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N-ACETYLTRANSFERASE 2 ENZYME GENOTYPE-PHENOTYPE DISCORDANCES IN BOTH HIV-NEGATIVE AND HIV-POSITIVE NIGERIANS

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Abstract

Background: The N-acetyltransferase 2 (NAT2) enzyme has been understudied in Nigerians including genotype-phenotype association studies.

Objective: Study objective was NAT2 haplotype identification and genotype-phenotype investigations in HIV-positive and HIV-negative Nigerians.

Method: Phenotypes included self-reported sulphonamide hypersensitivity survey, experimental and computational NAT2 phenotyping. NAT2 gene was amplified via polymerase chain reaction. Gene sequencing employed ABI 3730 and Haploview 4.2 for haplotype reconstruction. Genotype-phenotype analyses used Chi square p-value and odds ratio with 95% confidence interval.

Results: Self-reported sulphonamide hypersensitivity revealed prevalence of 3.1% and 12.4% in HIV-positive and HIV-negative Nigerians respectively. NAT2 genetic variants 191G>A, 282C>T, 341T>C, 481C>T, 590G>A, 803A>G and 857 G>A were not significantly different between both groups (OR 0.87 95%CI 0.54–1.38 $P = 0.55$). Nine haplotypes: *NAT2*4*, *NAT2*12A*, *NAT2*13A*, *NAT2*5B*, *NAT2*6A*, *NAT2*7B*, *NAT2*5C*, *NAT2*14B* and *NAT2*14A* had frequencies > 1% while *NAT2*12B* had 1.1% in HIV-positive and 0.4% in HIV-negative group. Overall, slow acetylator haplotypes made up 68%. *NAT2*12* signature SNP (sSNP) was in high linkage

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disequilibrium with sSNP for *NAT2*13* (D′ = 0.97, r^2 = 0.61) and *NAT2*5* (D′ = 0.98, r^2 = 0.64). Genotype-phenotype association analysis showed haplotypes *NAT2*13A*, *NAT2*5C*, *NAT2*7B* and *NAT2*14A* strongly associated with slow metabolic phenotype (*P* = 0.002, 0.029, 0.032, and 0.050 respectively). Computational phenotypes were similar with 30.9%, 66% and 3.1% for slow, intermediate and rapid acetylators respectively among HIV-positive Nigerians and 31.2%, 66.3% and 2.5% among HIV-negative group. Overall, slow phenotypes made up 31%.

Conclusions: NAT2 haplotype frequencies are similar in Nigerians, irrespective of HIV status, but genotype-phenotype discordances exist.

Keywords

N-acetyltransferase 2; Sulphonamide hypersensitivity; HIV-positive Nigerians; Genotype-phenotype; discordance

INTRODUCTION

Use of anti-retroviral drugs (ARVs) for management of HIV/AIDS are often associated with drug-induced adverse effects that may lead to discontinuation in some patients or change to alternative therapies. The degree to which the different classes of ARVs induce hypersensitivity reactions varies. Nucleoside reverse transcriptase inhibitors (NRTIs) induce reactions in 2–17%, non-nucleoside reverse transcriptase inhibitors (NNRTIs) in 0.1–32%, PIs in 1–19%, and rarely do Entry inhibitors induce such reactions. Sulphonamides (cotrimoxazole in particular) used for treatment of opportunistic infections top the range with hypersensitivity reactions induced in 30–60% of HIV-positive patients [1,2]. Sulphonamide hypersensitivity is characterized by several reactions such as fever, pruritus, maculopapular rash, erythema multiforme, and when severe, Stevens-Johnson syndrome and Toxic Epidermal necrolysis (TEN) [3–5]. Sulphonamide drugs include sulphamethoxazole-trimethoprim combination (cotrimoxazole), which is an antibacterial agent used mostly for treatment of bacterial infections, and taken both prophylactically and for treatment against *Pneumocystis carinii* pneumonia (PCP) in HIV-positive patients; sulphadoxine-pyrimethamine (an antimalarial drug) commonly prescribed for malarial treatment in Nigeria, and several others which can be applied either topically for treatment of burns or given as non-absorptive medications for local therapeutic effects in the bowel.

