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Pharmacogenetic testing for NAT2 genotypes in a Tanzanian population across the lifespan to guide future personalized isoniazid dosing

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Abstract

Despite updated recommendations for weight-based isoniazid dosing in children with drug-susceptible tuberculosis (TB) and higher dose isoniazid in regimens for adults with drug-resistant TB, individual pharmacokinetic variability can lead to sub-target isoniazid exposure. Host pharmacogenetics and isoniazid exposure remain understudied, especially in the East African population. We therefore employed a real-time polymerase chain reaction (qPCR) assay system to test genomic DNA extracted from saliva samples targeting the NAT2 gene responsible for isoniazid metabolism to describe the frequency of human single nucleotide polymorphisms in NAT2 within populations of children and adults in Tanzania, ascribe those polymorphisms to acetylator phenotype, and correlate to serum isoniazid exposures. In adults treated with higher dose isoniazid, genotypes with a predicted allelic phenotype of slow or intermediate acetylation were able to achieve a 0.41 μg/mL higher C_{max} ($p = 0.018$) and a 2.9h*μg/mL higher AUC_{0-12} ($p = 0.003$) per mg/kg increase in isoniazid dosage versus adults with rapid acetylation phenotype. A similar relationship was not found in the younger age population as predicted by timing of NAT2

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Contributions of authors

All authors declare no competing interests. SKH, DJO and MVM conceptualized and designed the study. SGM and EM managed patient recruitment and oversaw the study sites. MHA and CAP performed pharmacokinetic analyses. MS, TW, AM, DM and JG contributed to data collection. SKH, JG and ERH verified the source data. MVM, DM, DJO and SKH performed statistical analysis. MVM, DJO, AT, PB, ERH and SKH interpreted the data. DJO, MVM and SKH wrote the first draft of the manuscript and DJO produced the figures. All authors revised and edited the final version of the manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tube.2022.102246.

maturation. This saliva based qPCR assay was fieldable to guide personalized isoniazid dosing in adults but not young children that may not have full NAT2 maturation and activity.

Keywords

Isoniazid; Pharmacogenetics; Pharmacokinetics; Acetylation; *N*-acetyltransferase

1. Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* is among the top 10 causes of death globally and routinely ranks as the number one cause of global infectious disease mortality. In 2020, TB incidence was approximately 10 million, including a decline a notification of new cases as a result of COVID-19 related disruptions in routine services, yet mortality was estimated to have increased at ~1.5 million deaths [1]. Despite the global burden of mortality attributed to TB, curative antibiotic regimens have existed for decades. Increasingly pre-clinical and clinical studies have determined that a considerable portion of TB treatment failure and mortality is driven by suboptimal pharmacokinetics/pharmacodynamics (PK/PD) [2].

A cornerstone drug to the conventional anti-TB regimen is isonicotinic acid hydrazide (isoniazid, INH). INH is selective in spectrum to mycobacteria, primarily inhibiting mycolic acid synthesis and disrupting cell wall construction, and exerting bactericidal effect against populations of *M. tuberculosis* in rapid growth phases [3]. The bactericidal activity of INH is concentration-dependent whereby higher serum peaks (C_{max}) or total area under the concentration time curve (AUC) associate with improved microbial kill. However, oral INH is rapidly absorbed and peaks early in the daily dosing interval [4], and much of the total exposure relates to its rapidity of metabolism by the arylamine *N*-acetyltransferase encoded on the human NAT2 gene [5]. NAT2 genotypes can vary considerably by population of study, and NAT2 maturation may not reach full activity until later childhood [6,7].

A new abundance of work has determined target serum thresholds for minimum exposure for anti-TB drugs including INH [2,8]. Yet despite WHO recommended weight-based dosing for INH in both drug-susceptible TB and higher dose INH for MDR-TB, we and others have observed considerable pharmacokinetic variability [9,10]. For instance, we previously reported that in a study of Tanzanian children undergoing TB treatment, children receiving increased dosing per revised World Health Organization (WHO) recommendations saw increased serum exposure of rifampin and pyrazinamide but not INH or ethambutol as compared to children receiving non-revised dosages [11]. Given that very little is known with regard to the pharmacogenetic epidemiology of NAT2 genotype distribution in East Africa more broadly and Tanzania specifically, we sought to describe the frequency of human single nucleotide polymorphisms (SNPs) in NAT2 within populations of children and adults in Tanzania, ascribe those polymorphisms to acetylator phenotype, and correlate to serum INH exposures.

Leveraging existing molecular diagnostics capacities at the Kilimanjaro Clinical Research Institute, we employed a real-time polymerase chain reaction (qPCR) based platform for

characterizing high fidelity SNPs within NAT2 and leveraging DNA extraction from simple saliva collection. This pharmacogenetics work was nested within a larger study of the effects of pharmacokinetic variability and TB drug resistance on treatment outcomes which conveniently allowed comparison of populations with a wide range of mg/kg dosing treated for both drug- susceptible and MDR-TB (clinicaltrials.gov identifier NCT03559582).

