Distribution of allelic and genotypic frequencies of \textit{NAT2} and \textit{CYP2E1} variants in Moroccan population

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Abstract

\textbf{Background:} Several pathogenesis and genetic factors influence predisposition to antituberculosis drug-induced hepatotoxicity (ATDH) especially for isoniazid (INH). However, the major susceptibility genes for ATDH are \textit{N-acetyltransferase 2} (\textit{NAT2}) and \textit{cytochrome P450 2E1} (\textit{CYP2E1}). \textit{NAT2} gene determines the individual’s acetylator status (fast, intermediate or slow) to metabolize drugs and xenobiotics, while \textit{CYP2E1} c1/c1 genotype carriers had an increased risk of ATDH. Polymorphisms of the \textit{NAT2} and \textit{CYP2E1} genes vary remarkably among the populations of different ethnic origins. The aim of this study was to determine, for the first time, the frequency of slow acetylators in Moroccan population by genotyping of \textit{NAT2} gene variants and determining the genotype c1/c1 for \textit{CYP2E1} gene, in order to predict adverse effects of Tuberculosis treatment, particularly hepatotoxicity.

\textbf{Results:} The frequencies of specific \textit{NAT2} alleles were 53%, 25%, 2% and 4% for \textit{NAT2*5}, \textit{NAT2*6}, \textit{NAT2*7} and \textit{NAT2*14} respectively among 163 Moroccan studied group. Genotyping of \textit{CYP2E1} gene, by real-time polymerase chain reaction using TaqMan probes, revealed frequencies of 98.5% for c1/c1 and 1.5% for c1/c2 among 130 Moroccan studied group.

\textbf{Conclusion:} The most prevalent genotypes of \textit{NAT2} gene in Moroccans are those which encode slow acetylation phenotype (72.39%), leading to a high risk of ATDH. Most Moroccans are homozygous for c1 allele of \textit{CYP2E1} gene which aggravates hepatotoxicity in slow acetylators.

This genetic background should be taken into account in determining the minimum dose of INH needed to treat Moroccan TB patients, in order to decrease adverse effects.

\textbf{Keywords:} Tuberculosis, \textit{CYP2E1} gene, \textit{NAT2} gene, Polymorphism, Acetylators, Adverse effects, Moroccans

\textbf{Background}

Pharmacogenetics refers to genetic differences in metabolic pathways which can affect individual responses to drugs, both in terms of therapeutic effect as well as adverse effects. Pharmacogenetics is generally regarded as the study or clinical testing of genetic variation that gives rise to differing responses to drugs. Its purpose is to optimize the therapeutic decisions based on the genome of the individual and the target molecule. Medicines are developed and used together with pharmacodiagnosis tools to achieve desired drug efficacy and safety.

Tuberculosis (TB) is an infectious disease caused by the \textit{Mycobacterium tuberculosis}. TB remains to date one of the major public health problems in the world, with 8.6 million of incident cases, 12.0 million prevalent cases and 1.3 million deaths in 2012 \cite{1}. In Morocco, 27,429 new cases of TB were reported in 2012, an incidence of 83 new cases per 100,000 inhabitants, according to the epidemiological services of the Moroccan Ministry of Health (Unpublished data).

The main drugs to treat TB are isoniazid (INH), rifampicin (RMP) and pyrazinamide (PZA), ethambutol (EMB) and/or streptomycin used in combination for 6 months or
more [2]. Tuberculosis treatment cause adverse drug reactions (ADRs), including hepatitis, gastrointestinal intolerance, kidney failure, cutaneous and hematological reactions, which can lead to therapy discontinuation or more serious morbidity and mortality [3]. Among first-line anti-TB drugs, INH is the most effective but also the one that can easily cause hepatotoxicity.