Immunogenic activities via metabolic products and genetic mechanisms have been suspected to be responsible for sulphonamide hypersensitivity reactions [1]. Metabolism of sulphonamides involve the activities of N-acetyltransferase 2 (NAT2) and CYP450 isozymes leading to acetylation and oxidation reactions in the liver [6–8]. Metabolism of sulphonamides can lead to formation of reactive hydroxylamine and nitroso metabolites by oxidation of the N^4 nitrogen atom. Formation of these metabolites by cytochrome P450-mediated reaction has been demonstrated *in vitro* using human liver microsomes [8]. It has been found that relatively a smaller fraction (approximately 2%) undergoes oxidation and the hydroxylamine and nitroso metabolites formed are responsible for the known adverse reactions. It is postulated that the mechanism by which these reactions occur is that the oxidative metabolic products form haptens with cellular molecules, which then induce delayed immunogenic reactions (Type IV hypersensitivity reaction) thereby resulting in

varied host-dependent adverse reactions [9,10]. These reactions occur between 7–14 days after intake of drugs and symptoms resolve on withdrawal of the drug. This process of sulphonamide oxidation is a minor route of metabolism. The major metabolic pathway for sulphonamides is detoxification via formation of N-acetyl moieties, which are conjugated with glucuronic acid and then eliminated as glucuronides.

Genetically, sulphonamide hypersensitivity is explained via activity of N-acetyltransferase 2 (NAT2). The enzyme exhibits polymorphism and populations are characteristically subdivided into three groups: rapid acetylators, intermediate acetylators and slow acetylators [3]. In subjects with decreased capacity for N-acetylation (slow acetylators), the balance between the different routes of metabolism (oxidation vs acetylation) is disturbed, allowing more of the parent drug to become available for oxidative metabolism thereby leading to hypersensitivity reactions.

Many studies on sulphonamide hypersensitivity in both HIV-negative and HIV-positive populations have shown very low prevalence (3–5%) in the former group and over ten times (30–60%) this degree in HIV-positive patients [1,2,11]. Similarly, varied studies centered on NAT2 with respect to drug disposition in HIV-positive patients or prevalence of hypersensitivity reactions to cotrimoxazole have been performed and reported [2,5,8,12,13]. However, such studies in Nigerian populations are scarce. Nigeria has a population of about 170 million with over 3.4 million people living with HIV/AIDS [14] yet this vulnerable group are under-represented in genetic studies with respect to N-acetyltransferase 2. Sulphonamides are often administered to HIV-positive patients, especially to those with CD4+ count < 200 cells/μL [1,15]. In a previous study [16] an epidemiological survey in the general population on self-reported hypersensitivity reaction to sulphonamides among Nigerians resident in Ibadan was performed and findings reflected deviation from what obtains in other populations [2,5].

This study was then designed to characterize NAT2 enzyme in Nigerians (HIV-negative and HIV-positive) by sequencing the gene in the two groups and observing for how it relates to self-reported sulphonamide hypersensitivity and other defined phenotypes. This included quantifying the acetylator activity of the enzyme in a small population size, and analyzing for genotype-phenotype association. Finally, computational inference of NAT2 phenotype using the genotype data acquired was also performed.

METHODS

Survey

This is as previously reported [16]. The survey was carried out in the general community and also at the PEPFAR clinic, University College Hospital (UCH), Ibadan. Self-reported hypersensitivity to oral intake of sulphonamides among HIV-positive patients who were on daily intake of cotrimoxazole as against those not on daily intake of the drug was analyzed.

Subjects for NAT2 Genotyping

Target populations of volunteers consisted of 106 HIV-positive patients and 441 HIV-negative volunteers who were unrelated and predominantly from the Yoruba-speaking tribe

in Ibadan, Nigeria. The HIV-positive population was composed of registered patients at the PEPFAR clinic of the leading tertiary health institution in the country, the University College Hospital (UCH), Ibadan. Total number of male volunteers was 255 and females were 292. Ethical approval for the study was obtained from the University of Ibadan/University College Hospital (UI/UCH) Institutional Review Board (IRB) and informed consent was obtained from volunteers.

Genomic DNA extraction

Peripheral blood (5 ml) was collected into EDTA bottles and DNA was extracted following the Qiagen Blood kit (USA) protocol. The samples were re-suspended in Tris-EDTA buffer, quantified using the Nanodrop® Spectrophotometer (ThermoScientific, USA) and stored at −20 °C.

