Diabetes is associated with genotypically drug-resistant tuberculosis

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Diabetes is associated with failure of tuberculosis (TB) treatment, but it is unclear whether this is related to genotypic drug resistance of the infecting mycobacteria. We used whole genome sequencing (WGS) to examine 1,365 known drug resistance mutations in 896 *Mycobacterium tuberculosis* isolates from TB patients that were screened for diabetes using HbA1c testing as part of the TANDEM project [1]. Ethical approval was received from the London School of Hygiene and Tropical Medicine and institutional review boards in Indonesia and Peru. In Peru we selected all available *M. tuberculosis* isolates from TANDEM patients (44 with and 445 without diabetes), and in Indonesia we selected all available isolates from diabetic patients (n=115) plus a subset of isolates from non-diabetic patients (n=292) from the same clinics, during the same time period, frequency-matched by age. We used TB Profiler version 0.3.8 [2] to determine *M. tuberculosis* lineage and drug resistance. A phylogeny was constructed using PhyML version 3.0 [3], and the minimum pairwise distance for isolates was calculated separately for patients with and without diabetes, stratified by country. We examined if diabetes was associated with genotypic drug resistance against individual drugs or with MDR-TB for the two countries separately and combined, with multilevel multivariable logistic regression, taking into account the country of origin and adjusting for age, gender, HIV-infection, previous TB treatment, and *M. tuberculosis* lineage. In additional analyses we examined the effect of HbA1c level, and stratified results for new versus known diabetes. For comparison of specific drug resistance mutations we used univariate and country-stratified analysis because of small numbers. We also addressed the hypothesis that isolates from patients with diabetes would have fewer mutations compensating for loss of fitness associated with drug resistance, noting that diabetes leads to lower host immune defence against *M. tuberculosis* [4]. For this analysis, all non-synonymous SNPs in *rpoA*, *rpoB* outside the rifampicin resistance-determining region (RRDR), *rpoC*, *ahpC* promoter region and *ubiA* were considered as mutations potentially compensating for the loss of fitness caused by drug resistance mutations [5]. More detailed methods are provided as supplementary material (10.6084/m9.figshare.9884303).

Diabetes was more common among Indonesian patients, and drug resistance more common in Peru. TB patients with diabetes were older, more often female, and slightly heavier than TB patients without diabetes, and fewer diabetic patients reported a previous history of TB treatment compared to those without diabetes (Table). Diabetes was not associated with a particular *M. tuberculosis* lineage or with genotypic clustering; the median minimum pairwise distance for isolates from diabetic and non-diabetic patients was 164 respectively 161 SNPs in Indonesia, and 98 respectively 67 SNPs in Peru. Drug resistance mutations were found in isolates of 21 / 115 (18%) diabetic and 44 / 292 (15%) non-diabetic patients in Indonesia, and

17 / 44 (39%) diabetic and 88 / 445 (20%) non-diabetic patients in Peru. In multilevel multivariable logistic regression, diabetes was the only factor significantly associated with genotypic drug resistance against at least one drug (OR 1.8; 95% CI 1.1-2.9). The association between diabetes and drug resistance was similar for patients with new (adjusted OR 2.0; 95% CI 0.9-4.4) and previously diagnosed diabetes (OR 1.7; 95% CI 0.98-2.9), and not dependent on the HbA1c level (not shown). The relation between diabetes and resistance was still present after exclusion of 19 HIV-infected patients (adjusted OR 1.8; 95% CI 1.1-2.9). At the level of individual drugs, diabetes was significantly associated with rifampicin resistance (OR 2.5; 95% CI 1.2-5.3; Supplementary Figure (10.6084/m9.figshare.9884303)), also among patients not previously treated for TB (OR 3.1; 95% CI 1.2-8.3, data not shown). We also found more fluoroquinolone resistance in diabetic TB patients, and this difference reached statistical significance for Peru (OR 6.69; 95% CI 1.37-32.68; Supplementary Figure). We did not find evidence of interaction between rifampicin and fluoroquinolone resistance in the multilevel multivariable model ($P_{interaction}$ = 0.232, data not shown). Finally, although this association did not reach statistical significance, the odds of MDR-TB were twice as high in diabetic versus non-diabetic patients (OR 2.09; 95% CI 0.92-4.77; Supplementary Figure).

Examining individual drug resistance mutations (Table), diabetes among TB patients in Peru was associated with more mutations in *Rv1482c-fabG1* (p<0.01), which confers resistance to isoniazid and ethionamide, and *gyrA* (P<0.05), which accounts for fluoroquinolone resistance; *rpoB* mutations leading to rifampicin resistance also appeared more common in diabetes patients (15.9% vs. 9.4%; p=0.069). In Indonesia, drug resistance was less common and no significant association was found between diabetes and specific resistance mutations. With regard to compensatory mutations, no differences were found in the association between isoniazid or rifampicin resistance mutations and potential compensatory mutations between patients with and without diabetes, and no interaction was found between diabetes and the presence of compensatory mutations (data not shown).