The incidence of anti-tuberculosis drug-induced hepatotoxicity ranges from 1% to 36% [3-6]. Genetic factors have been reported as a risk for hepatotoxicity [7-10]. These factors were attributed to genetic variability in arylamine N-acetyltransferase2 (NAT2) gene, a cytosolic phase II conjugation enzyme primarily responsible for the deactivation of INH [7-11]. INH is metabolized to acetylsalicylic acid via hepatic NAT2 [12]. On the other hand, acetylsalicylic acid is hydrolyzed to acetylsalicylic acid, which is oxidized by cytochrome P450 2E1 (CYP2E1) to form some hepatotoxic intermediates [13,14]. Disposal of acetylsalicylic acid also depends on further acetylation by NAT2 to form a non-toxic metabolite, diacetylhydrazine [15,16].

NAT2 gene on 8p22 is a key human enzyme in drug detoxification and elimination. Variants in NAT2 gene affect the activity of anti-tuberculosis drugs and result in three different phenotypes: rapid (RA), intermediate (IA) and slow acetylators (SA). Most SNPs reported to date are found within the 873 bp intronless coding region of NAT2 gene. Among the seven most common SNPs, four result in amino acid changes leading to a significant decrease in acetylation capacity and are associated to slow acetylator phenotype rs1801280 (c.341 T > C; NAT2*5), rs1799930 (c.590G > A; NAT2*6), rs1799931 (c.857 G > A; NAT2*7), and rs1801279 (c.191G > A; NAT2*14) [17,18]. The three others rs1041983 (c.282C > T; NAT2*13A), rs1799929 (c.481C > T; NAT2*11A), rs1208 (c.803A > G; NAT2*12A) are synonymous SNPs or do not alter the phenotype [17,18]. They have been identified as fast alleles [19,20].

NAT2*4 is considered as the reference allele in the case of absence of all the known SNPs, and is designated as a fast allele [19,20]. A heterozygous compound genotype (NAT2*4/*14 or NAT2*4/*5 or NAT2*4/*6 or NAT2*4/*7) is considered as intermediate acetylator. The pharmacogenetic interest of these data lies in adjusting the dose of isoniazid based on genotype and phenotype found in the patient in order to prevent hepatotoxicity [21].

In humans, the CYP2E1 enzyme is encoded by the CYP2E1 gene on 10q24.3qter [22]. Various polymorphisms have been identified in the CYP2E1 gene, of which the CYP2E1 RsaI/PstI polymorphism (rs2031920; -1053 C>T (Rsa I c1 > c2)) in its 50-flanking region may affect the activity or inductibility of the enzyme [23,24].

Polymorphisms of the NAT2 and CYP2E1 genes vary remarkably among the populations of different ethnic origins. Acetylators phenotypes in Moroccan population were previously reported to have a higher frequency of phenotypic slow acetylators [25].

The aim of this study was to determine the distribution of allelic and genotypic frequencies of NAT2 and CYP2E1 variants in Moroccan controls in order to estimate the prevalence of slow acetylators among Moroccans, who are facing the risk to develop hepatotoxicity after TB treatment.

Results

In this study we identified twelve different genotypes for NAT2 gene. The most of them encode for the slow acetylator phenotype. Genotypes of the four variants of NAT2 gene and their corresponding phenotypic profiles are presented in Table 1. With regard to phenotype, the results show that 72.39% [95% CI 65.39-79.39] of the studied group were SA, 21.48% [95% CI 15.04-27.91] were IA and 6.13% [95% CI 2.38-9.88] were RA. For the four variants NAT2*5, NAT2*6, NAT2*7 and NAT2*14 of NAT2 gene, the allelic frequencies in the studied group were 53% [95% CI 47-58], 25% [95% CI 20-29], 2% [95% CI 1-3] and 4% [95% CI 2-6], respectively (Table 2). NAT2 alleles frequencies of Moroccans compared with other populations are shown in Table 2.

The allelic and genotypic distribution of the rs2031920 variant of CYP2E1 in the studied group is reported in Table 3. The c2/c2 genotype was not found in the studied group. It seems that the majority of Moroccans are carriers of c1/c1 genotype with a frequency of 98.5% [95% CI 96-100]. CYP2E1 genotypic frequencies of Moroccans compared with different populations of the world are shown in Table 4.