NAT2 Genotyping

NAT2 genotyping was performed using PCR-direct sequencing method. Seven known single nucleotide polymorphisms (SNPs) were investigated in the NAT2 gene: G191A (rs1801279), C282T (rs1041983), T341C (rs1801280), C481T (rs1799929), G509A (rs1799930), A803G (rs1208), and G857A (rs1799931). Two oligonucleotides (primers) were designed to amplify a single amplicon size of 1,077 base pairs (bp) encompassing the entire 870 bp coding region of the NAT2 exon. The designed primer sequences were: forward primer (F1) 5'-AACATTAACTGACATTCTTGAG-3' and reverse primer (R1) 5'-GTTTTCTAGCATGAATCACTC-3' both obtained from Integrated DNA Technologies (IDT), USA. PCR was performed using an Applied Biosystems 2720 thermal cycler with a reaction volume of 15 μl containing 24 ng of DNA template, 1× PCR buffer, 2.5 mM $MgCl_2$, 200 μM dNTPs, 125 nM each of the primers (F1 and R1), and 0.375U Taq Gold enzyme (Thermo Scientific Fisher, USA) at cycling conditions of 95°C for 5 min, 40 cycles of denaturation at 95°C for 15 s, annealing at 56°C for 15 s, elongation at 72°C for 60 s followed by 72°C for 10 min. The PCR product was purified by isopropanol precipitation and DNA sequencing was performed using Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, USA) in accordance with manufacturer's recommendations on an ABI PRISM 3730 DNA Analyser. Nucleotide substitutions were identified and analyzed using the Sequencher 4.2.2™ software. Polymorphisms were named according to their position along NAT2 flanking sequences from UCSC (Feb. 2009 assembly, GRCh37/hg19), the A of ATG translation initiation codon corresponding to position 1. Haplotype reconstruction was performed with the Haploview 4.2 program [17] and named in accordance with the consensus gene nomenclature of human NAT2 alleles (http://nat.mbg.duth.gr/background_2013.html).

NAT2 Experimental and Computational Phenotyping

1. Forty-nine (49) HIV-negative volunteers who formed part of another study centered on pharmacokinetic profiling of dapsone in Nigerians, were administered a single tablet of Dapsone 50 mg. Venous blood collected 3h post drug administration was analyzed by high performance liquid chromatography (HPLC) for plasma concentration of dapsone and its metabolite, monoacetyldapsone. The ratio of the metabolite to the parent drug was used to

determine the acetylation phenotype of these individuals. This subset of study volunteers was also genotyped for NAT2.

2. Following the acquisition of genotype data, computationally inferred phenotype of both the HIV-positive and the HIV-negative groups were separately determined using an online software programme, NAT2PRED [18,19]. Genotype data were formatted according to author's instructions for processing and subsequent returning of results.

Statistical analysis

From the allele frequencies, genotype frequencies were determined using the Hardy-Weinberg equation ($p^2 + 2pq + q^2 = 1$). Data were analyzed statistically using odds ratio and the two tailed χ^2 test with Yates' correction.

RESULTS

Figure 1 captures information on HIV-positive patients self-reporting on hypersensitivity to cotrimoxazole, comparing between those on daily intake of the drug and others not on daily intake. The survey outcome on self-reported hypersensitivity to sulphonamides was analysed between the two groups for odds of occurrence of observed sulphonamide hypersensitivity reactions among HIV-positives as compared to the control and there was no significant difference (OR 0.83 95%CI 0.55–1.26 $P = 0.39$).

Five hundred and four (504) DNA samples comprised of 407 from the HIV-negative volunteers and 97 from HIV-positive volunteers had the NAT2 gene amplified and sequenced. Frequencies of alleles for all 7 SNPs [G191A (rs1801279), C282T (rs1041983), T341C (rs1801280), C481T (rs1799929), G509A (rs1799930), A803G (rs1208), and G857A (rs1799931)] were computed individually for both HIV-positive patients and HIV-negative volunteers. All genotypes fell within Hardy-Weinberg equilibrium (HWE). No significant differences were found between the two study populations (Table 1). The genotype data obtained for both groups was analyzed for the odds of obtaining slow acetylation alleles among the disease cohort as compared to the control and there was no significant difference (OR 0.87 95%CI 0.54–1.38 $P = 0.55$).

Haplotype reconstruction using Haploview 4.2 was performed separately for the two groups and frequencies of the identified haplotypes are as shown in Table 2. Haplotype reconstruction in both groups revealed a total of ten haplotypes with frequencies > 1% (Table 2). Nine of these (*NAT2*4*, *NAT2*12A*, *NAT2*13A*, *NAT2*5B*, *NAT2*6A*, *NAT2*7B*, *NAT2*5C*, *NAT2*14B* & *NAT2*14A*) had frequencies ranging from 1.3% - 27.9% while the tenth one, *NAT2*12B* had a frequency of 1.1% in the HIV-positive cohort and 0.4% in the HIV-negative group. Overall, the most abundant haplotype was *NAT2*6A* (27%), followed by *NAT2*5B* (18.4%), both of which are noted for slow acetylation. The third most prevalent haplotype was *NAT2*13A* (14.2%), classified as a rapid acetylator. *NAT2*14*, peculiar to African populations, had a total frequency of 12.3%. The linkage disequilibrium (LD) of NAT2 SNPs was analyzed (Figure 2). C282T was in strong but not complete LD with A803G ($D' = 0.97$, $r^2 = 0.61$). Similarly, A803G was in strong but not

complete LD with T341C ($D' = 0.98$, $r^2 = 0.64$) and T341C with C481T ($D' = 0.96$, $r^2 = 0.63$).

Computational inference of NAT2 phenotype utilized 6 of the 7 SNPs (G191A was excluded in the computation). The inferred phenotypes for both HIV-positive patients and HIV-negative persons showed no significant difference in the two groups (Fig. 3).

