saliva depends for example on food consumption, which we did not monitor.

Another point of discussion is that we studied polymorphisms in the NAT2*5B and NAT2*7 gene, whereas polymorphisms at other sites in the coding region or in the non-coding regulatory regions of NAT2 can cause a difference in the acetylating phenotype. Currently there are 61 alleles associated with the acetylating phenotype according to 'Consensus Human Arylamine N-Acetyltransferase Gene Nomenclature' [1]. However, it is unlikely that this has led to significant confounding, since the NAT2*5B and NAT2*7 polymorphisms are most common in the Caucasian population. Furthermore, gender was not evenly distributed in slow and fast acetylators, but we do not think that gender differences have a significant impact on acetylating activity. Also these results cannot be extrapolated to other ethnicities since interethnic difference can result in different outcomes. Finally, in some cases assumptions had to be made in order to obtain reliable graphs due to practical errors. In this situation, values representative for a healthy individual were used. In subject 2, the half-life of sulfadimidine was taken instead of the metabolic ratio to determine acetylating activity, because of too many missing values. The time-concentration curve of subject 2 was comparable to that of the other slow acetylators.

Recommendations

Further studies with a larger number of individuals are required to demonstrate the correlation between phenotype and genotype for NAT2 in students. To acquire more accurate data, these studies should be performed with whole genome screening instead of focusing on two polymorphisms. Also the use of a trimodal (slow/intermediate/fast) may be beneficial, because this model might represent the clinical situation better. Furthermore, other drugs than sulfadimidine may be used to give a better representation of the acetylation of drugs such as isoniazid. In conclusion, this study shows that there might not be a relation between the genotype of two common polymorphisms in the NAT2 gene and acetylating phenotype, although the small sample size limits our ability to draw conclusions.

Acknowledgements

We would like to thank Roos Masereeuw for her knowledge and supervision.

References

1. Taja-Chayeb L, Gonzalez-Fierro A, Miguez-Munoz C, Trejo-Becerril C, Cruz-Hernandez Ede L, Cantu D, et al. Acetylator status and N-acetyltransferase 2 gene polymorphisms; phenotype-genotype correlation with the sulfamethazine test. Pharmacogenetics and genomics. 2011;21(12):894-901. Epub 2011/09/29.
2. Chen B, Zhang WX, Cai WM. The influence of various genotypes on the metabolic activity of NAT2 in a Chinese population. European journal of clinical pharmacology. 2006;62(5):355-9. Epub 2006/03/30.
3. Selinski S, Blaszkewicz M, Ickstadt K, Hengstler JG, Golka K. Improvements in algorithms for phenotype inference: the NAT2 example. Current drug metabolism. 2014;15(2):233-49. Epub 2014/02/15.
4. Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, Delongchamp R, et al. Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 1999;8(8):683-92. Epub 2000/04/01.
5. Fuselli S, Gilman RH, Chanock SJ, Bonatto SL, De Stefano G, Evans CA, et al. Analysis of nucleotide diversity of NAT2 coding region reveals homogeneity across Native American populations and high intra-population diversity. The pharmacogenomics journal. 2007;7(2):144-52. Epub 2006/07/19.
6. Garcia-Martin E. Interethnic and intraethnic variability of NAT2 single nucleotide polymorphisms. Current drug metabolism.

2008;9(6):487-97. Epub 2008/08/06.
7. Meisel P, Arndt D, Scheuch E, Klebingat KJ, Siegmund W. Prediction of metabolic activity from genotype: the gene-dose effect of N-acetyltransferase. Therapeutic drug monitoring. 2001;23(1):9-14. Epub 2001/02/24.
8. Fischer E. Renal excretion of sulphadimidine in normal and uraemic subjects. Lancet. 1972;2(7770):210-2. Epub 1972/07/29.
9. Ylitalo P, Auterinen L, Marttinen A, Koivula T. Acetylator phenotyping with sulphadimidine in patients receiving isoniazid. Int J Clin Pharmacol Res. 1984;4(2):141-4.
10. Drayer DE, Reidenberg MM. Clinical consequences of polymorphic acetylation of basic drugs. Clinical pharmacology and therapeutics. 1977;22(3):251-8. Epub 1977/09/01.
11. Davies EC, Green CF, Mottram DR, Rowe PH, Pirmohamed M. Emergency re-admissions to hospital due to adverse drug reactions within 1 year of the index admission. British journal of clinical pharmacology. 2010;70(5):749-55. Epub 2010/11/03.
12. Benard-Laribiere A, Miremont-Salame G, Perault-Pochat MC, Noize P, Haramburu F, the ESGobotFnopc. Incidence of hospital admissions due to adverse drug reactions in France: the EMIR study. Fundamental & clinical pharmacology. 2014. Epub 2014/07/06.
13. Rihs HP, John A, Scherenberg M, Seidel A, Bruning T. Concordance between the deduced acetylation status generated by high-speed: real-time PCR based NAT2 genotyping of seven single nucleotide polymorphisms and human NAT2 phenotypes determined by a caffeine assay. Clinica chimica acta; international journal of clinical chemistry. 2007;376(1-2):240-3. Epub 2006/10/03.
14. Straka RJ, Burkhardt RT, Lang NP, Hadsall KZ, Tsai MY. Discordance between N-acetyltransferase 2 phenotype and genotype in a population of Hmong subjects. Journal of clinical pharmacology. 2006;46(7):802-11. Epub 2006/07/01.
15. Djordjevic N, Carrillo JA, Ueda N, Gervasini G, Fukasawa T, Suda A, et al. N-Acetyltransferase-2 (NAT2) gene polymorphisms and enzyme activity in Serbs: unprecedented high prevalence of rapid acetylators in a White population. Journal of clinical pharmacology. 2011;51(7):994-1003. Epub 2010/08/31.
16. Vree TB, Hekster YA, Nouws JF, Baakman M. Pharmacokinetics, metabolism, and renal excretion of sulfadimidine and its N4-acetyl and hydroxy metabolites in humans. Therapeutic drug monitoring. 1986;8(4):434-9. Epub 1986/01/01.
17. Sim E, Abuhammad A, Ryan A. Arylamine N-acetyltransferases: from drug metabolism and pharmacogenetics to drug discovery. Br J Pharmacol. 2014;171(11):2705-25.
18. Huang YS. Recent progress in genetic variation and risk of antituberculosis drug-induced liver injury. J Chin Med Assoc. 2014;77(4):169-73..
19. Sim E, Abuhammad A, Ryan A. Arylamine N-acetyltransferases: from drug metabolism and pharmacogenetics to drug discovery. Br J Pharmacol. 2014;171(11):2705-25.
20. Singla N, Gupta D, Birbian N, Singh J. Association of NAT2, GST and CYP2E1 polymorphisms and anti-tuberculosis drug-induced hepatotoxicity. Tuberculosis (Edinb). 2014;94(3):293-8.
21. Winter HR, Unadkat JD. Identification of cytochrome P450 and arylamine N-acetyltransferase isoforms involved in sulfadiazine metabolism. Drug Metab Dispos. 2005;33(7):969-76.