Table 1 Observed frequency of NAT2 genotypes encoding fast (RA), intermediate (IA) and slow (SA) acetylation phenotypes among Moroccan population studied

| Genotype | Genotype frequency | Phenotype |
|----------|--------------------|-----------|
| NAT2*4/*4 | 0.0613             | RA        |
| NAT2*4/*5 | 0.1166             | IA        |
| NAT2*4/*6 | 0.0859             | IA        |
| NAT2*4/*14 | 0.0123             | IA        |
| NAT2*5/*5 | 0.3313             | SA        |
| NAT2*5/*6 | 0.2209             | SA        |
| NAT2*5/*7 | 0.0184             | SA        |
| NAT2*5/*14 | 0.0368             | SA        |
| NAT2*6/*6 | 0.0797             | SA        |
| NAT2*6/*7 | 0.0184             | SA        |
| NAT2*6/*14 | 0.0123             | SA        |
| NAT2*14/*14 | 0.0061             | SA        |
Discussion

This results show that slow acetylators are the most frequent in our Moroccan population, and the NAT2*5 allele is the most represented. Comparing the allele frequencies of the four NAT2 variants reported in Table 2, the distribution pattern of NAT2*14 in Tunisian and NAT2*6, NAT2*7 in Caucasians populations did not vary significantly from our population (p > 0.05). On the other side, the allelic distribution in our population is different from other populations as Tunisians for the three alleles NAT2*5, NAT2*6 and NAT2*7 and from Caucasians for two alleles NAT2*5 and NAT2*14, this difference is statistically significant (p < 0.05) [26,32]. This difference with Tunisian neighbors could be explained by the origins of the Moroccan and Tunisian populations. Since about 8000 years ago native Berbers have been the major population group in all the North African regions, but through the centuries, Berbers have mixed differently with many other ethnic groups, Phoenicians, Carthaginians, Romans, Vandals, Byzantines, and Arabs whereas Ottoman rule reached Tunisia only [41,42]. Other explanation of the difference between our population and Tunisians could be a selection bias, and the limited size of their group controls.

Table 2 NAT2 alleles frequencies among Moroccan population and other ethnic groups

| Ethnic groups          | n    | NAT2*5 | NAT2*6 | NAT2*7 | NAT2*14 |
|------------------------|------|--------|--------|--------|---------|
| Caucasians [26]        | 3531 | 0.46   | 0.285  | 0.029  | 0.00    |
| Germanians [27]        | 844  | 0.425  | 0.278  | 0.013  | 0.001   |
| Americans [27]         | 387  | 0.437  | 0.266  | 0.019  | 0.001   |
| Southern Korean [27]   | 288  | 0.01   | 0.224  | 0.132  | 0.00    |
| Spanish [27]           | 258  | 0.47   | 0.25   | 0.006  | 0.004   |
| Southern Brazil [28]   | 254  | 0.289  | 0.104  | 0.021  | 0.014   |
| Egyptian [29]          | 199  | 0.497  | 0.26   | 0.028  | -       |
| Argentine [30]         | 185  | 0.37   | 0.256  | 0.08   | 0.013   |
| Omanians [31]          | 127  | 0.44   | 0.27   | 0.04   | 0.00    |
| Senegalese [27]        | 101  | 0.322  | 0.188  | 0.00   | 0.084   |
| Tunisians [32]         | 100  | 0.315  | 0.175  | 0.15   | 0.05    |
| Spanish [27]           | 97   | 0.361  | 0.17   | 0.067  | 0.103   |
| South Africa [27]      | 79   | 0.019  | 0.23   | 0.011  | -       |
| Japanese [33]          | 79   | 0.33   | 0.38   | 0.03   | -       |
| Indians [33]           | 61   | 0.53   | 0.25   | 0.02   | 0.04    |
| Moroccans (This study) | 163  | 0.53   | 0.25   | 0.02   | 0.04    |

n: sample size.