Experimental exploration of metabolic ratio of monoacetyldapsone to dapsone as an indicator of NAT2 phenotype, revealed 32 (65.3%) volunteers as rapid acetylators and 17 (34.7%) volunteers as slow acetylators (Fig. 4). Genotype-phenotype association analysis in this subset showed a rapid haplotype, *NAT2*13A* ($p = 0.002$), with slow ones *NAT2*5C* ($p = 0.028$), *NAT2*7B* ($p = 0.031$) and *NAT2*14A* ($p = 0.049$) significantly associated with slow acetylation phenotype (Table 3).

DISCUSSION

The importance of NAT2 activity in management of immunocompromised patients cannot be over emphasized. Cotrimoxazole, dapsone (an alternative to cotrimoxazole) and isoniazid, which are key drugs given for treatment of *Pneumocystis carinii* pneumonia (PCP) and tuberculosis in HIV-positive patients, undergo metabolism via acetylation reaction that is fostered by the enzyme. Persons who are known to be slow acetylators are highly prone to developing hypersensitivity reactions when taking sulphonamides [1,5]. Therapeutic outcomes in terms of cure, sub-therapeutic plasma concentration levels, or development of drug-induced adverse effects are supposedly hinged on the efficacy of the enzyme. Until now, the Nigerian population has been under-represented in genetic studies of NAT2. The choice of investigating NAT2 genotype frequencies in HIV-positive Nigerian patients was to obtain preliminary genomic data pertinent to this vulnerable group and to compare with those not having the disease. Also, studies on NAT2 phenotypes in Nigerians, whether HIV-positive or not are yet to be adequately captured.

Self-reporting on sulphonamide hypersensitivity in the general population as well as among outpatients attending the PEPFAR clinic was a separate study [16]. What was observed was very low prevalence of self-reported sulphonamide hypersensitivity among the HIV-positive group, despite having over 80% of them on daily intake of cotrimoxazole. Generally, 3.1% of this group reported reacting adversely to intake of cotrimoxazole, of which for those on daily intake of the drug, only 10% (18/181) attested to having experienced adverse reaction(s). These two values are very low as compared to what was expected, knowing that reports in literature and/or publications show very high incidence of adverse reactions to drugs by this vulnerable group [1,2,20,21]. A much higher prevalence was gotten in the HIV-negative group. This value, though higher than expected, however seems relatable to that reported for an African population where 8.8% self-reported on adverse reaction to cotrimoxazole [22]. Other surveys that have captured self-report in the general public have collectively attributed fairly higher values to antibiotic-induced adverse reactions [23–26]. It is however noteworthy that irrespective of diversity in population, there is the trend that self-reported adverse reaction to sulphonamides shows higher prevalence than the 3–5% commonly determined clinically [1,11,27,28].

Genotype profiling of NAT2 showed similarity in both HIV-positive and HIV-negative Nigerians therefore, polymorphism in the gene might not significantly impact on predisposition to the disease as has been established for other chronic illnesses such as cancer [29]. Whether this similarity translates to same disposition of substrate drugs in the two groups (HIV-positive vs HIV-negative), will have to be further investigated [30]. Genetic differences among populations from the same continent contribute 6% of genetic variation, and differences among populations from different continents contribute 9% to 13% [31]. For highly heterogeneous populations such as African populations, employing multi-locus analysis may therefore be essential for deriving accurate genotype information, unlike for European populations where 1 or 2 tag SNPs can be monitored for determining genotypes and/or inferring phenotypes [32–34]. The observed strong, but incomplete linkage disequilibrium, obtained in this study between NAT2 SNPs attests to this. For two SNPs to be regarded as being in complete disequilibrium, and therefore able to serve as tag SNP, the $r^2 \geq 0.80$, which none of the pairwise correlation in this study had. Genotype data for this study was derived by employing all seven signature SNPs within the NAT2 gene recognized for accurately typing an individual's acetylator status [3,34]. It makes this study more comprehensive than the genotype information reported by Ebeshi *et al*, which employed only 5 SNPs [35]. While Ebeshi *et al* could simply identify four haplotypes (*NAT2*4*, *NAT2*5*, *NAT2*6* and *NAT2*7*), this work adequately characterized NAT2 haplotypes up to the distinct isozymes thereby offering a richer dataset. Studies have established specific relationships between distinct NAT2 haplotypes with diseases such as cancer, hepatotoxicity in TB patients, poor cognitive functions in the elderly [34,36–38] or drug induced adverse reactions [38]. Therefore, acquisition of the frequencies of NAT2 haplotypes in Nigerians can be further employed in investigating whether similar relationships exist for the populace. In addition, the combined frequencies of *NAT2*14A* and *NAT2*14B* haplotypes, peculiar to Africans alone, and as captured in our study, exceeds that reported by Sabbagh *et al* [39].