2. Materials and methods

Patient population:

Patients and/or guardians signed written informed consent for a protocol approved by the institutional review boards for human subjects research at the National Institute for Medical Research in Tanzania (NIMR/HQ/R.8a/Vol.IX/2120) and the University of Virginia (UVA-HSR-IRB #18452). Adults starting treatment for MDR-TB were recruited from Kibong'oto Infectious Diseases Hospital in the Kilimanjaro region of Northern Tanzania, and children less than 15 years of age starting treatment for drug-susceptible TB were recruited from Haydom Lutheran Hospital in the Manyara region of Northern Tanzania. All adults were confirmed to have pulmonary disease from *M. tuberculosis* with *rpoB* mutation by GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA USA) and putative rifampin resistance before starting on an individualized MDR-TB regimen. Children either had microbiologically confirmed *M. tuberculosis* by Xpert MTB/RIF or unconfirmed TB defined by the updated National Institutes of Health Consensus Clinical Case Definitions for TB research in children, and were started on weight based fixed dose combinations of rifampin, INH, pyrazinamide and ethambutol in an initiation phase of 2 months followed by rifampin and INH for another 4 months of continuation phase [12].

Nucleic Acid Extraction from Saliva:

Nucleic acid extractions and quantitative PCRs (described below) were carried out at the Kilimanjaro Clinical Research Institute in Moshi, Tanzania. DNA from saliva samples was collected using DNA Genotek Oragene kits (DNA Genotek Inc., Kanata, Ontario, Canada) and extracted using the PrepIT-L2P kit (DNA Genotek) at enrollment and within 3 days of treatment initiation, with a procedure modified from that of the manufacturer. Briefly, saliva samples were first incubated at 50 °C in a dry bath for 1 h 500 μL of heated sample was then mixed with 20 μL PrepIT-L2P reagent. Samples were then centrifuged and resulting supernatants were mixed with 100% ethanol before pelleting DNA by further centrifugation. DNA pellets were then washed with 70% ethanol before allowing the pellet to dry before resuspension with 50 μL of TE solution (10 mM Tris, 1 mM EDTA, buffered to pH8). An "extraction blank" in which nuclease-free water was utilized as the sample was included with each extraction set was used in order to monitor for contamination.

2.1. qPCR-based genotyping

Assays for ADME SNP targets were procured from Thermo Fisher (Thermo Fisher, Waltham, MA, USA) in a custom-designed plate with primers and probes pre-assembled in each well. NAT2 targets included: c.341 (C/T; rs1801280), c.590 (A/G; rs1799930), and c.803 (A/G; rs1208), high fidelity SNP targets deemed most predictive based on prior studies and in order to incorporate into a fieldable and later scalable assay [13].

Each 10 μL qPCR reaction included 5 μL 2x TaqPath ProAmp Master Mix (Thermo Fisher), 1 μL Nuclease-Free water, and 4 μL extracted nucleic acid. One column in each plate was reserved for no-template control (nuclease-free water as sample) and per testing session, one column was reserved for positive controls consisting of pooled control DNAs obtained from the Coriell Institute (Coriell Institute for Medical Research, Camden, New Jersey, USA). Control DNAs included NA20294, NA19189, NA19023, and NA19351. Cycling conditions were as follows: 30 s 60 °C, 5 min 95 °C, 40 cycles 15 at 95 °C and 1 min at 60 °C, with a final incubation of 30 s at 60 °C. Reactions were carried out in the Applied Biosystems ViiA 7 Real-Time PCR system (Thermo Fisher).

2.2. Conversion of NAT2 genotypes into NAT2 haplotypes and predicted phenotypes

Individual NAT2 genotypes at positions c.803, c.341 and c.590 were combined into haplotypes and then converted to a NAT2 predicted acetylator phenotypes of “rapid” or “slow” according to the indicated phenotypes available at the database of human NAT2 alleles hosted at the Democritus University of Thrace (https://nat.mbg.duth.gr/). Phenotypes derived from c.341 and c.590 haplotypes were predicted as driving phenotype over the c.803 haplotype in the individuals from this study. Thus, only pairs of c.341 and c.590 haplotype derived phenotypes thereafter were combined to give an overall predicted acetylator phenotype for each individual with <Rapid, Rapid ≥ Rapid, <Slow, Slow> = Slow, and <Rapid, Slow ≥ Intermediate.