Studies have shown variation in the distribution of NAT2 alleles among different populations, where four major groups could be distinguished according to the frequency of NAT2*5 and NAT2*6 alleles and according to the presence of NAT2*7 and NAT2*14 alleles. NAT2*5 allele is the most common among Caucasian, Egyptian and Omani populations as in our population [26,29,31], while Asians such as South Korean and Japanese populations, have less NAT2*5 and more NAT2*7 [26,27,33]. NAT2*6 variant is at the second position in our population similarly to Caucasians [26]. NAT2*14 allele, at the third position in our population, is rare in Caucasians and absent in Omani and Southern Korea populations [26,27,31].

Junichi Azuma et al. 2012 [21], proposed in their recent study about NAT2 genotypes and impact on doses of INH to increase the recommended dose of 5 mg/kg by the World Health Organization (WHO) to 7.5 mg/kg in rapid acetylators, maintain it in intermediate acetylators, and reduce it to 2.5 mg/kg in slow acetylators. As Moroccans are mainly slow acetylators, we propose according to our results to reduce the dose of INH in TB patients carrying slow acetylators genotypes, in order to prevent hepatotoxicity and to decrease the cost of managing adverse events.

For the polymorphism rs2031920 of the CYP2E1 gene, our studied group consisted of 130 controls, originated from different regions of Morocco. From our results, it appears that there is a high frequency of c1/c1 genotype in Moroccan population (Table 3), which aggravates hepatotoxicity in slow acetylators patients under TB treatment. In Table 4, we compared our results with
those of other reported populations. The frequencies of c1/c1, c1/c2 and c2/c2 genotypes in Moroccans were close to those found in Caucasians [35,37,38].

**Conclusion**

In conclusion, this preliminary study shows that more than 70% of Moroccan subjects are carriers of NAT2*5, NAT2*6, NAT2*7 and NAT2*14 genotypes compatible with a slow acetylators status, and therefore, they are sensitive to lower doses of TB treatment. We should take into account this high prevalence of slow acetylators in order to decrease adverse effects, especially knowing that a vast majority of Moroccans are also homozygous for the c1 allele of CYP2E1 gene, which aggravates hepatotoxicity.

**Methods**

**Studied population**

Blood samples were collected from umbilical cords of 163 unrelated newborns. They originated from different regions of Morocco and the Moroccan origin of their parents and grandparents was confirmed. Informed consent for DNA analysis was obtained from the parents. Ethics approval was obtained from the local committee of National institute of Hygiene in Rabat for this study.

**Genotyping protocol**

Genomic DNA was extracted from three mL of blood using the salting-out method [43]. The quality and quantity of the DNA were controlled by A260/A280 using a Nanodrop spectrophotometer (2000/2000c Nanodrop; Fisher Scientific, Wilmington, DE, USA) and aliquots (50-100 μL) of packed blood cells were stored at 4°C until analyses. One hundred nanograms of extracted DNA was amplified in a final volume of 20 μL. Real time PCR mixture contained 10 μL of Master Mix (2X, TaqMan Genotyping Master Mix, Applied Biosystems), 0.5 μL of a specific probe (40 X, TaqMan, Applied Biosystems). The amplification protocol involves three steps, PCR which includes activation of Taq polymerase heating at 95°C for 10 minutes, followed by 40 cycles of amplification of 75 seconds each cycle (DNA denaturation for 15 seconds at 92°C, Hybridization for 1 minute at 60°C) and ends with Post-PCR Read at 60°C for 1 minute.

We genotyped 163 DNA samples for the four SNPs with strongest impact on the acetylation profile: rs1801280 (c.341 T > G; NAT2*5), rs1799930 (c.590G > A; NAT2*6), rs1799931 (c.857 G > A; NAT2*7), and rs1801279 (c.191G > A; NAT2*14) polymorphisms of NAT2 gene and 130 DNA samples for the rs2031920 polymorphism of CYP2E1 gene.