Inferred acetylation phenotype showed minority of slow acetylators in the overall population (31%), which is discordant to the genotype data that showed predominance of slow acetylator haplotypes (68.8%). Such NAT2 genotype-phenotype association has not been mentioned for any Nigerian population so far. However, it is notable that the SNP191G>A, responsible for *NAT2*14A* and *NAT2*14B*, which had combined frequencies above 10%, was not included in the software (NAT2PRED) used for computation. This is a recognized limitation in this study and gives credence to the need to develop phenotype-inference tools and/or diagnostic devices that incorporate all signature SNPs pertinent to specific populations when analyzing such populations [40].

The genotype-phenotype discordance was further corroborated in the phenotype outcome determined using dapsone as probe drug in a subset of the population, with 35% phenotypically slow acetylators compared to 71% genotypically slow acetylators. This result joins the league of discordant outputs reported for NAT2 genotype-phenotype association [12,13,41,42] as compared to studies that show correlation [5,43,44]. This means that for Nigerians, it might be preferable that acetylation phenotype is employed for classification of the populace rather than using only genotype.

Furthermore, statistical analysis showed four haplotypes significantly correlated with slow acetylation phenotype, with a haplotype that codes for rapid acetylation (*NAT2*13A*) being most significantly associated with slow acetylation phenotype. While this again adds value to the above highlighted discordances, it however may be too early to conclude on the impact of this relationship on drug disposition of NAT2 substrate drugs in Nigerians. This study identified that the most prevalent SNP was C282T, which though is a silent polymorphism, is the signature SNP for *NAT2*13A* cluster that leads to rapid acetylation. This polymorphism was not included in Ebeshi *et al*'s study. The same goes for the second most prevalent SNP, A803G, designated with *NAT2*12A* cluster that also leads to rapid acetylation.

In conclusion, NAT2 haplotype and phenotype dispositions in Nigerians have been investigated in both HIV-negative and HIV-positive volunteers. Genotype and inferred phenotype frequencies show no significant differences in the two groups but for the first time reveals Nigerians as being predominantly intermediate acetylators phenotypically, reveals high level of genotype-phenotype discordance and shows distinct difference in the frequency of *NAT2*12B* between the two groups. There may therefore be the need to phenotype Nigerians, before commencing on drugs that are substrates of NAT2.

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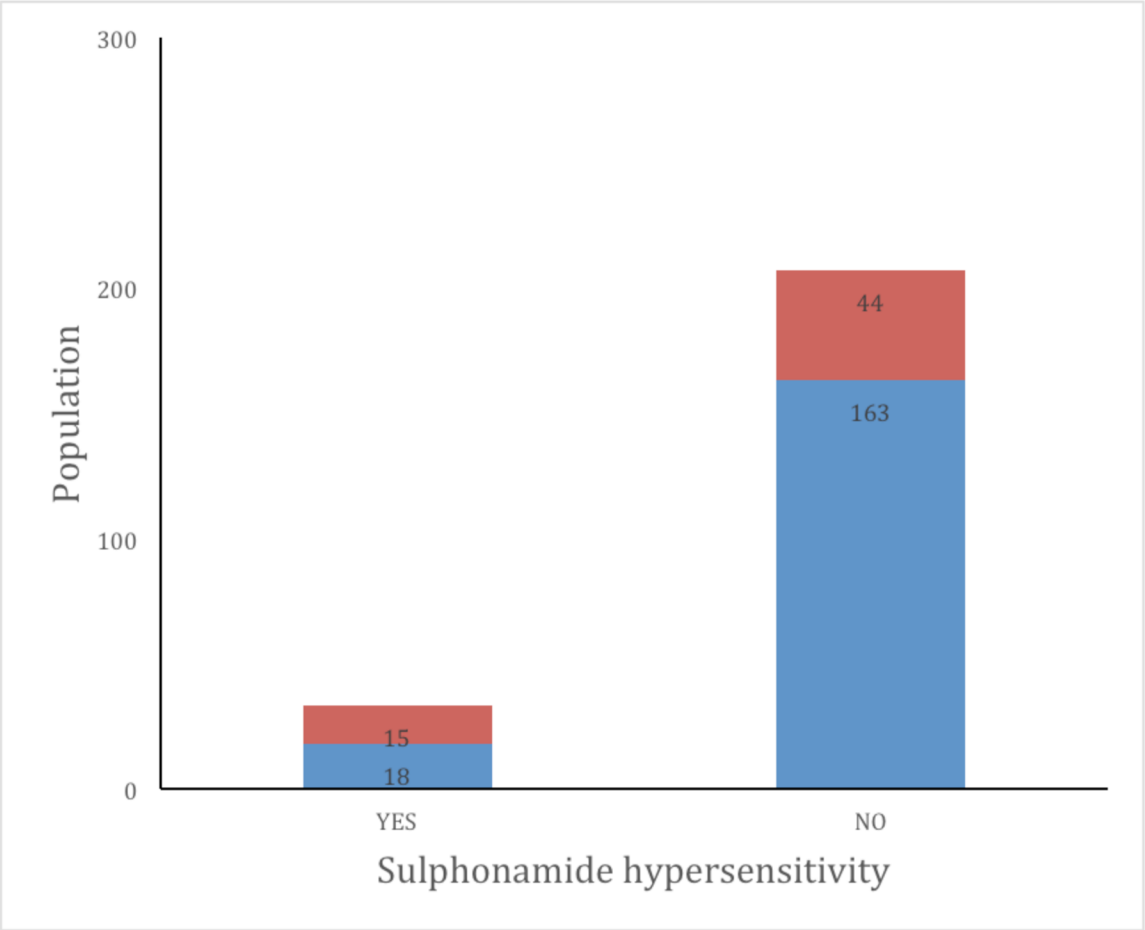