2.3. Assaying for pharmacokinetic exposure

Blood collections occurred at weeks 2, 4, 8, and 24 after the start of medication. Collections were performed at 1, 2, 6 and 12 h after observed medication administration for adults and 1, 2, and 6 h after observed medication administration for children. Serum was stored at −80 °C prior to transport and testing. Serum drug concentrations were measured at the University of Florida Infectious Diseases Pharmacokinetics Laboratory. INH drug concentrations were measured by a validated LC MS MS assay [11]. C_{max} and estimated AUC over 12 h ($AUC_{0–12}$) were determined by noncompartmental analysis using Phoenix WinNonlin version 8.0 (Certara USA, Princeton, New Jersey), which allowed $AUC_{0–12}$ estimates in those with 6-h values within the elimination phase. Minimum targets for C_{max} (3 μg/mL) and AUC (52 h*μg/mL) were drawn from studies in drug-susceptible TB and have not been fully established for varying levels of INH resistance as occur in RR/MDR-TB [8].

Prior to this study, the expected drug content was validated for each of the fixed-drug concentration formulations. Briefly, individual tablets were crushed, weighed, and diluted in 1:1 methanol/water. The contents of each tablet were spiked into serum samples and assayed using high performance liquid chromatography as described above [11].

2.4. Comparison of NAT2 genotypes against INH pharmacokinetic exposures

C_{max} and $AUC_{0–12}$ were only considered for the time period in which the participant’s prescribed mg/kg dosage remained constant during the period in which there was also no significant weight change prompting a change in dosage. The C_{max} and $AUC_{0–12}$ values were averaged over that time and compared to their genotype distribution at each individual NAT2 locus. Further analyses were carried out by combining genotypes at c.341 and c.590

into their associated NAT2 alleles (i.e. *NAT2*4*, *NAT2*5*, or *NAT2*6*, [14]) and combining alleles into a predicted overall acetylation phenotype as detailed above. The pharmacokinetic data were stratified according to whether the participant possessed a predicted Rapid phenotype or a Slow/Intermediate phenotype. Data were further stratified by "age category" as a predictor of NAT2 enzyme maturity [15]. Participants were classified as age category 1 if they were younger than 5.3 years old (NAT2 enzyme immature) at baseline and age category 2 if they were 5.3 years old or older at baseline (NAT2 enzyme mature). Analyses were carried out in Microsoft Excel v16.16.27 and R 4.0.2.

3. Results

3.1. Genotype/Allele frequencies and predicted NAT2 acetylator phenotypes

The parent study aimed to enroll 50 children receiving standard anti-tuberculosis treatment for drug-susceptible TB and 125 adults with MDR-TB. Nucleic acids were successfully extracted from all saliva samples collected from 50 children and 124 adults. The characteristics of the source populations are described in Table 1. Briefly, the median age of adults was 42 years while that of children was 2.3 years. The majority of the adult population were males while the pediatric population was nearly evenly split between males and females. Both populations reported having approximately 90 days of preceding illness at the time of TB diagnosis. Underlying health conditions were largely experienced by the adults with 38.3% living with human immunodeficiency virus (HIV) and 32.5% reporting ever having smoked (Table 1).

Successful NAT2 genotyping was achieved for all extracted DNA samples. Despite geographically proximal sites of enrollment, there were some differences between the pediatric and adult populations when examining NAT2 genotype frequencies. At c.341, the pediatric population had a higher proportion of individuals homozygous for the reference T variant as compared to the adult population (54.0% vs. 41.9%, respectively; Table 2a). The pediatric population also had a lower proportion of heterozygous individuals (genotype CT, 30.0%) versus the adults (46.8%). The two populations also differed from one another at the c.590 locus, with the pediatric population having fewer individuals homozygous for the reference G variant (38.0% vs. 52.4%) and more individuals heterozygous AG at this position (60.0% vs. 47.6%) as compared to the adult population. Despite the differences in genotype proportions at individual NAT2 loci, differences nearly disappeared upon conversion to predicted acetylator phenotypes with 14.5% and 16% having a predicted Rapid phenotype and 85.5% and 84% having a Slow or Intermediate phenotype in the adult and pediatric populations, respectively (Table 2b). These proportions were somewhat similar to those previously documented in Africa (Table S1).

3.2. Relationship of NAT2 genotype alone and INH pharmacokinetics by age category

Among the 124 adults with MDR-TB and genotyping data, there were 30 participants treated with high dose INH and included in the comparative pharmacokinetic analysis. The median INH dosage for the pediatric population was 5.9 mg/kg (range 2.1–18.4) while the median dosage for the adult population was 18.3 mg/kg (range 8.1–36.8). As shown in Fig. 1A, the majority of participants (n = 55, 66.3%) achieved a minimum C_{max} target of 3

μg/mL averaged over the treatment duration. Yet 36/55 (65.5%) of participants achieving this target were from Age Category 2. Only 19 of 80 (23.8%; Fig. 1B) of participants were able to achieve a 52 h*μg/mL target AUC value, with 17/19 (89.5%) coming from Age Category 2. However there were no statistically significant differences in C_{max} or $AUC_{0–12}$ values across individual loci (Tables S2A and S2B).