Genotyping of SNPs of both genes was performed with an allele-specific probe of SNP using allele-specific real-time polymerase chain reaction (StepOne Real-Time PCR System; Applied Biosystems7500, Foster City, CA, USA) using TaqMan (Applied Biosystems, Warrington, UK) probes. This method combines PCR and mutation detection in a single step. A hybridization probe is cleaved by the 5’ nuclease activity of Taq DNA polymerase only if the specific sequence is successfully amplified. Two TaqMan probes are used, one for each allele. TaqMan probes consist of a 18–22 bp oligonucleotide probe which is labeled with a reporter fluorophore at the 5’ end and a quencher fluorophore at the 3’ end. Allelic discrimination was obtained by the post-PCR read of fluorescence intensity.

For the CYP2E1 polymorphism the primers sequences were from assays-by-designs SM of the manufacturer’s (Applied Biosystems). Alleles of rs2031920 were assessed using primers rs2031920_F: TGACTTTTATTTCTCATTCTTCTCATATCATTCTGCTTTATCACAT and rs2031920_R: GTTTTTTTATTCTTGTCTGCTTGCTTACTGTAATAT and the Taqman probes rs2031920_V: VIC AGTTGCAATTGCTACTTT and rs2031920_F: FAM GTTGCAATTGCTACTTT (SNP position highlighted). For the four polymorphisms of NAT2 gene, the primers sequences were from Drug metabolism genotyping assay of the manufacturer’s (Applied Biosystems) which reference is (p / n 4362038).

Genotype frequencies in our population were calculated in accordance with the Hardy-Weinberg equilibrium. Intervals confidence 95% were calculated for phenotypic genotypic and allelic frequencies.

**Statistical analysis**

Statistical analysis was performed using the SPSS (Statistical Package for the Social Sciences) version 17.0 for

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### Table 4 Genotypic frequencies of rs2031920 polymorphism of CYP2E1 gene in different populations of the world

|          | Turkish [34] | Germanians [35] | Taiwanese [9] | Serbians [36] | French [37] | English [38] | Brazilians [28] | Chinese [26] | Indians [39] | Spanish [40] | Moroccans (This study) |
|----------|--------------|-----------------|--------------|--------------|-------------|--------------|----------------|--------------|--------------|--------------|------------------------|
| c1/c1    | 0.947        | 0.949           | 0.55         | 0.904        | 0.916       | 0.968        | 0.908          | 0.598        | 0.98         | 0.879        | 0.985                  |
| c1/c2    | 0.053        | 0.044           | 0.401        | 0.09         | 0.047       | 0.032        | 0.592          | 0.374        | 0.02         | 0.121        | 0.015                  |
| c2/c2    | 0.000        | 0.007           | 0.048        | 0.006        | 0.000       | 0.000        | 0.000          | 0.028        | 0.00         | 0.000        | 0.000                  |

n: sample size.
windows. The chi-square test was used to determine whether there is a significant difference between the expected frequencies and the observed frequencies relative to NAT2 gene distribution in the studied group versus other populations. Statistical significance was assumed at the p < 0.05.

Abbreviations
ATDH: Antituberculosis drug-induced hepatotoxicity; INH: Isoniazid; NAT2: N-acetylsulfate 2; CYP2E1: Cytochrome P450 2E1; TB: Tuberculosis; INH: Isoniazid; RMP: Rifampicin; PZA: Pyrazinamide; EMB: Ethambutol; ADRs: Adverse drug reactions; RA: Rapid acetylator; IA: Intermediate acetylator; SA: Slow acetylator; WHO: World Health Organization; SPSS: Statistical package for the social sciences; n: Sample size.

Competing interests
The authors declare that they have no competing interests.