Figure 1.
Prevalence of self-reported hypersensitivity to cotrimoxazole among HIV-positive patients Blue colour = HIV-positive volunteers on daily intake of sulphonamide; orange colour = HIV-positive volunteers not on daily intake of sulphonamide

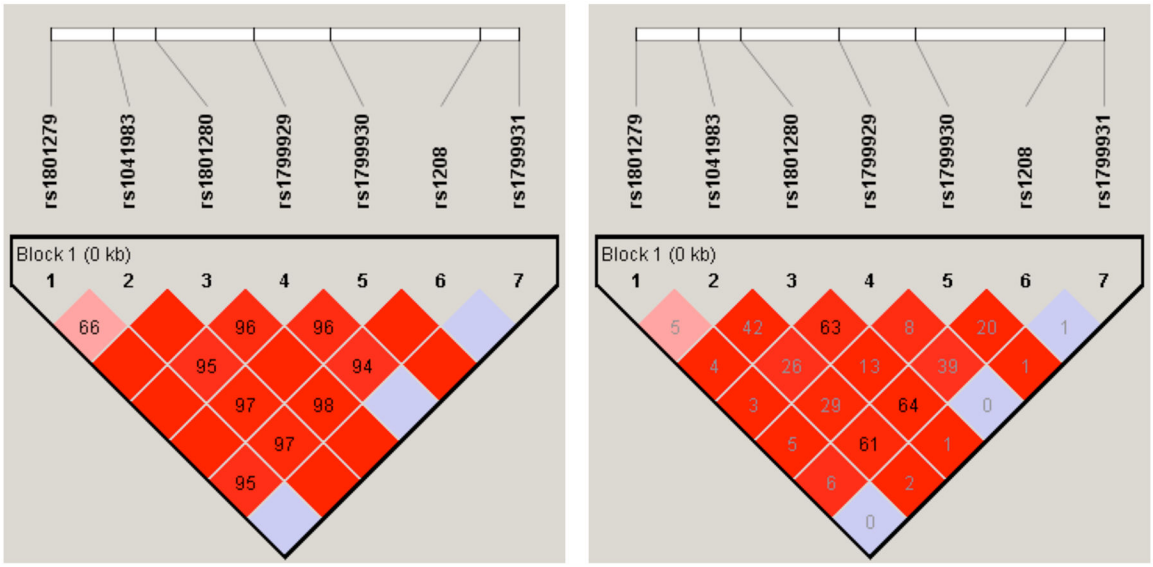

Figure 2.