Our findings are in line with previous studies that used phenotypic drug susceptibility testing (DST) and focused on isoniazid and rifampicin (reviewed in Tegegne *et al*. [6]). Several factors might account for the observed association between diabetes and drug resistance mutations. First, people with diabetes might be at higher risk of nosocomial transmission of drug-resistant tuberculosis in low-resource settings [7]; unfortunately we did not have the data to compare prior hospitalization for diabetes compared to non-diabetes tuberculosis patients. Second, lower rifampicin plasma concentrations among diabetes patients that were found in some [8] but not all [9] studies might lead to acquisition of drug resistance. However, all isolates in our study were collected before start of treatment, only 26% of patients with a drug-resistant

isolate reported an episode of previous TB treatment that might have resulted in acquired drug resistance mutations, and differences were also present among patients with a first episode of TB. We cannot exclude misclassification of TB treatment history, as this was self-reported and as some patients previously treated for TB may in fact have had another illness. However it is unlikely that possible misclassification of patients regarding TB treatment history explains the association between drug resistance mutations and diabetes, as it was evident both among patients with and without a history of previous TB treatment. Third, recent papers have found an interaction between drug resistance and cellular immunometabolism [10], and this interaction might be altered by diabetes. Fourth, similar to HIV, reduced host defence in people with diabetes might increase the risk of developing active TB caused by *Mycobacterium tuberculosis* strains with drug resistance mutations associated with loss of fitness [11], although we could not confirm this when looking at previously reported resistance-compensating mutations. The trend towards more fluoroquinolone resistance in TB patients with diabetes could be related to frequent use of fluoroquinolones for respiratory infections, which may be more common for those with diabetes [12].

In contrast to studies using phenotypic DST, WGS allowed us not only to investigate the association between diabetes and drug resistance at the gene level, but also to take the diverse *M. tuberculosis* genetic background into account. However, our study could not prove if diabetes is associated with more transmission of drug-resistance, as the sampling fraction of *M. tuberculosis* isolates was probably too low to identify transmission clusters. Besides higher rates of transmission, diabetes may also lead to more TB reactivation caused by drug-resistant strains.

In summary, for the first time, we used *M. tuberculosis* whole genome sequencing data from two countries to study the association between diabetes and genotypic drug resistance in TB patients. Diabetes was associated with an increased risk of disease caused by strains with resistance mutations, particularly against rifampicin, but also against isoniazid, ethionamide and fluoroquinolones. Higher rates of resistance mutations among diabetic TB patients could not be explained by previous TB treatment; the association between diabetes and resistance was also evident among patients with a first episode of TB. TB patients with diabetes should be prioritized for DST in settings where it is not performed for all patients, and more (molecular) epidemiological and mechanistic studies are needed to unravel the factors explaining the association between diabetes and TB drug resistance.

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Table. Patient characteristics and drug-resistance mutations stratified for country and diabetes

		INDONESIA		PERU	
		Diabetes (N=115)	**No diabetes (N=292)**	**Diabetes (N=44)**	**No diabetes (N=445)**
PATIENT CHARACTERISTICS					
Male gender		52 (45%)	168 (58%)	25 (57%)	268 (60%)
Age (years) - median (IQR)		50 (45-58)	39 (33-48)	52 (42-59)	28 (22-39)
Previous TB treatment		24 (21%)	79 (27%)	6 (14%)	96 (22%)
Previously diagnosed diabetes		83 (72.2%)	NA	32 (72.7%)	NA
Newly diagnosed diabetes		32 (27.8%)	NA	12 (27.3%)	NA
HIV infection		0	0	1 (2.3%)	18 (4.0%)
M. tuberculosis lineage					
East-Asian		40 (34.8%)	90 (30.8%)	7 (15.9%)	59 (13.3%)
Euro-American		69 (60.0%)	178 (61.0%)	37 (84.1%)	386 (86.7%)
Indo-Oceanic		5 (4.3%)	24 (28.2%)	0	0
M. bovis		1 (0.9%)	0	0	0
DRUG RESISTANCE MUTATIONS					
Any drug resistance		21 (18.3%)	44 (15.1%)	17 (38.6%)	88 (19.8%)
Drug	***Gene***				
Isoniazid	*katG*	8 (7.0%)	19 (6.5%)	6 (13.6%)	48 (10.8%)
	Rv1482c-fabG1	3 (2.6%)	10 (3.4%)	8 (18.2%)**	23 (5.2%)**
	ahpC	0	0	1 (2.3%)	0
Rifampicin	*rpoB*	9 (7.8%)	10 (3.4%)	7 (15.9%)	42 (9.4%)
Ethambutol	*embB*	3 (2.6%)	6 (2.1%)	5 (11.4%)	30 (6.7%)
	embC-embA	0	0	1 (2.3%)	6 (1.3%)
	embR	0	1 (0.3%)	0	0
Streptomycin	*rpsL*	4 (3.5%)	5 (1.7%)	1 (2.3%)	16 (3.6%)
	rrs	1 (0.9%)	2 (0.7%)	0	5 (1.1%)
Pyrazinamide	*pncA*	7 (6.1%)	14 (4.8%)	3 (6.8%)	22 (4.9%)
Ethionamide	*Rv1482c-fabG1*	3 (2.6%)	10 (3.4%)	8 (18.2%)**	22 (4.9%)**
	ethA	0	0	0	1 (0.2%)
Fluoroquinolones	*gyrA*	3 (2.6%)	3 (1.0%)	4 (9.1%)*	7 (1.6%)*
	gyrB	0	0	0	2 (0.4%)
Amikacin	*rrs*	0	0	0	5 (1.1%)
Capreomycin	*tlyA*	0	1 (0.3%)	0	7 (1.6%)
	rrs	0	0	0	5 (1.1%)
Kanamycin	*eis-Rv2417c*	1 (0.9%)	1 (0.3%)	1 (2.3%)	1 (0.2%)
	rrs	0	0	0	5 (1.1%)
Para-aminosalicylic acid	*folC*	0	2 (0.7%)	1 (2.3%)	2 (0.4%)
	thyA	0	0	0	1 (0.2%)

Drug resistance data represent the number (%) of isolates with at least one mutation in the respective gene.

* P-value <0.05; ** P-value <0.01 (p-values are Chi-square p-values unless the expected number of resistant isolates equalled less than 5, in which case the Fisher's Exact Test p-value was calculated).

IQR: interquartile range; HIV: human immunodeficiency virus; NA: not applicable.

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