Authors’ contributions
SG molecular study, redaction of the manuscript, statistic study. JR analysis of data, redaction of the manuscript. FZL contribution to molecular study. SCA preparation of the controls data. CJI contribution to statistic study. AB recruitment of controls. AS conception of the study, analysis of data. All authors read and approved the final manuscript.

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References
1. Data WHO/HLA C-P: Global tuberculosis report 2013. In: Book Global tuberculosis report, 2013. Edited by: Global tuberculosis report 2013. Switzerland: World Health Organization (WHO), 2013.
2. WHO: Anti-Tuberculosis Drug Resistance in the World: The WHO/IUATLD Global Project on Anti-Tuberculosis Drug Resistance Surveillance Report No. 3. Geneva: WHO, 2004.
3. Forget EJ, Menzies D: Adverse reactions to first-line antituberculosis drugs. Expert Opin Drug Saf 2006, 5:231–249.
4. Mara F, Mara CA, Bruchet N, Richardson K, Moadebi S, Elwood RK, Fitzgerald JM: Adverse drug reactions associated with first-line anti-tuberculosis drug regimens. Int J Tuberc Lung Dis 2007, 11:866–875.
5. Saukkonen JJ, Cohn DL, Jasmer RM, Schenker S, Jereb JA, Nolan CM, Peloquin CA, Gordin FM, Nunes D, Strader DB, Bernardo J, Venkataramanan R, Sterling TR: An official ATS statement: hepatotoxicity of antituberculosis therapy. Am J Respir Crit Care Med 2000, 167:935–952.
6. Tossmann A, Boeree MJ, Aarnoutse RE, de Lange WC, van der Ven AJ, Dekhuijzen R: Antituberculosis drug-induced hepatotoxicity: concise up-to-date review. J Gastroenterol Hepatol 2008, 23:192–202.
7. Ohno M, Yamaguchi J, Yamamoto I, Fukuda T, Yokota S, Maeda K, Igarashi T, Azuma J: Slow N-acetylsulfate 2 genotype affects the incidence of isoniazid and rifampicin-induced hepatotoxicity. Int J Tuberc Lung Dis 2000, 4:256–261.
8. Hussain Z, Kar P, Hussain SA: Antituberculosis drug-induced hepatitis: risk factors, prevention and management. Indian J Exp Biol 2003, 41:1226–1232.
9. Huang YS, Chen HD, Su WJ, Wu JC, Chang SC, Chiang CH, Chang FY, Lee SD: Cytochrome P450 2E1 genotype and the susceptibility to antituberculosis drug-induced hepatitis. Hepatology 2003, 37:924–930.
10. Sharma SK, Balamurugan A, Saha PK, Pandey RM, Mehra NK: Evaluation of clinical and immunogenic response factors for the development of hepatotoxicity during antituberculosis treatment. Am J Respir Crit Care Med 2002, 166:916–919.
11. Shimizu Y, Dobashi K, Mita Y, Endou K, Moriya S, Osano K, Koike Y, Higuchi S, Yabe S, Utsugi M, Ishizuka T, Hisada T, Nakazawa T, Mori M: DNA microarray genotyping of N-acetylsulfate 2 polymorphism using carbamidoine as the linker for assessment of isoniazid hepatotoxicity. Tuberculosis (Edinb) 2008, 88:374–381.
12. Mitchell JR, Zimmerman HH, Ishak KG, Thorgerisson UP, Timirell JA, Snodgrass WR, Nelson SD: Isoniazid liver injury: clinical spectrum, pathology, and probable pathogenesis. Ann Intern Med 1976, 84:181–192.
13. Farrell GC: Drug-induced acute hepatitis. In Drug-induced liver disease. Edited by Farrell G. Edinburgh Churchill Livingstone; 1994. 247–299.
14. Ryan DE, Ramanathan L, Iida S, Thomas PE, Haniu M, Shively JE, Lieber CS, Levin W: Characterization of a major form of rat hepatic microsomal cytochrome P-450 induced by isoniazid. J Biol Chem 1985, 260:6385–6393.
15. Mitchell JR, Thorgerisson UP, Black M, Timirell JA, Snodgrass WR, Potter WZ, Jollow HR, Keiser HR: Increased incidence of isoniazid hepatitis in rapid acetylators: possible relation to hydrazine metabolites. Clin Pharmacol Ther 1975, 18:70–79.