Linkage disequilibrium plot of 7 notable SNPs located in the NAT2 gene

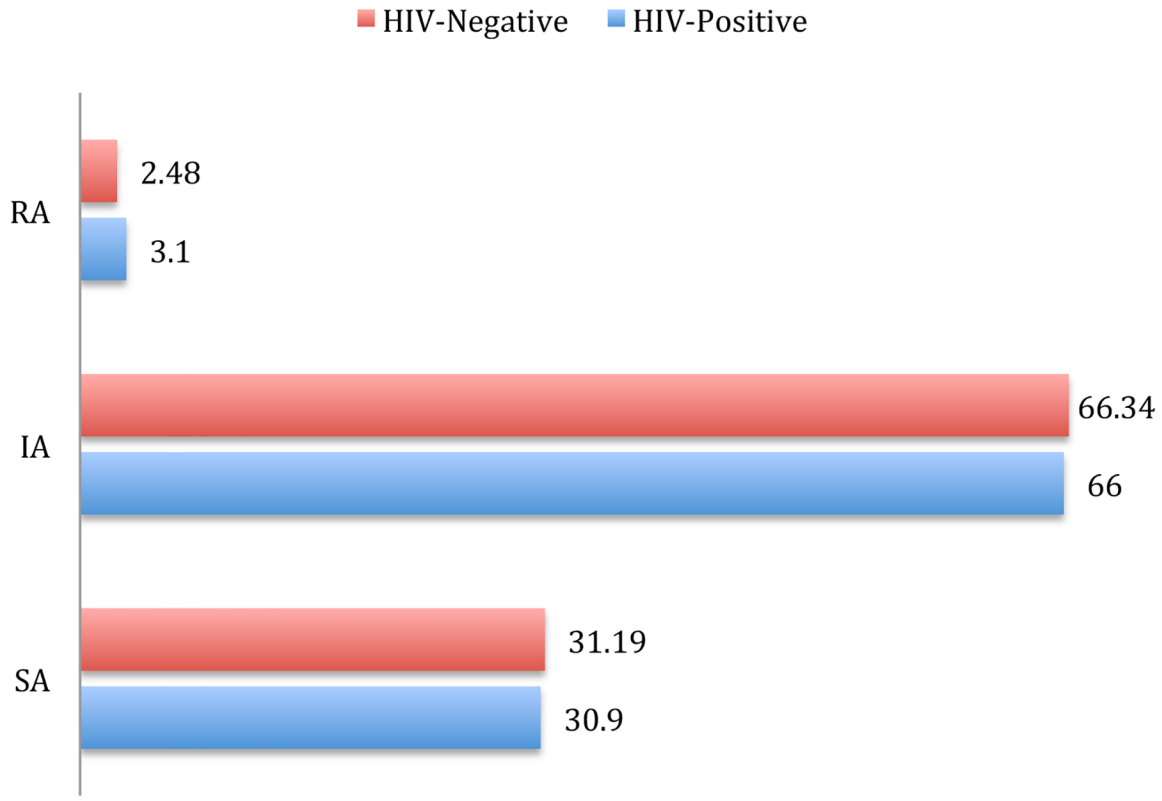

Figure 3.
Computationally inferred phenotype activity in HIV-positive and HIV-negative Nigerians
IA = Intermediate acetylator, RA = rapid acetylator, SA = slow acetylator

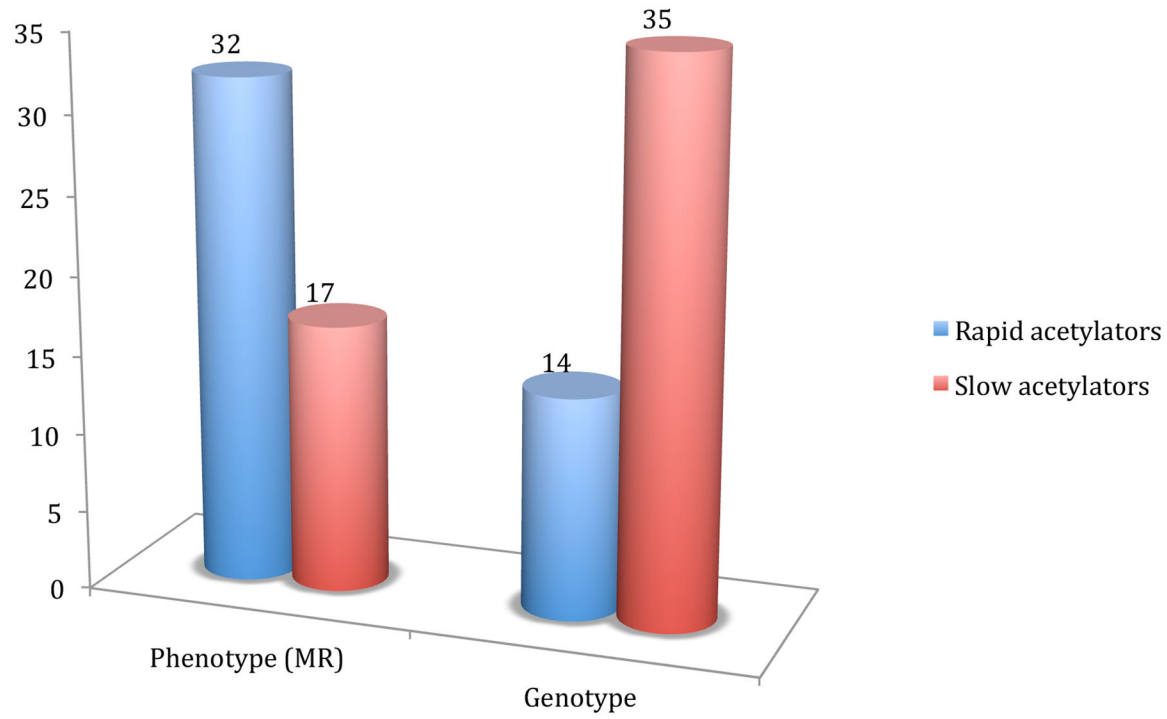

Figure 4.