3.3. Relationship of predicted acetylator phenotype and INH pharmacokinetics

When analyzing the entire population without age categorization, the median C_{max} for participants with a Rapid acetylation phenotype compared to Slow/Intermediate phenotypes (4.41 vs. 3.98 μg/mL, respectively), and median $AUC_{0–12}$ (20.74 vs. 22.93 h*μg/mL, respectively) demonstrated no statistically significant differences. However, as shown in Fig. 2A and B, the median C_{max} and $AUC_{0–12}$ values for those individuals in Age Category 2 were higher with both acetylation types. These differences were statistically significant (Tables 3A and 3B).

Understanding that one of the primary drivers of PK exposure is mg/kg dosing, we incorporated the mg/kg dosage along with visualizing the age category to determine if NAT2 alleles and predicted phenotypes influenced the slope of expected increase in PK exposure with increasing mg/kg dosage. When C_{max} vs. mg/kg dosage and $AUC_{0–12}$ vs. mg/kg dosage are stratified by predicted phenotype, as shown in Fig. 3A and B, the slope of the lines generated by Slow and Intermediate acetylators are significantly steeper than those for Rapid acetylators. As visualized in Fig. 3A and B, these differences are driven largely by individuals in the older age category. Slow and Intermediate acetylators achieve 0.41 μg/mL higher C_{max} ($p = 0.018$) and a 2.9h*μg/mL higher $AUC_{0–12}$ ($p = 0.003$) per mg/kg increase in INH dosage compared Rapid acetylators (Table 4).

4. Discussion

The major findings of this study were that qPCR NAT2 genotypes from saliva samples found a predicted acetylator phenotype distribution similar to those in other locations previously reported from Africa (Table S1), and that predicted allelic phenotypes of Slow/Intermediate or Rapid influenced the slope increase in INH C_{max} and $AUC_{0–12}$ with increasing mg/kg dosage. Importantly however, age also had a significant impact on INH exposure, likely related to higher mg/kg doses in adults and NAT2 maturation status, whereby acetylator phenotype contributed most to INH exposure for adults receiving higher mg/kg dosages. These results are consistent with prior studies in adult populations including those in South Africa, India, Poland, and China with respect to the influence of genotype/phenotype and INH exposure parameters [16–19].

We suspect that the specific sub-analysis of the pediatric cohort failing to show that predicted acetylator phenotype to have a significant effect on INH exposures was due to the median childhood age of 2.2 years, which was below the 5.3 year old cutoff previously demonstrated to be associated with NAT2 maturity and adult-like activity [15]. These results from our pediatric population would appear to be consistent with two studies of South African children in which age, NAT2 maturation status, and dosage had a larger influence on INH kinetics than NAT2 genotype and predicted acetylator status alone [16,20].

Interestingly, a study of Ghanaian children showed a pharmacogenetic/pharmacokinetic relationship more akin to that of adults [21]. This might be explained by the higher median age in the Ghanian study and increased INH mg/kg dosage compared to that of the children in the present study [22].

Given that inter-individual differences in ADME physiology and other external environmental or host factors make it difficult to successfully implement a one-size-fits-all strategy to weight-based dosing, understanding an individual's pharmacogenetic background can importantly account for one fixed factor when considering the dose and medication composition of long-term regimens. Furthermore, while sub-target PK exposure can adversely impact treatment efficacy, exposure-related adverse events are also major causes of side effects and treatment interruption. Notably, individuals classified as having the slow acetylator phenotype are at increased risk of INH-induced hepatotoxicity [23]. Yet with pre-dosing knowledge of NAT2 genotype, investigators from Japan were able to demonstrate that adjusting INH dosage according to NAT2 genotype resulted in minimizing treatment failures while avoiding INH-induced liver injury [24]. Given the increasing availability of qPCR capacity, as well as the ease and non-invasive nature of saliva collection, NAT2 genotype-based dosing may ultimately obviate the need for assays requiring more invasive serum collection or labor-intensive PK assays that utilize chromatography or mass spectrometry. This would cut down the need for cold transport and shipment and provide a simpler screening mechanism to identify individuals requiring more specialized testing.