16. Lauterburg BH, Smith CV, Todd EL, Mitchell JR: Pharmacokinetics of the toxic hydrazino metabolites formed from isoniazid in humans. J Pharmacol Exp Ther 1985, 235:566–570.
17. Fretland AJ, Leff MA, Doll MA, Hein DW: Functional characterization of human N-acetylsulfate 2 (NAT2) single nucleotide polymorphisms. Pharmacogenetics 2001, 11:207–215.
18. Zang Y, Doll MA, Zhao S, Streat JC, Hein DW: Functional characterization of single-nucleotide polymorphisms and haplotypes of N-acetylsulfate 2. Carcinogenesis 2007, 28:1665–1671.
19. Parkin DP, Vandenplas S, Botha FJ, Vandenplas ML, Seifert HI, van Helden FO, van der Walt BJ, Donald PR, van Jaarsveld PP: Trermalidity of isoniazid elimination: phenotype and genotype in patients with tuberculosis. Am J Respir Crit Care Med 1997, 155:717–722.
20. Cascorbi I, Brockmoller J, Bauer S, Reum T, Roots I: Cytochrome P450 2E1 genotype and chlorozoxazone metabolism in healthy and alcoholic Caucasian subjects. Pharmacogenetics 1996, 5:257–259.
21. Azuma J, Ohno M, Kubota R, Yokota S, Nagai T, Tsuyuguchi K, Okuda Y, Takashina T, Kamiura S, Fujio Y, Kagawa I: NAT2 genotype guided regimen reduces isoniazid-induced liver injury and early treatment failure in the 6-month four-drug standard treatment of tuberculosis: a randomized controlled trial for pharmacogenetics-based therapy. Eur J Clin Pharmacol 2013, 69:1001–1110.
22. Kilibi K: Regional mapping of short tandem repeats on human chromosome 10: cytochrome P450 gene CYP2E1, D10S1196, D10S2290, and D10S2252. Genomics 1993, 18:202–204.
23. Lucas D, Menez C, Girre C, Berthou F, Bodenez P, Joannet I, Hispard E, Baranova H, Bathum L, Bernou F, Boffetta P, Bouchardy C, Bresky K, Brockmoller J, Cascorbi I, Clapper ML, Couteille C, Daly A, Dell’Orso M, Dolzan V, Dieder CM, Feyer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hispel EL, Ingelmann-Sundberg M, Kalina L, Kang D, Khara M, et al.: Cytochrome P450 2E1 genotype and chlorozoxazone metabolism in healthy and alcoholic Caucasian subjects. Pharmacogenetics 1995, 5:298–304.
24. Neafsey P, Ginsberg G, Hattis D, Johns DO, Guyton KZ, Sonawane B: Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. J Toxicol Environ Health B Crit Rev 2009, 12:362–398.
25. Att Moussa L, Khasooua CE, Hube B, Jana M, Bedug M, Soulymani R: Determination of the acetylator phenotype in Moroccan tuberculosis patients using isoniazid as metabolic probe. Int J Clin Pharmacol Ther 2002, 40:548–553.
26. Gate S, Gaspari L, Alexandre AK, Ambrosone C, Atrup H, Atrup JL, Baranova H, Bathum L, Bernou F, Boffetta P, Bouchardy C, Bresky K, Brockmoller J, Cascorbi I, Clapper ML, Couteille C, Daly A, Dell’Orso M, Dolzan V, Dieder CM, Feyer A, Haugen A, Hein DW, Hildesheim A, Hirvonen A, Hispel EL, Ingelmann-Sundberg M, Kalina L, Kang D, Khara M, et al.: Evaluation of single-nucleotide polymorphisms and haplotypes of N-acetylsulfate 2. Cancer Epidemiol Biomarkers Prev 2006, 15:257–259.
27. Sabbagh A, Langenay A, Darlu P, Gerard N, Krishnamoorthy R, Poloné ES: Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. J Toxicol Environ Health B Crit Rev 2009, 12:362–398.
30. Chamorro JG, Castagnino JP, Musella RM, Frías A, Aranda FM, De Llanaraga GF: The distribution of allelic and genotypic frequencies of N-Acetyltransferase-2 variants in an Argentine population. J Infect Dev Ctries 2012, 6:271–274.