Genotype-phenotype discordance in a subset of the HIV-negative population

Phenotype here was derived from the metabolic ratio (MR) of monoacetyldapsone to dapsone obtained from hplc analysis of the drug in plasma for the 49 volunteers. Values ≤ 0.30 were classified as slow acetylators and values > 0.30 as rapid acetylators. This chart relates this NAT2 derived phenotype for these individuals compared with their NAT2 genotype. The genotype-phenotype discordance is seen here where phenotypically there are more rapid acetylators (blue color) than slow acetylators (red color), whereas when examined genotypically, there are less rapid acetylators (blue color) than slow acetylators (red color).

Table 1.

Comparison of the wild-type (wt) alleles and mutant (mut) alleles found for the different SNPs in HIV-negative (N = 407, alleles = 814) and HIV-positive volunteers (N = 97, alleles = 194).

	SNP	wt alleles	mut alleles	χ^2 value	χ^2 p-value	Remark
HIV-negative	191	709	105	0.744	0.3885	NS
HIV-positive		174	20			
HIV-negative	282	365	449	0.033	0.8569	NS
HIV-positive		89	105			
HIV-negative	341	602	212	0.003	0.9543	NS
HIV-positive[1]		143	49			
HIV-negative	481	659	155	0.09	0.9543	NS
HIV-positive[1]		153	39			
HIV-negative	590	592	222	0.007	0.934	NS
HIV-positive[1]		137	53			
HIV-negative	803	533	281	0.179	0.62727	NS
HIV-positive[1]		122	70			
HIV-negative	857	789	25	0.267	0.6053	NS
HIV-positive		190	4			

[1]There were missing data for a very few of the HIV-positive patients. NS = Not significant

Table 2.

Comparison of NAT2 Haplotype distribution between HIV-positive and HIV-negative Nigerians

Phenotype	Haplotype ID	Frequency in overall population	Frequency in HIV-positive	Frequency in HIV-negative	Chi Square	p Value
Slow	NAT2*6A	0.270	0.279	0.268	0.098	0.7548
Slow	NAT2*5B	0.184	0.186	0.184	0.003	0.955
Fast/rapid	NAT2*13A	0.142	0.140	0.143	0.009	0.9232
Slow	NAT2*14B	0.101	0.086	0.105	0.628	0.4281
Fast/rapid	NAT2*12A	0.083	0.093	0.080	0.328	0.5668
Fast/rapid	NAT2*4	0.082	0.091	0.079	0.29	0.5902
Slow	NAT2*5C	0.073	0.064	0.075	0.274	0.6007
Slow	NAT2*7B	0.027	0.020	0.029	0.445	0.5048
Slow	NAT2*14A	0.022	0.013	0.024	0.847	0.3575
Fast/rapid	NAT2*12B	0.005	0.011	0.004	1.294	0.2553
Slow	NAT2*6O	0.002	0.003	0.002	0.048	0.8274
Slow	NAT2*5A	0.002	0.005	0.001	1.125	0.2888
Slow	NAT2*6E	0.002	0.005	0.001	1.24	0.2654
Slow	NAT2*6N	0.002	0.002	0.002	0.043	0.8351
Unknown	NOT DESCRIBED	0.001	0.000	0.002	0.162	0.687

Total slow haplotypes: 66.3% HIV-positive, 69.1% HIV-negative (overall = 68.8%)

Table 3.

Association of NAT2 haplotypes to slow acetylation phenotype in a subset (49 HIV-negative volunteers) of the study population

Haplotype	Haplotype ID	Frequency	Chi Square	P Value
GTTCAAG	NAT2*6A	0.214	0.786	0.3753
GCCTGGG	NAT2*5B	0.214	3.69	0.0547
GTTCGAG	NAT2*13A	0.153	9.409	0.0022
ATTCGAG	NAT2*14B	0.133	0.869	0.3513
GCTCGGG	NAT2*12A	0.082	0.361	0.5478
GTTCGAA	NAT2*7B	0.082	4.628	0.0315
GCTCGAG	NAT2*4	0.051	2.799	0.0943
GCCCGGG	NAT2*5C	0.051	4.773	0.0289
ACTCGAG	NAT2*14A	0.02	3.843	0.0499

The Haploview software program is a computational tool for performing genomic association studies. Yellow highlights are identified haplotypes showing a strong association (p value ≤ 0.05) with slow acetylation phenotype. The acetylation phenotype was derived from the metabolic ratio of monoacetyldapsone to dapsone when dapsone was administered to the volunteers and then quantified in their plasma. Slow phenotypes are individuals whose metabolic ratio was ≤ 0.30 and rapid acetylators with ratios > 0.30.