Instructively, our initial examinations of individual NAT2 loci or even whole NAT2 haplotypes did not reveal any statistically significant relation to pharmacokinetic parameters. While the trend of increased C_{max} and $AUC_{0–12}$ INH levels amongst Slow and Intermediate acetylators versus Rapid acetylators was consistent with prior studies in South African [16] and Polish populations [18], only after integrating mg/kg dosage did we demonstrate statistically significant differences in the rates of change of both C_{max} and $AUC_{0–12}$ per mg/kg increase in INH. These lower rates of change in both C_{max} and $AUC_{0–12}$ parameters would be consistent with prior research demonstrating that individuals with a predicted Rapid acetylation phenotype clear INH at an increased rate as compared with predicted Intermediate or Slow acetylation phenotypes [25]. In addition, Kinzig-Schippers et al. previously demonstrated that as much as 88% of INH clearance variability could be explained by NAT2 genotypes, albeit this study was performed in adult Caucasians [26]. We chose to compare predicted acetylator phenotypes to C_{max} and AUC rather than clearance, as C_{max} and AUC are most associated with microbial kill and prevention of resistance, and target exposures have previously been described.

A key limitation of the study was the sample size drawn from a convenient cohort. Post-hoc power calculations based on observed distributions of Rapid and Slow/Intermediate acetylators for prediction of $AUC_{0–12}$ revealed a power of 0.72 which is lower than the often targeted 0.80 beta-value. Furthermore, the relatively small number of genetic loci examined could have been explored more comprehensively with a sequencing approach.

In summary, NAT2 genotyping utilizing a qPCR platform from saliva samples found a genotype distribution in Tanzania where predicted allelic phenotype significantly influenced

INH PK exposure for adults on higher mg/kg doses. A screening test for NAT2 genotype may therefore guide personalized dosing of INH now currently used in numerous regimens for drug-susceptible and MDR-TB.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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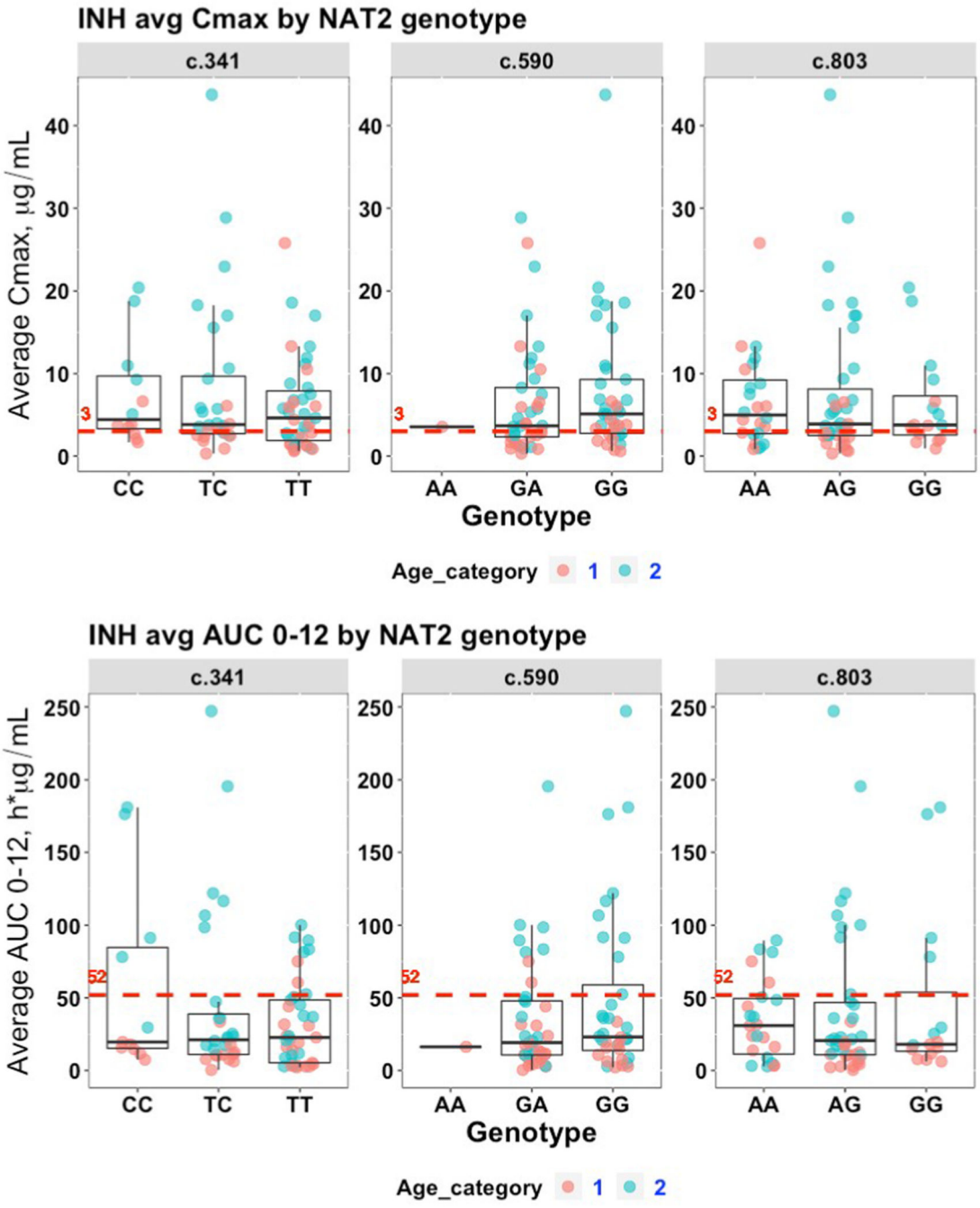