31. Tanira MO, Simsek M, Al Balushi K, Al Lawasta K, Al Barawani H, Bayoumi RA: Distribution of aryamine N-acetyltransferase 2 (nat2) genotypes among Omani. J Sci Res Med Sci 2003, 5:9–14.

32. Bendjemana K, Abdennebi M, Gara S, Jmal A, Ghanem A, Touati S, Bouussen H, Ladgham A, Guemira F: Genetic polymorphism of glutathione-S transferases and N-acetyl transferases 2 and nasopharyngeal carcinoma: the Tunisia experience. Bull Cancer 2006, 93:297–302.

33. 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.

34. Kunak SC, Ada AO, Karacaoglan V, Soydas E, Bilgen S, Iscan M: Drug/xenobiotic metabolizing enzyme polymorphisms in a Turkish population. African Journal of Pharmacy and Pharmacology 2012, 6(7):2068–2074.

35. Neuhaus T, Ko YD, Lorenzen K, Fischhoff S, Harth V, Brode P, Vetter H, Bolt HM, Porch B, Bruning T: Association of cytochrome P450 2E1 polymorphisms and head and neck squamous cell cancer. Toxicol Lett 2004, 151:273–282.

36. Brocic M, Supic G, Zeljic K, Jovic N, Kozomara M, Zagorac S, Zlatkovic M, Majic Z: Genetic polymorphisms of ADH1C and CYP2E1 and risk of oral squamous cell carcinoma. Otolaryngol Head Neck Surg 2011, 145:586–593.

37. Bouchardy C, Hinvonon C, Couteau G, Ward PJ, Diaper P, Benhamou S: Role of alcohol dehydrogenase 3 and cytochrome P4502E1 genotypes in susceptibility to cancers of the upper aerodigestive tract. Int J Cancer 2000, 87:734–740.

38. Yang B, O'Reilly DA, Demaine AG, Kingsnorth AN: Study of polymorphisms in the CYP2E1 gene in patients with alcoholic pancreatitis. Alcohol 2001, 23:91–97.

39. Roy B, Ghosh SK, Sutradhur D, Sikdar N, Mazumder S, Barman S: Predisposition of antituberculosis drug induced hepatotoxicity by cytochrome P450 2E1 genotype and haplotype in pediatric patients. J Gastroenterol Hepatol 2006, 21:784–786.

40. Leiro-Fernandez V, Valverde D, Vazquez-Gallardo R, Constenla L, Fernandez-Villar A: Genetic variations of NAT2 and CYP2E1 and isoniazid hepatotoxicity in a diverse population. Pharmacogenomics 2010, 11:1205–1206. author reply 1207-1208.

41. Sefiani A: Genetic disorders in Morocco. In Genetic disorders Among Arab populations. Edited by Teebi A. Springer-Verlag Berlin Heidelberg; 2010: 455-472.

42. L C: Genetic Disorders in Tunisia. In Genetic Disorders Among Arab. 2nd edn. Edited by Teebi SA: Springer-Verlag Berlin Heidelberg; 2010: 613-638.

43. Sambrook JFE, Maniatis T: Molecular cloning: a laboratory manual. In Molecular Cloning: A Laboratory Manual. Edited by Sambrook J. New York: Cold Spring Harbor Laboratory Press; 1989.