Fig. 1. INH exposure analyzed by genotypes at three NAT2 loci.

Available average C_{max} (µg/mL) and average $AUC_{0–12}$ (h*µg/mL) values were analyzed by genotype at each of three NAT2 SNP loci. Each marker represents an individual participant. Those having a non-mature NAT2 phenotype as measured by age less than 5.3 years of age ("Age Category 1") represented by red markers and blue markers representing individuals 5.3 years of age or older ("Age Category 2"). A) C_{max}; B) $AUC_{0–12}$. The red, dashed line in each subplot represents the target expected exposure level for adult patients.

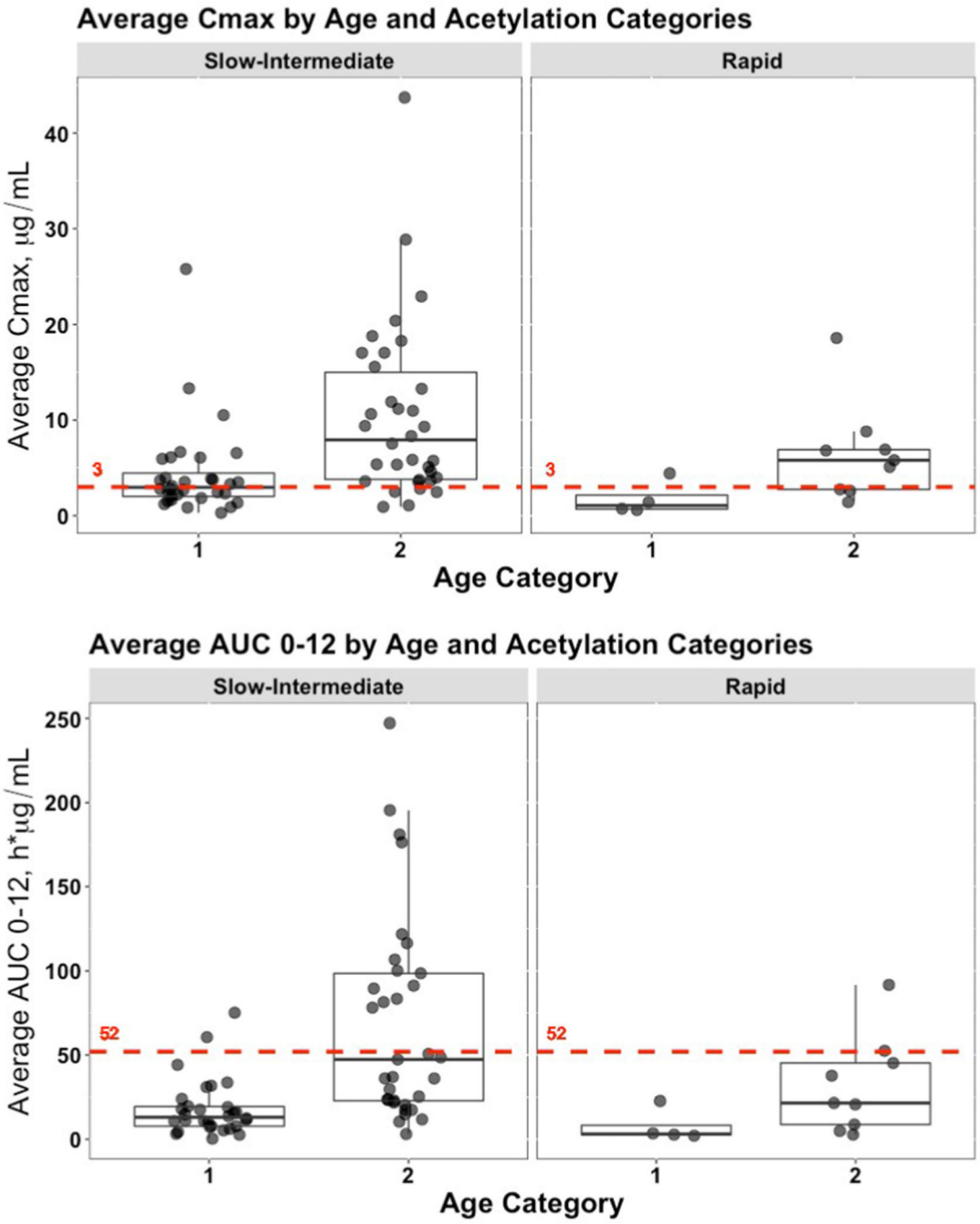

Fig. 2. INH exposure analyzed by predicted NAT2 acetylator phenotype.

Genotype information at c.341 and c.590 were converted to NAT2 haplotypes/alleles and predicted NAT2 acetylator phenotypes and then used to analyze available average C_{max} (µg/mL) and average $AUC_{0–12}$ (h*µg/mL) values. Data was further stratified according to the same age categories used in Fig. 1. Each marker represents an individual participant. A) C_{max}; B) $AUC_{0–12}$. The red, dashed line in each subplot represents the target expected exposure level for adult patients.

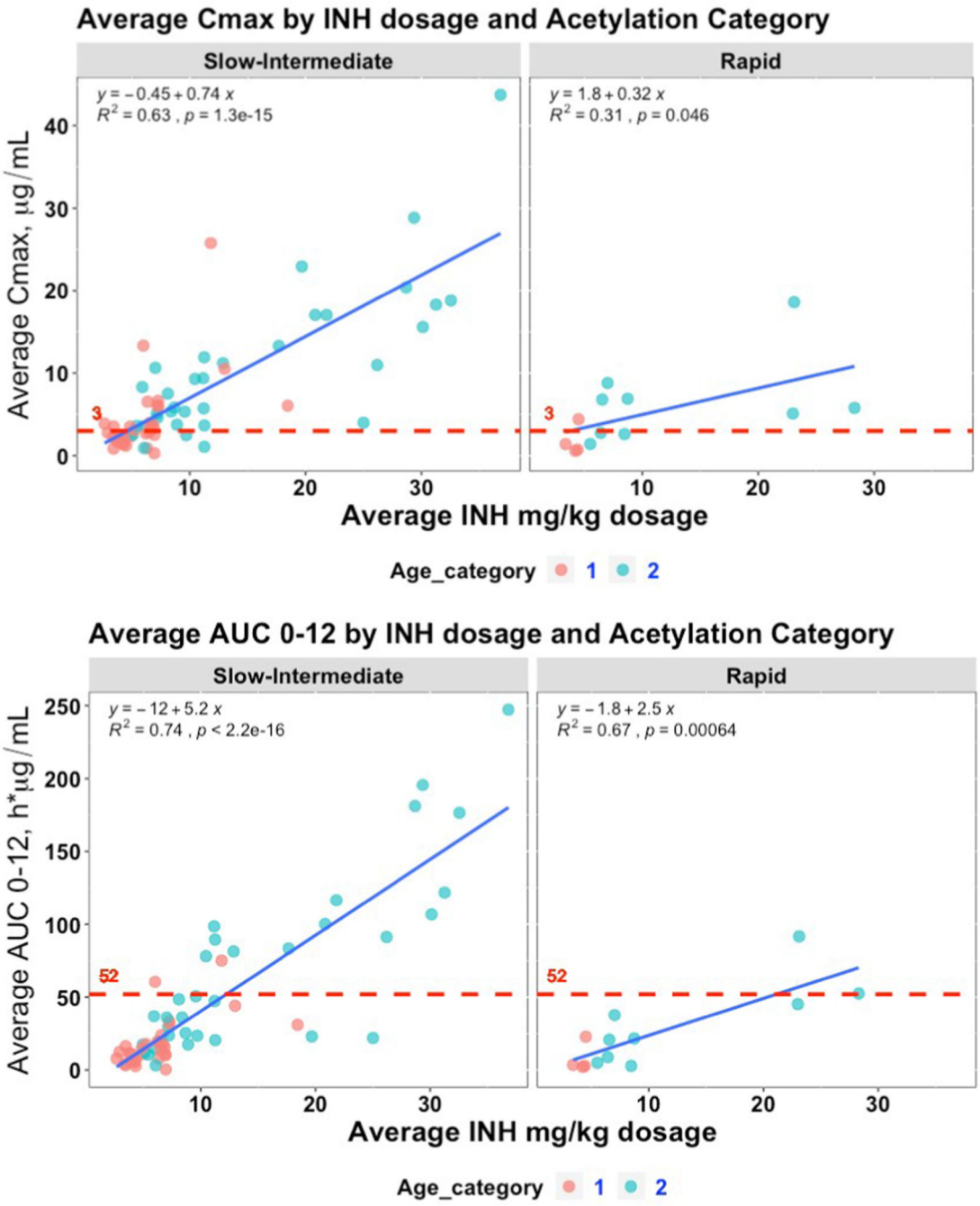

Fig. 3. INH exposure analyzed by mg/kg dosage and predicted NAT2 acetylator phenotype.

Average C_{max} (µg/mL) and average $AUC_{0–12}$ (h*µg/mL) values were first stratified by predicted NAT2 acetylator phenotype and then plotted against the averaged mg/kg INH dosage given to each patient. Each marker represents an individual participant with individuals with a non-mature NAT2 phenotype as measured by age less than 5.3 years of age (“Age Category 1”) represented by red markers and blue markers representing

individuals 5.3 years of age or older ("Age Category 2"). A) C_{max}; B) $AUC_{0–12}$. The red, dashed line in each subplot represents the target expected exposure level for adult patients.

Table 1

Demographic information for the study participants.

	Overall	Kilimanjaro Tanzania, Adult MDR	Manyara Tanzania, Pediatric
Participants	174	124	50
Median Age at baseline, years (range)	34 (0.3–80)	42 (18–80)	2.3 (0.3–14.8)
Sex, %M	60.7	65.3	48
Median Height at baseline, cm (range)	157 (59.5–184)	164.5 (133.6–184)	79.9 (59.5–151)
Median Mass at baseline, kg (range)	44.1 (5–81)	49 (27–81)	8.6 (5–29.3)
Median days ill at baseline (range)	90 (5–400)	91 (7–370)	90 (5–400)
HIV+, %	27.2	38.3	0
Diabetes, %	1.7	2.4	0
Ever smoked, %	23.7	32.5	2.0

Table 2A

Genotype frequenciesat NAT2 loci tested.

	Kilimanjaro Tanzania, Adult MDR n = 124	Manyara Tanzania, Pediatric n = 50
rs1801280, c.341 T- > C		
TT	41.9	54.0
TC	46.8	30.0
CC	11.3	16.0
rs1799930, c.590 G- > A		
GG	52.4	38.0
GA	47.6	60.0
AA	0	2.0
rs2282143, c.803 A- > G		
AA	29.8	34.0
AG	50.0	46.0
GG	20.2	20.0

Table 2B

Frequencies of predicted NAT2 acetylation phenotypes.

NAT2 Phenotype	Northern Tanzania, Adult, MDR, n = 124	Northern Tanzania, Pediatric, n = 50
Rapid[a]	18 (14.5)	(16.0)
Intermediate-Slow[a]	106 (85.5)[b]	42 (84.0)[b]

[a]NAT2 *4/*4 = Rapid; *4/*5 and *4/*6 = Intermediate; *5/*5, *5/*6 and *6/*6 = Slow.

[b]Includes participants whose exact allelic makeup could not be determined due to heterozygosity at both c.341 and c.590.

Table 3A

Frequencies and summary statistics for c_{max} as analyzed by predicted acetylator phenotype and age category.

Predicted Acetylator Phenotype	Age Category	Participants achieving <3 μg/mL	Participants achieving ≥3 μg/mL	Median C_{max}, μg/mL	IQR, μg/mL	p-value, Wilcoxon Rank Sum Test for Age Category
Slow-Intermediate	1	16	16	2.97	2.44	8.89×10^{-5}
	2	5	29	7.91	11.19	
Rapid	1	3	1	1.07	1.46	0.020
	2	3	6	5.78	4.14	

The p-value for the Wilcoxon Rank Sum Test comparing the overall Cmax difference between predicted acetylation categories is 0.35.

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Table 3B

Frequencies and summary statistics for $AUC_{0–12}$ as analyzed by predicted acetylator phenotype and age category.

Predicted Acetylator Phenotype	Age Category	Participants achieving <52 h*µg/mL	Participants achieving ≥52 h*µg/mL	Median $AUC_{0–12}$, µg/mL	IQR, µg/mL	p-value, Wilcoxon Rank Sum Test for Age Category
Slow-Intermediate	1	28	2	13.10	11.71	1.30×10^{-6}
	2	19	14	47.33	75.63	
Rapid	1	4	0	3.15	5.70	0.076
	2	7	2	21.56	36.46	

The p-value for the Wilcoxon Rank Sum Test comparing the overall AUC0–12 difference between predicted acetylation categories is 0.14.

Table 4

Comparison of Pharmacokinetic Exposures Explained by mg/kg Dosage and Predicted NAT2 Acetylation Phenotype.

Site	Slope	P value, ANCOVA Dosage*phenotype
C_{max}, μg/mL per mg/kg change		
Overall	0.67	
Slow-Intermediate	0.73	0.018
Rapid	0.32	
AUC_{0-12}, h*μg/mL per mg/kg change		
Overall	5.0	
Slow-Intermediate	5.4	0.003
Rapid	2.5	